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Sustainable Development: Approaches to long-term sustainability in economic, environmental, and social sectors.
Education for Sustainability: Research on how education systems can foster sustainability awareness and action.
Technological Innovations: Advances in science and technology that support sustainable solutions.
Interdisciplinary Approaches: Bridging multiple fields to address complex sustainability challenges.

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ABOUT THE COVER

Nephelium ramboutan-ake locally called "duro" is a tropical fruit-bearing plant in the family Sapindaceae that is native to several Southeast Asian countries, including the Philippines. Both its fruits and seeds are traditionally consumed as food, while its medicinal potential remains largely unexplored. In the study by Villaluz et al. featured in this issue, phytochemical screening of "duro" leaf extracts revealed the presence of alkaloids, flavonoids, saponins, and tannins. The extracts also exhibited moderate antibacterial activities.

Photo credits: Olive Jane T. Villaluz et al.

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EDITORIAL

Bridging Research and Policy for Sustainable Development in the Philippines

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Dear Readers,

This issue presents a collection of studies that reflect the growing breadth and depth of research addressing pressing environmental, socio-economic, and educational challenges in the Philippines. Taken together, these works collectively underscore a critical imperative: the need to more effectively translate scientific research into actionable policies. Across diverse fields, these contributions provide evidence-based insights that can directly inform decision-making, program design, and institutional reform.

In the domain of coastal and marine resource management, several studies point to both opportunities and urgent risks. Research on alternative aquaculture practices, such as bamboo substrates for abalone culture and improved survival strategies for blue swimming crab, offers practical, low-cost innovations that can be scaled through targeted government support and extension services. At the same time, findings on harmful algal species, such as *Pyrodinium bahamense*, along with assessment of mangrove biodiversity and reports of dugong strandings, highlight the need for strengthened coastal monitoring systems, stricter environmental regulation, and sustained investment in ecosystem conservation. Complementing these efforts are studies on aquatic animal health and regeneration, including evidence that *Aloe vera* extracts enhance wound healing and fin regeneration in zebrafish. Such findings demonstrate the potential of plant-based, environmentally friendly interventions to support the aquaculture and ornamental fish industries while reducing dependence on synthetic products. These are not isolated scientific observations, but are policy signals requiring coordinated national and local action.

Equally significant are studies that explore agriculture and food systems, and consumer behavior, which further reveal structural gaps that demand policy attention. Evidence on consumer preferences across income groups points to inequalities in access and affordability that must be addressed through inclusive market policies and value chain interventions. Meanwhile, documented challenges in agricultural extension services, particularly in geographically and politically complex areas such as Basilan, underscore the need to strengthen institutional capacity, improve coordination among agencies, and ensure that frontline officers are adequately supported. Without such reforms, even the most well-designed agricultural programs risk limited impact. Research on the phenotypic characterization of the Paraoakan chicken, a unique native breed of Palawan, likewise underscores the importance of preserving local genetic resources that support rural livelihoods and food security. The study provides baseline information that can guide breeding, conservation, disease management, and livestock development programs, emphasizing the role of science in supporting resilient local food systems.

This issue also showcases advances in plant science and natural product research, which highlight the untapped potential of the Philippines' biological resources. Studies examining agromorphological and phytochemical variations of *Orthosiphon aristatus*, as well as antibacterial and antioxidant properties of indigenous plants such

as “duro” and Tabon-tabon, highlight the importance of documenting and valorizing local biological resources. However, realizing this potential requires clear policy frameworks on bioprospecting, intellectual property rights, and sustainable resource management. Strategic investment in research and development, coupled with support for local industries, will be essential to translate laboratory findings into real-world applications. Similarly, investigations into medicinal plants such as *Aloe vera* continue to expand opportunities for developing locally sourced, science-based health and aquaculture products, reinforcing the value of biodiversity-driven innovation.

Beyond the natural sciences, this collection brings attention to education and the human dimension of development, particularly in the context of rapid technological change. The documented “digital divide” between educators and students signals an urgent need for policy reforms in teacher training, curriculum design, and digital infrastructure. Similarly, findings on the reading competencies of pre-service teachers raise concerns about foundational skills that directly affect the quality of education delivery. At the same time, evidence demonstrating the positive role of artificial intelligence as a scaffold for language learning and critical thinking among university students illustrates how emerging technologies can be harnessed to improve educational outcomes when integrated through sound pedagogical frameworks. Such findings support the development of policies that promote responsible, inclusive, and evidence-based adoption of AI in higher education. These studies remind us that technological advancement must be matched with adaptive educational strategies and capacity building. Addressing these gaps requires systemic interventions, ranging from curriculum standardization to continuous professional development and targeted literacy programs.

The issue also contributes important insights into wildlife health and conservation. Findings on tick infestations in free-ranging Philippine pangolins provide valuable baseline information on parasite prevalence in one of the country’s most threatened endemic mammals. Such studies reinforce the importance of wildlife health surveillance, rescue and rehabilitation programs, and stronger conservation policies that integrate animal health into biodiversity management strategies. As emerging diseases and environmental pressures continue to threaten wildlife populations, science-based monitoring becomes increasingly essential for effective conservation planning.

Across these diverse contributions, a central message emerges: evidence must drive policy, and policy must enable implementation. The research presented here offers concrete, context-specific insights, but their value ultimately depends on whether they are integrated into planning processes, funding priorities, and governance frameworks. This calls for stronger linkages between academia, government agencies, and local communities, as well as mechanisms that facilitate the uptake of research into policy and practice.

As the Philippines confronts the intersecting challenges of environmental degradation, economic inequality, and social transformation, the role of science in policymaking cannot remain peripheral. The studies in this issue provide not only knowledge, but direction. The task ahead is clear: to move decisively from evidence to action.

CONTENTS

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Efficacy of bamboo as an alternative substrate for cage culture of abalone *Haliotis asinina* Linnaeus, 1758

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ABSTRACT

Abalone is a highly valued marine gastropod with a declining wild population due to increased fishing pressure. To meet market demand, there is growing interest in the cage culture of abalone juveniles. However, the most commonly used substrate in cage culture, polyvinyl chloride (PVC), has been linked to environmental and health risks. This study examined the efficacy of bamboo as an alternative substrate for the bottom and suspended cage culture of tropical abalone *Haliotis asinina* Linnaeus, 1758. Two culture experiments were conducted using (1) sea bottom cages and (2) suspended sea cages in Binduyan, Puerto Princesa City, and Pamantolon, Taytay, Palawan, respectively. Abalone juveniles grown with bamboo substrate (BS) had significantly higher weight gain, specific growth rate, and shell length growth rate compared to those reared with PVC substrate (PS) ($P < 0.05$). In addition, after 90 days and 150 days of culture, abalone juveniles on BS exhibited positive allometric growth compared to those on PS, which showed isometric growth. Moreover, the survival rates of abalone juveniles with BS were not significantly different from those with PS at $P < 0.05$. These findings suggest that BS is a viable alternative to PS for abalone juvenile culture, as it is indigenous, inexpensive, and environmentally friendly. The study's results can promote sustainable aquaculture practices for abalone while raising awareness of the potential environmental and health risks associated with PVC for cultured abalone and humans consuming cultured abalone.

Keywords: allometric, bottom sea cage, growth rate, isometric, PVC, suspended sea cage

INTRODUCTION

The tropical abalone *Haliotis asinina* Linnaeus, 1758, is not only palatable but also considered a nutraceutical due to its many health benefits. It contains various bioactive molecules, such as polysaccharides, proteins, and fatty acids that provide many health benefits, including antioxidant, anti-cancer, anti-inflammatory, anti-thrombotic, anti-microbial, anti-aging, anti-arthritic, and anti-hypertensive properties (Suleria et al. 2017a, b). Moreover, its shells can be used to make jewelry and ornaments (Surtida 2000). Strong domestic and overseas demand has made abalone a highly valued marine gastropod (Setyono 2005). The high market value of abalone has led to increased fishing pressure, causing a dramatic decline with wild population (Smith 2022; Gonzales 2015). Consequently, significant interest has emerged in the cage culture of

abalone juveniles to meet market demands and their high export potential.

Since the early 1990's, *H. asinina* broodstock has been successfully bred in Philippine hatcheries to support the increasing demand for abalone juveniles (Fermin 2001). Growth and spawning occur year-round, with fast growth and high survival rates in both land-based and sea-based culture systems (Fermin 2001). Despite advancements in abalone juvenile culture in the Philippines, continuous improvements are needed for optimal results. One aspect worth examining is the use of polyvinyl chloride (PVC) substrates. An appropriate substrate provides a suitable space for abalone juveniles to shelter and forage for food, day and night (Setyono 2015). Several studies have examined the use of PVC substrates (PS) in both on land-based and sea-based abalone juvenile culture, and is currently the most commonly used substrate in commercial production (Capinpin et al. 1999; Setyono 2015; SEAFDEC/AQD 2000, 2022). However, PVC plastic has been reported to pose potential risks to human health and the environment (Osmanski 2020; Health Care Without Harm Europe 2021). Given that abalone graze on its surface for periphytic algae, the use of PVC in their culture should be reconsidered.

Previous studies on the effect of substrates in abalone juvenile culture have primarily focused on synthetic materials, such as PVC pipes, plastic baskets, corrugated fiberglass, plexiglass, rubberized canvas, and fibro-cement board (Avilles and Shepherd 1996; Gapasin and Polohan 2005; Setyono 2007). No studies have been conducted on the impact of indigenous materials, such as bamboo, as alternative substrates to synthetic materials in abalone cage culture except for abalone conditioning, as reported by De Guzman and Creencia (2014). The present study investigates the use of bamboo (*Bambusa blumeana* Schult.f.) as an alternative substrate. Bamboo substrate (BS) is an indigenous material usually abundant in rural coastal areas, costing PHP 100-150 (USD 1.72-2.58) per pole (5 m), and is environmentally friendly. Considering the environmental benefits of these indigenous materials, elucidating their effects on the growth and survival of abalone juveniles in cage culture is needed. As the Philippines' abalone juvenile culture continues to expand, this study's results have significant applications. The current generation must rise to the challenge of adopting eco-friendly products for a sustainable future. A favorable outcome from this study could encourage small-scale aquaculturists to use inexpensive, indigenous, and environmentally friendly substrates for abalone juvenile culture in both the bottom and suspended sea cages.

This study investigated the potential of BS as an alternative to PS. Specifically, this study determined the following: (1) weight growth rates, shell length (SL) growth rates, and survival rates of reared abalone in bottom sea cage culture and suspended sea cage culture experiments; (2) food conversion efficiency (FCE) of reared abalone in suspended sea cage culture experiment; and (3) length and weight (LW) relationship of reared abalone in bottom sea cage culture and suspended sea cage culture experiments, which can be used in stock evaluation models to predict weight from length.

METHODS

Study Site

This study included two experiments conducted from 2019 to 2021. The first study site was the Western Philippines University Binduyan Marine Research Station (WPU-BMRS), located at Barangay Binduyan, Puerto Princesa City, 79 km north of the capital of Palawan province. Here, the researchers conducted the bottom sea cage culture, where weight, SL, and FCE data were collected. The second study site was in Barangay Pamantolon, Taytay, Palawan, 147 km from WPU-BMRS (Figure 1), where the suspended sea cage culture experiment was conducted, with data collected on weight and SL only.

Experiment 1: Bottom Sea Cage Culture

A total of 216 abalone juveniles, with a mean (±SE) weight of 1.52 ± 0.77 g and a mean SL of 20.37 ± 3.21 mm, were obtained from the hatchery of WPU-BMRS for this experiment. This study applied two treatments (T), each with three replicate cages: T1 = BS (bamboo substrate) and T2 = PS (PVC substrate). Two sets of BS and PS, each measuring approximately 0.30 m × 0.30 m with a total surface area of 0.36 m^2, were placed in each cubical cage. Each replicate meshed (3.2 mm) cages (30 cm^3) were positioned on a platform at a depth of 8-9 m, 100 m from the shoreline, following a completely randomized design (CRD). Thirty-six (36) abalone juveniles were stocked in each cage were tagged using a laminated label pasted with Mighty Bond® glue to the posterior side of the abalone shell (Figure 2). These tagged abalone samples were used for measuring weight and SL throughout the three-month culture period. A stocking density of 100 abalone juveniles per m^2 substrate surface area was maintained. Explicit acclimation was not conducted, as the tanks where the juveniles were kept used seawater freshly pumped from the open sea, which was approximately 100 m from the cage rearing site. A summary of Experiment 1 characteristics is provided in Table 1.

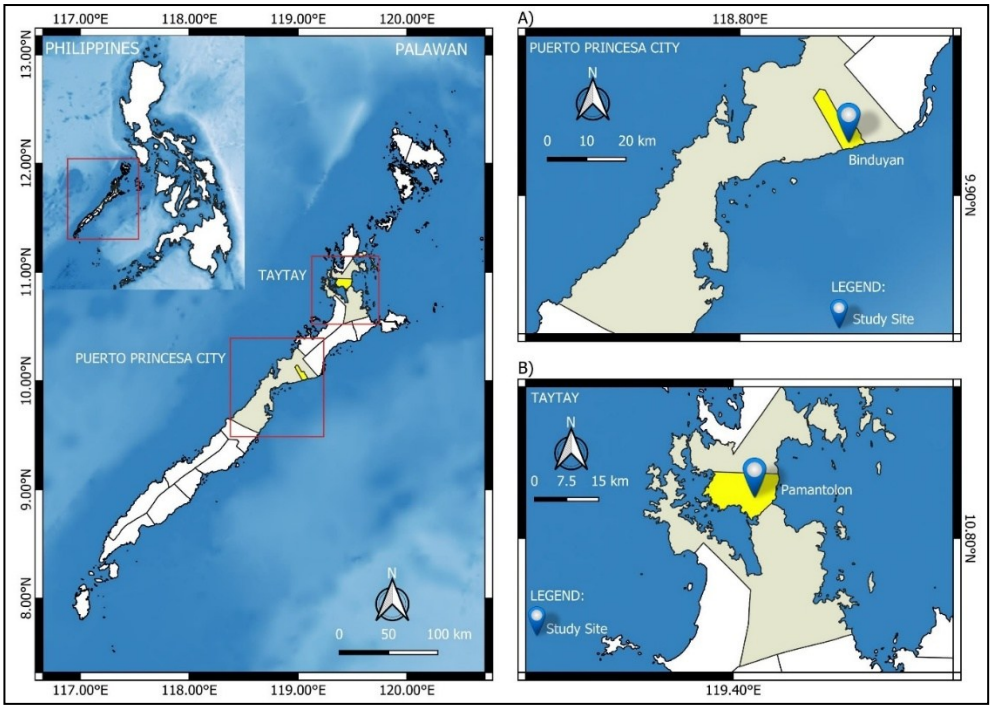

Figure 1. Map showing the study sites in (A) Binduyan, Puerto Princesa City and (B) Pamantolon, Taytay, Palawan, Philippines.

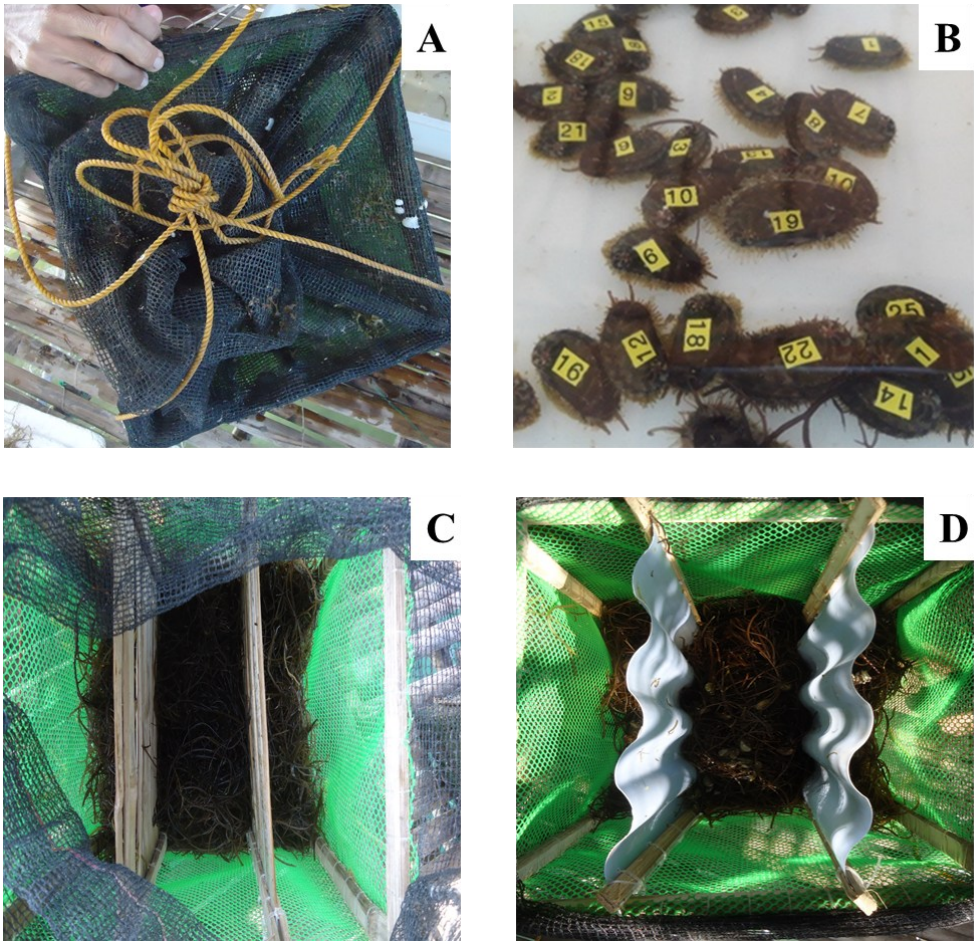

Figure 2. Photographs of the (A) covered meshed cage with rope for attachment to a bottom platform, (B) tagged abalone juveniles used in the experiment, and (C) bamboo substrates and (D) PVC substrates placed inside the cage.

Table 1. Summary of characteristics of the experiments on rearing abalone juveniles using sea cages at Palawan, Philippines.

Variables	**Experiment 1, Bottom Sea Cage Culture**		**Experiment 2, Suspended Sea Cage Culture**	
Treatment (Substrate)	1-bamboo	2-PVC	1-bamboo	2-PVC
Replicate cages	3	3	3	3
Abalone mean initial shell length (mm)	20.33	20.14	26.27	26.00
Abalone mean initial weight (g)	1.51	1.51	3.36	3.20
Dimension of cage used (cm^3)	30	30	30	30
Mesh size of cage net (mm)	3.2	3.2	3.2	3.2
Density/cage (0.36 m^{-2} surface area substrate)	36	36	60	60
Distance of cage from surface (m)	8-9	8-9	1-2	1-2
Interval of data measurement (days)	15	15	30	30
Method of retrieving the sea cages	SCUBA diving	SCUBA diving	Manual lifting	Manual lifting
Food provided in the cage (seaweed)	*Gracilaria*	*Gracilaria*	*Gracilaria*	*Gracilaria*
Interval of provision of seaweed food (days)	15	15	15	15
Feeding rate (% of body weight)	>180	>180	>100	>100
Culture period (days)	90	90	150	150
Study site	Binduyan Marine Research Station		Pamantolon, Taytay, Palawan	

Experiment 2: Suspended Sea Cage Culture

A total of 360 abalone juveniles were used in this culture, stocked at a density of 167 abalone juveniles per m^2 substrate surface area, with 60 individuals per cage. The juveniles had a mean weight of 3.26 ± 1.38 g and a mean SL of 26.13 ± 3.38 mm and were sourced from the hatchery of WPU-BMRS. The abalone juveniles were transported for approximately three hours in oxygenated bags containing cut bamboo pole substrates lined with a damp towel. Ice packs placed outside the bags in a styrofoam box kept them cool during transport. Upon arrival at the site, acclimation was conducted before placing the abalone juveniles in the suspended sea cages. Acclimation took place in a shaded hut near the longline for about one hour, during which ambient seawater was gradually sprinkled over them until they were fully submerged.

Similar to Experiment 1, the same cage materials and substrate sizes were used, along with two treatments, each with three replicate cages (T1 = BS, T2 = PS). The cages were suspended on a longline in a seaweed farm, approximately 1.5 m apart and 1-2 m below the sea surface. The juveniles were reared for five months. A summary of the characteristics of Experiment 2 is provided in Table 1.

Feeding and Cage Management

Proximate analysis of *Gracilaria firma* C.F. Chang & B.-M. Xia, 1976, the seaweed utilized as a primary food source for abalone in the experiments had 13.45% crude protein, 2.15% N, 66.26 mg/g amino acid, and 49.27% amino acid in protein. The seaweed was collected from the coastal areas of Tabon, Quezon, Palawan and maintained in concrete tanks with aeration at the WPU-BMRS hatchery. Before feeding, the seaweed was weighed, and any unconsumed portions were weighed again after 15 days. In Experiment 2, the typical seaweed provision per cage on an *ad libitum* basis per cage ranged from 600 g on day 0 to 1,500 g on day 135. Every 15 days, unconsumed seaweeds were removed and recorded to estimate food consumption. The feeding rates were maintained at >180% and >100% (both on an *ad libitum* basis) with seaweed supply monitored and recorded regularly.

Weekly cages inspections were carried out to check for predator attacks, such as tears or holes in the mesh. Any attached algae, fouling organisms, or debris were removed to ensure a constant water influx into the cage. Additionally, the cages were thoroughly cleaned during this process.

Weight Growth Rates

In Experiment 1, data were collected every 15 days with the assistance of SCUBA divers, who retrieved the cages from the sea bottom. Each abalone juvenile was manually removed from the substrate using a spatula and transferred to a basin filled with seawater and aeration. The juveniles were then placed on a dry towel before their weight was measured using a digital weighing scale (Salter, 200 g).

In Experiment 2, the weight of tagged abalone juveniles was measured every 30 days using a digital weighing scale. For consistency with Experiment 1, the weight values on days 15, 45, and 75 were estimated through interpolation based on the 30-day measurement data.

After measurements were taken in both Experiment 1 and Experiment 2, all juveniles were returned to their respective cages, which were then transported back to the culture site and securely tied in place.

Weight gain (WG) was calculated using the formula:

$$\boldsymbol{WG} = \left[\frac{WT - WI}{WI}\right] x\ 100\%.$$

Specific growth rate (SGR) was computed using the formula:

$$\boldsymbol{SGR} = \frac{\ln WT - \ln WI}{T}\ x\ 100\%.$$

Daily increase in body weight (DIBW) was computed using the formula:

$$\boldsymbol{DIBW} = \frac{WT - WI}{T} \text{ (g/day) * 1000mg/g,}$$

where: W_T = weight at time T (g); W_I= initial weight (g); *ln* = natural logarithm.

Shell Length Growth Rates

In Experiment 1, the SL of abalone juveniles was measured every 15 days using the Vernier caliper. After measurement, all juveniles were returned to their respective cages, which were then transported back to the culture site.

In Experiment 2, the SL of tagged abalone juveniles was measured every 30 days using a Vernier caliper. For consistency with Experiment 1, the SL values on days 15, 45, and 75 were estimated through interpolation based on the 30-day measurement data.

After measurements were taken in both Experiment 1 and Experiment 2, all abalone juveniles were returned to their respective cages, which were then transported back to the culture site and securely tied in place.

Shell length increment (SLI) was calculated using the formula:

$$\boldsymbol{SLI} = [(SL_T - SL_I) / SL_I] \times 100\%.$$

Daily increase in shell length (DISL) was computed using the formula:

$$\boldsymbol{DISL} = \frac{SLT - SLI}{T} \text{ (mm/day) * 1000 } \mu\text{m/mm,}$$

where: SL_T = shell length at time T (mm); SL_I = initial shell length (mm); T = number of rearing days.

Survival Rates

To measure the survival rates of abalone, the researchers first counted and recorded the initial abalone stock per cage. In Experiment 1, the number of surviving abalone in each treatment was recorded every 15 days, while in Experiment 2, counts were taken every 30 days. For consistency with Experiment 1, survival rate values in Experiment 2 on days 15, 45, and 75 were estimated through interpolation based on the 30-day measurement data.

For both Experiment 1 and Experiment 2, the survival rate for each cage was calculated by comparing the live count with the initial abalone stock.

The survival rate (SR) was computed using the formula:

$$\boldsymbol{SR} = \frac{Number\ of\ live\ abalone}{Total\ initial\ stock} \times 100\%.$$

The biomass of cultured abalone was calculated using the formula:

$$\boldsymbol{B} = \text{Total stock} \times \% \text{ Survival} \times W_M$$

where: W_M = mean body weight.

Statistical analysis was conducted on the collected data to identify trends in survival rates. Descriptive statistics, including mean and standard deviation, were used to summarize survival rates for each treatment. An independent samples t-test was performed to determine significant differences in survival rates between treatments in each experiment at $P < 0.05$. Additionally, repeated measures ANOVA was used to assess survival rate trends over time within each treatment.

Feed Conversion Efficiency

Feed conversion efficiency (FCE) is the ability of abalone to convert *Gracilaria* into body weight. In this study, FCE was calculated using data on food consumption and weight gain in Experiment 2. As detailed above in the feeding management section, feed consumption was estimated based on recorded weight of food before and after 15 days, assuming that the lost amount was consumed.

The feed conversion ratio (FCR) is calculated using the formula:

$$\boldsymbol{FCR} = \textit{Feed consumed / Weight gain.}$$

The feed conversion efficiency (FCE) is computed using the formula:

$$\boldsymbol{FCE} = \textit{(Weight gain / Feed consumed) x 100.}$$

Length-Weight Relationship

To analyze the length-weight (LW) relationship, all weight and SL data from Experiment 1 and Experiment 2 were grouped into three length classes (15-25, 26-35, 35-45 mm). The LW relationship was expressed using the equation: L = SL of the abalone in mm, W = aL^b, where W = weight of the abalone in g, a = intercept, b = slope (growth coefficient) (Le Cren 1951; Najmudeen 2015). The parameters a and b were calculated using linear regression in SPSS on log-transformed SL and weight data, with the coefficient of determination (R^2) used as an indicator of linear regression quality. A t-test was performed at a confidence level of 95% to confirm if the values of *br* (calculated *b* coefficient (or slope) from linear regression) obtained by linear regression were significantly different from the isometric value, expressed by the equations below (Najmudeen 2015).

Hypothesis: H_0: b0 = 3 (Isometric growth); H_A: $br \neq 3$ (Allometric growth)

The t-test formula to test the hypothesis: $\boldsymbol{t} = \frac{br - b0}{SE}$

where:

br – calculated *b* coefficient (or slope) from linear regression

b0 = 3

SE – standard error of *b* coefficient from linear regression

Data Analysis

Data analyses were conducted using SPSS version 26.0 for Windows. Descriptive statistics were applied to summarize and organize the dataset. An independent samples t-test was conducted to determine whether there were significant differences in WG, SGR, DIBW, SLI, DISL, SR, FCR, and FCE between treatments at $P < 0.05$.

To ensure consistency and reliability, the initial sample sizes for each treatment were standardized. The values of weight and SL were initially averaged across individual abalone juveniles within each cage, subsequently averaged across the three replicate cages per treatment to determine overall treatment effects. This method of averaging was maintained throughout the experiment.

In Experiment 1, the BS treatment utilized initial sample weights ranging from 0.41 to 3.31 g, with corresponding initial sample lengths between 13.60 and 26.60 mm. Meanwhile, the PS treatment had initial sample weights ranging from 0.45 to 3.29 g, with initial sample lengths from 14.10 to 26.20 mm. The final weights and lengths for each treatment group are detailed in Table 1.

In Experiment 2, the BS treatment featured initial sample weights between 1.00 and 5.95 g, with initial sample lengths ranging from 19.80 to 33.10 mm. The PS treatment, on the other hand, included initial sample weights between 1.30 and 5.65 g and initial sample lengths ranging from 20.00 to 32.20 mm.

RESULTS

Weight Growth Rates

Experiment 1, bottom sea cage culture. After 90 days of culture, abalone juveniles grown in bottom sea cages with BS and PS exhibited an increasing growth pattern (Figure 3). The weight mean of abalone juveniles with BS increased from 1.51 ± 0.70 g to 6.13 ± 2.17 g, while with PS increased from 1.51 ± 0.73 g to 4.77 ± 1.88 g (Table 2). A t-test revealed that abalone juveniles with BS gained weight significantly higher than those with PS. The mean weight gain of abalone juveniles with BS after 90 days was 358.13 ± 186.85%, compared to 279.71 ± 182.92% for those with PS (Table 2).

Abalone juveniles in both treatments exhibited the faster specific growth rate (SGR) after 45 days of culture, which then gradually declined. In general, the t-test showed that abalone juveniles with BS had significantly higher SGR ($P < 0.001$) compared to those with PS (Table 2).

Experiment 2, suspended sea cage culture. After 150 days of culture, abalone juveniles grown in suspended sea cages with BS and PS followed a similar increasing growth pattern, as shown in Figure 3. The mean weight of abalone juveniles with BS increased from 3.09 ± 1.19 g to 7.64 ± 2.20 g, while those with PS increased from 3.02 ± 1.04 g to 7.37 ± 2.28 g (Table 3). Abalone juveniles cultured with BS exhibited significantly higher mean weights from day 60 to day 120 ($P < 0.05$). Nevertheless, on day 150, a t-test showed no significant difference ($P = 0.27$) in mean weight between juveniles with BS and PS.

On day 60, abalone juveniles in the suspended sea cages reached their fastest SGR in both substrates, but this rate gradually declined. Based on the t-test results, there was no significant statistical difference ($P = 0.61$) in the growth rates of abalone juveniles with BS and PS.

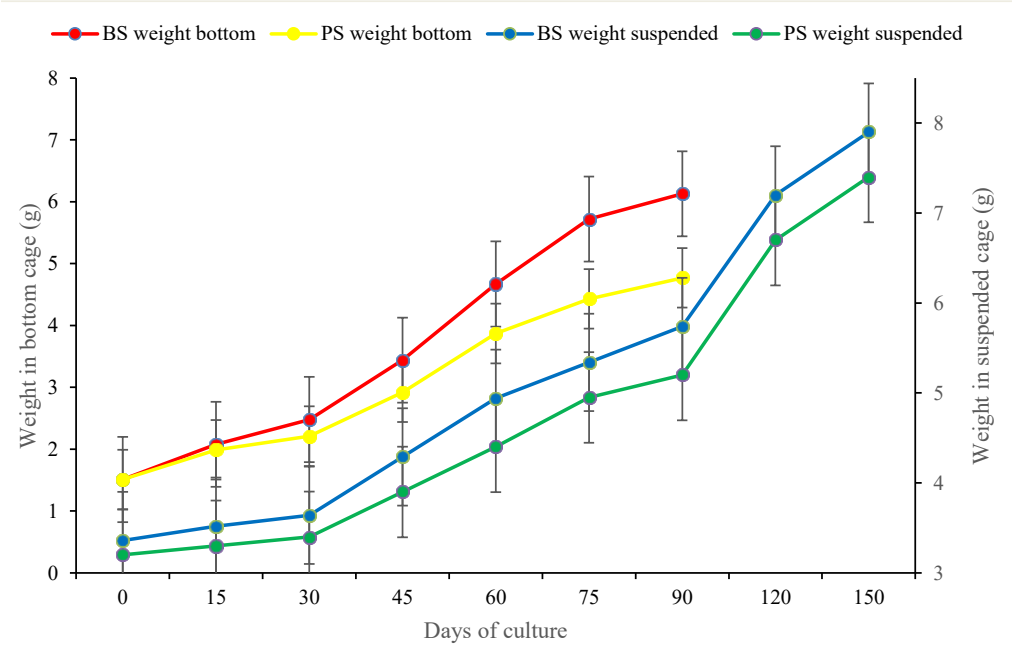

Figure 3. The mean (±SE) weight growth pattern of abalone juveniles with time across the two treatments: bamboo substrate (BS) and PVC substrate (PS) in the bottom and suspended sea cages. (Note: The BS and PS weights in suspended cages on days 15, 45, and 75 were estimated through interpolation.)

Table 2. Weight growth rates of abalone *Haliotis asinina* in bottom sea cages with bamboo substrate and PVC substrate fed with seaweed *Gracilaria firma* for 90 days (W_I – initial weight, W_F – final weight, W_G – weight gain, DIBW – daily increase in body weight, SGR – specific growth rate). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (t-test).

Treatment	Mean W_I (g)	Mean W_F (g)	W_G (%)	DIBW (mg/day)	SGR (%)	Biomass (g)
Bamboo substrate	1.51 ± 0.70	6.13 ± 2.17***	358.13 ± 186.85**	51.13 ± 19.67***	1.61 ± 0.43***	631.17 ± 2.16
PVC substrate	1.51 ± 0.73	4.77 ± 1.64	279.71 ± 182.92	36.43 ± 15.00	1.37 ± 0.50	486.41 ± 1.64

Table 3. Weight growth rates of abalone *Haliotis asinina* juveniles in suspended sea cages with bamboo substrate and PVC substrate fed with seaweed *Gracilaria firma* for 150 days (W_I – initial weight, W_F – final weight, W_G – weight gain, DIBW – daily increase in body weight, SGR – specific growth rate).

Treatment	Mean W_I (g)	Mean W_F (g)	W_G [a] (%)	DIBW (g/day)	SGR (%)	Biomass (g)
Bamboo substrate	3.09 ± 1.15	7.64 ± 2.20	161.83 ± 103.83	0.03 ± 0.01	0.60 ± 0.24	1,336.45 ± 2.43
PVC substrate	3.02 ± 1.04	7.37 ± 2.28	155.96 ± 95.35	0.03 ± 0.01	0.58 ± 0.23	1,290.57 ± 2.31

Shell Length Growth Rates

Experiment 1, bottom sea cage culture. The SL in both substrates increased over 90 days of sea cage culture (Figure 4). The mean SL increased from 20.33 ± 3.22 mm to 32.06 ± 3.49 mm with BS, and from 20.14 ± 3.35 mm to 29.57 ± 3.21 mm with PS. A t-test revealed that abalone juveniles with BS had a significantly higher increment than those with PS (Table 4).

After 60 days, abalone juveniles in the bottom sea cages reached their highest SL growth rate. Thereafter, the SL growth rate gradually declined across all substrates. In general, abalone juveniles with BS had significantly higher DISL than those with PS (Table 4).

Experiment 2, suspended sea cage culture. The SL of abalone juveniles increased over 150 days of sea cage culture (Figure 4). The mean SL increased from 25.71 ± 3.05 mm to 33.88 ± 2.96 mm with BS, and from 25.77 ± 2.76 mm to 33.22 ± 2.96 mm with PS (Table 5). A t-test revealed no statistically significant difference in SL increment between juveniles with BS and PS ($P = 0.76$).

The DISL of abalone juveniles was nearly identical for both BS and PS. The results of the t-test confirmed that there was no statistically significant difference in DISL between two treatments ($P = 0.24$).

Survival Rates

Experiment 1, bottom sea cage culture. The survival rates of abalone juveniles in bottom sea cages with BS and PS were relatively high, at 95.37% and 94.4%, respectively (Figure 5). Mortality in this study was primarily due to mishandling during the removal of abalone juveniles from the substrates. However, the t-test showed no significant difference in the survival rates between the two substrates ($P = 0.76$).

Experiment 2, suspended sea cage culture. Similarly, in suspended sea cage culture, survival rates of abalone juveniles with BS and PS were relatively high. Figure 6 shows that the survival rates for BS and PS were 92.81% and 95.93%, respectively. Mishandling was again the primary cause of mortality, as juvenile abalone are difficult to remove from substrates. However, a t-test showed no significant difference in the survival rates between the two substrates ($P = 0.21$).

Table 4. Shell length growth rates of abalone *Haliotis asinina* juveniles in bottom sea cages with bamboo substrate and PVC substrate fed with seaweed *Gracilaria firma* for 90 days (SL_I-initial shell length, SL_F-final shell length, SLI-shell length increment, DISL-daily increase in shell length). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (t-test).

Treatment	SL_I (mm)	SL_F (mm)	SLI (%)	DISL (µm/day)
Bamboo substrate	20.33 ± 3.22	32.06 ± 3.49	59.65** ± 22.37	129.35*** ± 34.52
PVC substrate	20.14 ± 3.35	29.57 ± 3.21	50.46 ± 22.67	105.84 ± 34.77

Table 5. Shell length growth rates of abalone *Haliotis asinina* juveniles in suspended sea cages with bamboo substrate and PVC substrate fed with seaweed *Gracilaria firma* for 150 days (SL_I-initial shell length, SL_F-final shell length, SLI-shell length increment, DISL-daily increase in shell length).

Treatment	SL_I (mm)	SL_F (mm)	SLI (%)	DISL (mm/day)
Bamboo substrate	25.71 ± 3.05	33.88 ± 2.96	31.76 ± 15.82	0.053 ± 0.021
PVC substrate	25.77 ± 2.76	33.22 ± 2.96	29.49 ± 16.91	0.049 ± 0.022

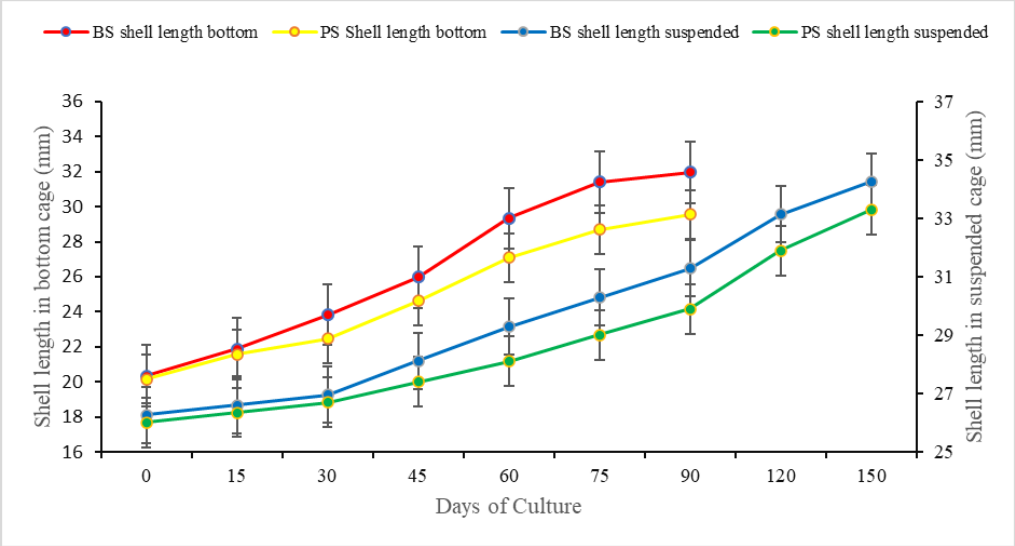

Figure 4. The mean (±SE) shell length growth pattern of abalone juveniles with time across the two treatments (bamboo substrate (BS) and PVC substrate (PS) in the bottom and suspended sea cages. (Note: The BS and PS shell length in suspended cages on days 15, 45, and 75 were estimated through interpolation.)

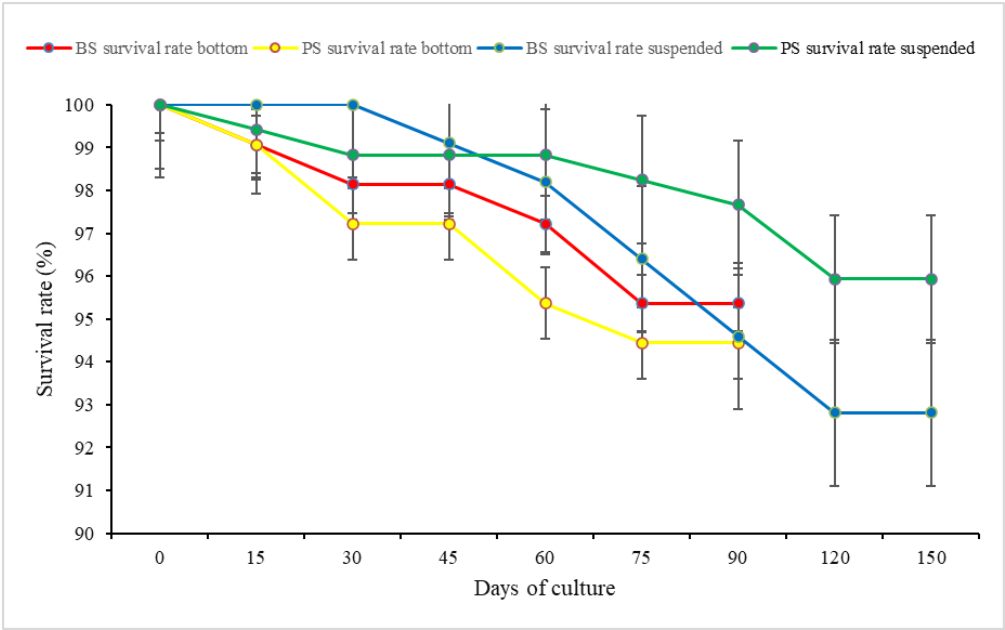

Figure 5. The survival rate (±SE) of abalone juveniles with time across the two treatments: bamboo substrate (BS) and PVC substrate (PS) in the bottom and suspended sea cages. (Note: The BS and PS survival rates in suspended cages on days 15, 45, and 75 were estimated through interpolation.)

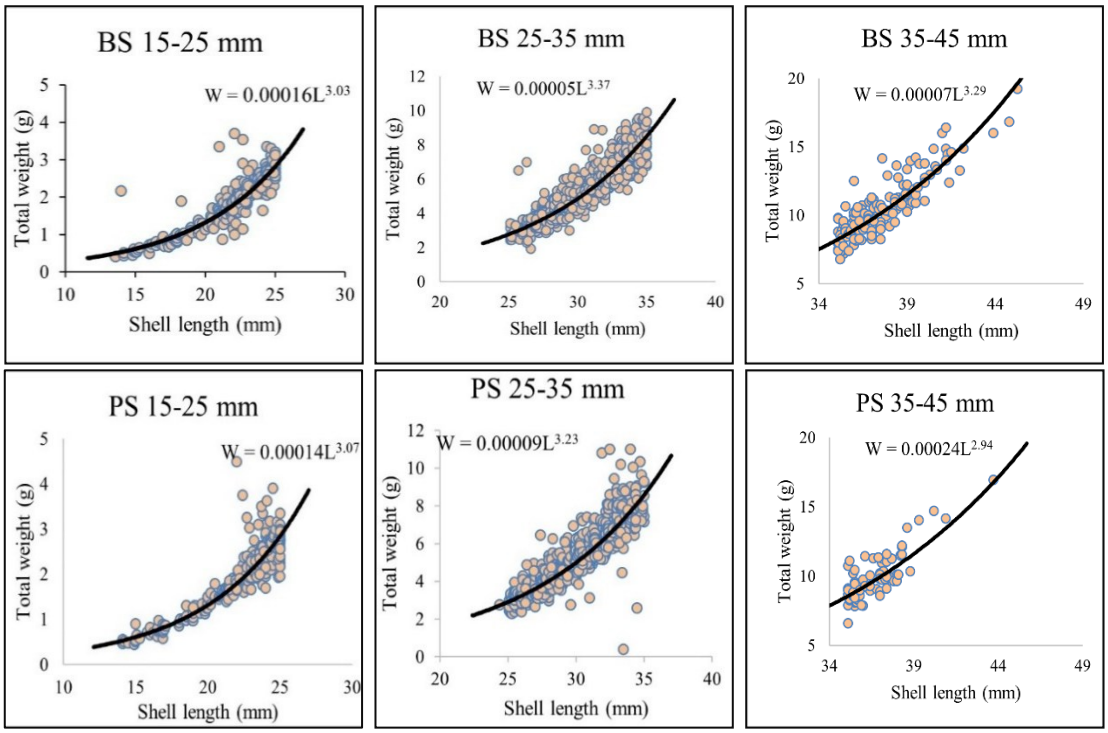

Figure 6. Length-weight relationship of abalone *Haliotis asinina* cultured with bamboo substrate (BS) and PVC substrate (PS) for 15-25 mm shell length (BS: $b = 3.03$, isometric, PS: $b = 3.07$, isometric), 25-35 mm shell length (BS: $b = 3.37$, allometric +, PS: $b = 3.23$, allometric +), and 35-45 mm shell length (BS: $b = 3.29$, isometric, PS: $b = 2.94$, isometric).

Food Conversion Efficiency

The results demonstrated that FCE was higher for abalone juveniles with BS (11.86%) than for those with PS (9.86%). In terms of FCR, approximately 10.10 kg of *Gracilaria* algal food was needed to produce 1 kg of abalone with PS, compared to a relatively lower FCR of 8.43 for abalone with BS (Table 6).

Length and Weight Relationships

Experiment 1, bottom sea cage culture. Abalone juveniles reared with BS ($b = 2.89$) and PS ($b = 3.00$) initially showed isometric growth. However, after 90 days, only abalone juveniles with BS ($b = 3.15$) transitioned to positive allometric growth, while those with PS ($b = 2.95$) continued to exhibit isometric growth. The LW relationship analysis showed a reasonably good fit for both substrates: BS (SL 24–42 mm, $R^2 = 0.93$) and PS (SL 22–38 mm, $R^2 = 0.779$). The relative condition factor (Kn) remained nearly unchanged from the start of the experiment (BS = 1.03, PS = 1.01) to the end of the experiment (BS = 1.0, PS = 1.02) (Table 7).

Experiment 2, suspended sea cage culture. Abalone juveniles reared with BS ($b = 2.61$) initially exhibited negative allometric growth, while those with PS ($b = 3.03$) showed isometric growth. However, by the end of the experiment, abalone juveniles with BS had transitioned to positive allometric growth, whereas those with PS continued to exhibit isometric growth. The LW relationship analysis indicated a reasonably good fit for both substrates: BS (SL 26–42.20 mm, $R^2 = 0.878$) and PS (SL 24.40–43.70 mm, $R^2 = 0.861$). The Kn at the beginning of the experiment showed good health conditions (BS = 1.02, PS = 1.04), and both treatments maintained relatively good health conditions by the end (BS = 1.00 and PS = 1.04) (Table 8).

Figure 6 illustrates the LW relationships of *H. asinina* across different size groups. The 15-25 mm size group showed isometric growth in both BS and PS. The Kn values were similar across substrates, indicating healthy growth. The R^2 values were high, indicating that the model was a good fit for the data (Table 8).

For the 25-35 mm size group, the growth shifted to a positive allometric+ for both substrates, with a higher *b*-value or allometric exponent in BS than in PS. A higher *b* indicates a faster growth rate for the BS. The Kn values continued to indicate healthy growth. The R^2 values remained high for both substrates, indicating a good fit of the model to the data (Table 8).

For the 35-45 mm size range, the growth reverted to isometric for both BS and PS. The *b*

coefficient was higher for BS than PS, which is supposed to indicate faster growth, but the t-test revealed that both groups still exhibited isometric growths. The Kn value remained 1.01 for both substrates, which still indicates healthy growth. The R^2 value was a good fit for BS, but lower for PS, indicating a weaker correlation among the data (Table 8).

Table 6. Food conversion ratio and food conversion efficiency of abalone *Haliotis asinina* juveniles in suspended sea cages with bamboo substrate and PVC substrate fed with *Gracilaria firma* for 45 days (FCR-feed conversion ratio, FCE-feed conversion efficiency).

Treatment	Length Range (mm)	Weight Gained (g)	Food Consumed (g)	FCR	FCE (%)
Bamboo substrate	1.16 – 4.68	54.6	460.32	8.43	11.86
PVC substrate	1.28 – 3.44	50.02	505.42	10.10	9.86

Table 7. Length-weight relationship parameters of the abalone *Haliotis asinina* juveniles between the two substrates: bamboo substrate and PVC substrate in bottom sea cages for 90 days culture (*a*–the line intercept, *b*–the slope/constant, Kn–the relative condition factor).

Substrate	N	Days of Culture	Length Range (mm)	*a*	*b*	Kn	SE (b)	R^2	P-value	Growth Type
Bamboo substrate (BS)	108	0	13.6 – 26.60	0.00023	2.89	1.03	0.113	0.861	< 0.001	Isometric
PVC substrate (PS)	108	0	14.10 – 26.20	0.00017	3.00	1.01	0.069	0.947	< 0.001	Isometric
Bamboo substrate (BS)	103	90	24.0-42.0	0.00011	3.14	1.0	0.089	0.930	< 0.001	Allometric +
PVC substrate (PS)	102	90	22.0-38.0	0.00021	2.95	1.02	0.166	0.779	< 0.001	Isometric

Table 8. Length-weight relationship parameters of the abalone *Haliotis asinina* juveniles between the two substrates: bamboo substrate and PVC substrate in suspended sea cages for 150 days culture (*a*–the line intercept, *b*–the slope/constant, Kn–the relative condition factor).

Substrate	N	Days of Culture	Length Range (mm)	*a*	*b*	Kn	SE (b)	R^2	P-value	Growth Type
Bamboo substrate	167	0	19.8 – 33.10	0.00061	2.61	1.04	0.150	0.646	< 0.001	Allometric -
PVC substrate	172	0	20.00 – 32.20	0.00015	3.03	1.02	0.082	0.890	< 0.001	Isometric
Bamboo substrate	155	150	26.0 - 42.20	0.00009	3.23	1.00	0.098	0.878	< 0.001	Allometric +
PVC substrate	165	150	24.40 - 43.70	0.00011	3.15	1.04	0.099	0.861	< 0.001	Isometric

DISCUSSION

Growth Rates

This study provides valuable insights into the use of bamboo substrate (BS) for rearing abalone juveniles, highlighting its potential as an effective alternative to PVC substrate (PS). The results showed that abalone juveniles raised with BS exhibited significantly higher weight gain and SL growth rates than those reared with PS. These findings are consistent with the previous study by Creencia et al. (2021), which suggested that BS could serve as a viable substrate for abalone juvenile culture in bottom sea cages.

In the two experiments, the researchers investigated the impact of BS on the growth of abalone juveniles in both bottom and suspended sea cage culture at different stages of development. Although periphytic algae abundance was not directly measured in this study, the periphyton presence on the bamboo slats was consistently observed throughout the experiment. Previous studies (e.g. Keshavanath et al. 2004; Zhang et al. 2013; Creencia et al. 2019) have supported the influence of periphytic algae in

enhancing abalone growth, which demonstrated higher periphyton biomass on BS than other materials. Future studies should quantify periphyton abundance to better understand its contribution to abalone growth performance. This periphyton biomass may have served as an additional food source, promoting the enhanced growth of abalone juveniles. This observation is consistent with the study of Keshavanath et al. (2004), which found that periphyton on bamboo poles could supplement fish feed in tilapia culture, resulting to increased yields compared to systems without bamboo poles. Similarly, Wahab et al. (1999) reported that the use of BS significantly ($P < 0.05$) increased growth and production of Indian carp in ponds culture.

Compared to the previous study on a 90-day culture period, the experiment using BS in bottom sea cage culture yielded higher DIBW (51.13 ± 19.67 mg/day) and SGR (1.61 ± 0.43%) than the findings of Bautista-Teruel and Millamena (1999), who used PS, tank culture, different algal (*Gracilaria bailinae* J.F.Zhang & B.M.Xia, 1994) feeds, and a smaller initial stocks (DIBW = 10 mg/day; SGR = 0.06 ± 0.05). In contrast, the results were lower than those reported by Capinpin and Corre (1996) (DIBW = 67.1 mg/day; DISL = 192.9 μm/day). The faster growth rate in Capinpin and Corre's (1996) study could be attributed to their smaller initial stock, different algal (*Gracilaria heteroclada* J.Feldmann & G.Feldmann, 1943) feed, and lower stocking density. Smaller juveniles generally grow faster due to higher feeding rates per unit biomass (Capinpin et al. 1999; Minh et al. 2010). Abalone juvenile growth decreases as stocking density increases due to density-dependent competition for space or food, which affects feeding rates and movement efficiency during feeding (Capinpin et al. 1999). The algal (*G. heteroclada*) feed used by Capinpin and Corre (1996) could also be a factor, which has a higher crude protein content of 17.32% compared to only 13.4% in the algal (*G. firma*) feed used in this study (Table 1). Although the protein content of the food may affect abalone growth rates, this may not always be the determining factor. Bautista-Teruel and Millamena (1999) reported lower growth rates despite using feed with higher protein content (*G. bailinae* – 17.56%) and a smaller size of initial stock. These results indicate that culture conditions, including initial size, stocking density, type of food, temperature, water quality, and substrate, significantly influence abalone juvenile growth (Capinpin et al. 1999; Steinarsson and Imsland 2003; Alcantara and Noro 2005; Minh et al. 2010). These factors need to be considered when comparing growth results across different studies. Differences in culture methods across studies makes it difficult to draw general conclusions about abalone juvenile growth performance. Therefore, it is essential to clearly describe rearing methods when comparing results to ensure consistency in evaluations. By doing so, the scientific community can better understand the factors influencing abalone juvenile growth and make informed decisions about abalone farming practices.

In the suspended sea cage experiment, abalone juveniles were grown in suspended cages near the water surface using BS and PS. The results showed that weight gain was significantly higher with BS than with the PS after 90 days of culture ($P < 0.01$). However, after 150 days, the difference became statistically insignificant ($P < 0.05$). In terms of SL, the abalone juveniles raised with BS maintained a significant advantage over those with PS throughout the 150-day culture period, consistent with the bottom sea cage experiment. These findings suggest that BS can serve as a cost-effective and locally available alternative to PS for the grow-out culture of abalone juveniles in long-line aquaculture systems.

Both experiments showed a notable decline in the growth rate of abalone juveniles towards the later stages of culture. This decline in daily growth rates is likely attributed to the onset of gonad maturation, which requires substantial energy allocation. At the end of the study, the SL range of the abalone juveniles ranged from 32.06 mm to 34.27 mm, approaching the size threshold for sexual maturity (Capinpin et al. 1998). It is well established that the growth rate of abalone juveniles decrease after reaching sexual maturity, as energy is redirected towards gonad development rather than somatic growth (Capinpin and Corre 1996; Mai et al. 1996).

Survival Rates

The handling of abalone juveniles during sampling may have affected their growth and immunity due to stress caused by manual detachment and placement in plastic containers without aeration (Hooper et al. 2011; Daw 2022). Mishandling during sampling was one of the causes of the mortality of abalone juveniles in this study. Additionally, fluctuations in water quality due to adverse weather conditions may further influence their survival rate. Despite these challenges, the survival rates for BS in both experiments remained relatively high, ranging from 92.78% to 99.17%. Another contributing factor to abalone mortalities in this study was the presence of crabs inside the cage. Aspe et al. (2019) reported that swimming crabs (*Thalamita crenata* Rüppell, 1830 and *Charybdis natator* Herbst, 1794*)* prey on abalone juveniles in the Pamantolon area of Taytay, Palawan, where the suspended cage experiment was conducted. Predators in enclosed systems, such as cages, pose a significant threat to abalone juvenile farming, as the cultured animals have no means of escaping. Additionally, cage damage may allow predators to enter or juveniles to escape, both of which negatively affect production. Schiel and Welden (1987), in their study on the response of red abalone (*Haliotis rufescens* Swainson, 1822) to predators, found that crabs and lobsters consumed more abalone than sea

stars. Furthermore, Tegner et al. (1989) identified predation as one of the leading causes of failed attempts to enhance natural abalone populations. The use of black netting or double netting in cages was observed in the experiments to be an effective deterrent against potential abalone juvenile predators. The high survival rates in cages with BS substrate suggest that BS may serve as a viable, cost-effective, and sustainable alternative for abalone juvenile culture.

Feed Conversion Efficiency

Regarding FCE, the presence of BS provided a significant advantage by promoting more efficient food utilization and growth in abalone juveniles compared to PS. The improved FCE of abalone juveniles observed with BS can be attributed to several factors. The BS provides attachment to facilitate feeding, maximizes accessibility to food resources (Setyono 2015), and serves as a habitat for microscopic food sources, such as benthic diatoms and other organisms that settle and attach to the substrate. These organisms contribute to the overall food availability and enhance the nutritional quality of the abalone diet (Wahab et al. 1999; Keshavanath et al. 2004; Zhang et al. 2013). Furthermore, the substrate creates a sheltered environment for abalone, reducing stress and providing a sense of security. This favorable environment likely enhances their feeding efficiency and overall growth performance (Setyono 2015). It has been suggested that the growth of *H. asinina* is influenced by the availability of attachment space or shelter (Fermin and Buen 2002). By offering a designated substrate for attachment, abalones have increased access to food resources, which may result in improved FCE.

Additionally, BS offers advantages in terms of its composition and degradation properties. As a biodegradable material, BS provides a natural and environmentally friendly option compared to the PS, a non-biodegradable synthetic material. The BS creates a more natural and suitable habitat for abalone, mimicking their natural environment and promoting their overall well-being. This may positively influence their feeding efficiency and subsequently improve FCE (Setyono 2015).

It is worth noting that PS has been widely employed in abalone culture due to its durability and practicality. However, this study's findings suggest that BS offers advantages in terms of FCE, promoting more efficient feed utilization and growth.

Length-Weight Relationships

The LW relationship analysis assessed the growth performance of abalone juveniles reared with BS compared to PS. This study found that abalone juveniles with both BS and PS initially showed isometric growth, meaning there was a proportional increase in weight and SL. However, after 90 days in the bottom cage experiment, abalone juveniles with BS showed a positive allometric growth pattern (b = 3.14), while abalone juveniles with PS continued to show isometric growth (b = 2.95) (Table 7). The isometric growth of abalone juveniles with PS (SL range = 22-38 mm, mean = 29.57 mm) is consistent with the study of Najmudeen (2015), which also reported isometric growth of abalone juveniles in the SL size range of 25–35 mm. Interestingly, the abalone juveniles with BS (SL range = 24–42 mm, mean = 32.06 mm) showed a positive allometric growth pattern in this study.

A LW model was created to further verify this growth pattern based on all the weight and length data in two experiments. The result of the LW model for abalone juveniles with a SL of 26-35 mm in BS ($W=0.00005L^{3.37}$) and PS ($W=0.00009L^{3.23}$) showed positive allometric growth (Table 8). The SL 26-35 mm LW model also showed a good fit for both substrates, with high R^2 values (BS = 0.87, PS = 0.75) indicating a strong correlation between weight and SL. Additionally, the Kn remained consistently good for both treatments from the initial day of culture until the end of the experiment (BS = 1.0, PS = 1.02) (Table 9).

Table 9. Length-weight relationship of reared abalone *Haliotis asinina* juveniles between the two substrates: bamboo substrate and PVC substrate in various size groups (*a*–the line intercept, *b*–the slope/constant, Kn–the relative condition factor).

Substrate	Length Range (mm)	*a*	*b*	Kn	SE (b)	R^2	P-value	Growth Type
Bamboo substrate	15 - 25	0.00016	3.03	1.03	0.102	0.76	< 0.001	Isometric
PVC substrate	15 - 25	0.00014	3.07	1.01	0.064	0.90	< 0.001	Isometric
Bamboo substrate	25 - 35	0.00005	3.37	1.01	0.043	0.87	< 0.001	Allometric +
PVC substrate	25 - 35	0.00009	3.23	1.05	0.065	0.75	< 0.001	Allometric +
Bamboo substrate	35 - 45	0.000068	3.29	1.01	0.194	0.65	< 0.001	Isometric
PVC substrate	35 - 45	0.00024	2.94	1.01	0.443	0.38	< 0.001	Isometric

The suspended cage experiment revealed that the abalone juveniles reared with BS (b = 2.61) exhibited negative allometric growth initially, while those reared with PS showed isometric growth (BS = 3.03). However, towards the end of the experiment, the abalone juveniles with BS displayed positive allometric growth, while those with PS remained isometric. The LW relationship analysis indicated a reasonably good fit for both substrates. The Kn at the beginning of the experiment showed good health conditions for both treatments. By the end of the experiment, both treatments maintained relatively good health conditions. One possible explanation for the observed differences in growth rates between the BS and PS treatments is the development of periphytic algae on the bamboo slats used in BS. Periphytic algae can provide an additional food source for abalone juveniles, which could contribute to faster growth rates (Zhang et al. 2013; Creencia et al. 2019). Additionally, the supply of drift algae, which can vary in composition depending on location and water movement, could also affect the growth rate of abalone juveniles in different treatments (Tutschulte and Connel 1988). Previous studies have shown that the composition of drift algae can influence the diet and growth of abalone juveniles (Poore 1972a, 1972b), and areas with higher water movement tend to have higher growth rates, although there is an optimal degree of water movement for feeding (Shepherd 1973; McShane et al. 1988).

Generally, the results suggest that both BS and PS are suitable for abalone juvenile culture, as the abalone maintained good health throughout the culture period. However, using bamboo slats may have contributed to faster growth rates due to the development of periphytic algae, highlighting the potential for using alternative substrates or modifying substrate design to improve abalone juvenile growth performance. These results suggest substrate choice can significantly impact abalone juveniles' growth and health. The BS may be more favorable for abalone juvenile growth and development than the PS. However, the suitability of the substrate may change as the abalone juveniles grow and develop. Therefore, a careful evaluation of substrate choice is necessary throughout the different stages of the abalone juvenile culture. Further studies are needed to investigate the underlying mechanisms of how substrates affect abalone juvenile growth and development.

This study highlights the potential of using BS as an alternative to PS for abalone juvenile rearing. The results show that abalone juveniles reared with BS had significantly higher weight and SL growth rates than those raised with PS. They also had comparable survival rates and feed conversion efficiency with PS. This suggests that BS can be a cost-effective and sustainable alternative for abalone juvenile culture in cages.

Overall, using BS in abalone juvenile rearing could be a promising approach for sustainable and cost-effective abalone juvenile culture. This could encourage farmers to use low-cost and locally available materials for abalone juvenile grow-out culture. The results of these experiments suggest that the choice of substrate can significantly impact the growth and health of abalone juveniles. Additionally, the LW relationship analysis can provide useful insights into the growth patterns of abalone juveniles under different rearing conditions.

These findings have significant implications for sustainable abalone juvenile culture development, as using locally available materials can reduce costs and improve efficiency while promoting environmental sustainability. Ultimately, these findings can contribute to the development of a sustainable and eco-friendly abalone culture, benefiting both the environment and the economy.

Further research is needed to confirm these findings and evaluate the effectiveness of BS in different ecological contexts. To address these limitations, future research should confirm the study's findings by measuring differences in the abundance and nutritional quality of periphytic algae in BS and PS. It should also include qualitative methods and involve stakeholders in similar systems to provide insights into unexpected interactions and feedback loops.

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GENERATIVE AI STATEMENT

Artificcial intelligence was not used in the conduct and writing of this study exceot for minor grammatical checking.

ETHICAL CONSIDERATIONS

This study followed all institutional and national ethical guidelines for the care and use of experimental abalone juveniles.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests.

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The language digiclash: Digital immigrants teaching digital natives in the Philippine context

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ABSTRACT

In education, the digital competence gap between the digital natives who learn technology use from early years and those digital immigrants who learn technology at a later stage has had a profound effect on the way teaching and learning is conducted. This phenomenology research examined the experiences of novice and experienced Filipino and English teachers referred as digital immigrants in using information and communication technology (ICT) tools in the virtual learning environment. The participants of this qualitative descriptive study were purposefully selected participants from a local school district in Bohol and a state university in Candijay, which is situated in the central Visayas, Philippines. Results revealed that digital immigrant teachers faced challenges such as poor internet connectivity, resistance to change, and gaps in ICT skills. These issues were exacerbated by the region's limited access to ICT resources, internet, and adequate ICT training programs. Teachers, however, adopted strategies like proactivity, seeking social support, and fostering peer relationships to cope with these challenges. The study highlights the need for a more integrated approach to ICT integration that includes skill development, infrastructure improvement, and a shift in mindset. It suggests that professional development, mentorship, and collaborative teaching strategies are essential for empowering digital immigrant teachers. Moreover, improved digital infrastructure, especially internet access, will be crucial in enabling teachers to completely adopt ICT in their language instruction during an era of rapid digital shift.

Keywords: coping strategies, digital competence, ICT challenges, technology integration, virtual learning

INTRODUCTION

The incorporation of information and communication technology (ICT) into the use of pedagogy is more dire than it is innovative. It presents real world challenges aimed at people like teachers who hardly encounter opportunities to use technology on both a personal and career level (Akram et al. 2022; Msambwa et al. 2024). This is even truer for teachers who are considered digital immigrants (Bennett 2012; Kesharwani 2020). These teachers encounter particular hurdles attempting to reconcile the chasm between outdated technological proficiency and the increasing technological educational landscape where

the role of ICT is ever growing. This generational divide is especially noticeable in classrooms where digital natives, students raised with technology are taught by educators who struggle to use these tools effectively (Prensky 2001; Tran et al. 2020; Elaoufy 2023). As indicated, the strangeness of the new technology is not only between the immigrants and the natives in relation to teaching but is a challenge to teaching, to student learning, to engagement, and to student achievements (Köse 2024). This issue has given rise to the concept of digiclash. The word digiclash came into existence due to a linguistic process of combining two words to form a new word called blending (Gao 2023). Digiclash describes the ongoing tension between digital immigrant teachers and their digital native students as they navigate the use of technology in education. This digital clash is observed in the workplace, such as in areas where language teachers are situated, where different generations struggle to align their expectations associated with digital transformation. The changes in the educational environment have created a range of dilemmas for teachers who were forced to move to remote instruction on short notice and without adequate training on how to employ technology to assist learning (Estigoy 2021). The integration of ICT in the classrooms has also created blended learning opportunities which makes teaching and learning of a language very important.

Furthermore, the absence of some forms of technology integration into the classroom has been associated with hindering pedagogy and student involvement; as such, the barriers teachers experience with concerning the use of ICT tools should be more expedient factors (Pérez-López and Ibarrondo-Dávila 2020). This highlights the importance of studies about the problems caused by the integration of ICT, especially for digital immigrant language teachers who usually feel less confident in the use of these devices and resources than digital native students.

Worldwide, both competence and exposure to ICT are necessary for educators who strive for successful integration of ICT in their teaching (UNESCO 2023). This is aligned with the United Nations' Sustainable Developmental Goal 4 which seeks to highlight the role of technology in improving the quality of education of people across the world society (Haleem et al. 2022; Varriale et al. 2024). In the field of language learning, ICT integration represents one of the major developments that can revolutionize the effectiveness of knowledge transfer. Although the potential for ICT to revolutionize education is well-understood, many digital immigrant teachers find it challenging to know how to best exploit these technologies (Guo et al. 2008; Raman and Yamat 2014; Basilotta-Gómez-Pablos et al. 2022). With a low ICT background, these teachers are not in position to use ICT for effective teaching and learning, hence weakening the quality of education at a time when there is ICT revolution (Asare et al. 2023). This highlights the need for specialized forms of support and continued professional development in order to assist digital immigrant teachers to reduce the digital competence divide and improve the teachers' use of ICT in language teaching.

It has been widely established in the literature that in order to achieve successful ICT integration, a solid digital infrastructure alone is not sufficient, and a coherent professional development policy that increases teachers` ICT competence and motivation must also be in place (Senkivska 2022; Obidovna and Rustambekovich 2024). The likelihood of successful and positive classroom learning is contingent upon a teacher's technology proficiency and expertise. This is an affirmation with earlier studies which suggest teachers need to receive adequate training to cope with different ICT issues (Amhag et al. 2019; Pratolo and Solikhati 2021; Koch and Fehlmann 2025).

In the Philippines, the use of ICT in education is complicated by infrastructure issues, particularly in remote areas. Many schools report slow internet connections, lack of digital tools, minimal opportunities for teacher training and professional development (Barrot et al. 2021; Malangen and Isorena 2022). Such challenges are heightened in rural, marginal regions like the Subcongressional district of Bohol, where physical seclusion and absence of regional support systems aggravate the digital chasm, hindering the integration of ICT. This highlights the need for research that would specifically focus on the problems associated with digital immigrant teachers in these areas and methods on increasing technology access and teacher readiness. These were exacerbated with the transition to online classes during the COVID-19 Pandemic. The transition was an uphill challenge for a lot of teachers, particularly in remote areas, in the context of deficient infrastructure and a lack of adequate ICT training and other rules (Joaquin et al. 2020; Selvaraj et al. 2021; Kaur 2023). The shift to virtual learning has revealed underlying systemic problems in the education system, particularly in underserved communities, and just how much is at stake in solving the problems of ICT access and teacher preparation.

Despite having multiple studies analyzing the integration of ICT in education, most of the literature has been centered around urban educators as topics of focus (Ghavifekr and Rosdy 2015; Shah 2022; Kennedy 2023). There remains, however, in the literature, a gap focused on the pedagogical difficulties of digital immigrant language teachers, particularly in rural and under-resourced regions, where there is a lack of infrastructure and the barriers to technology use are heightened. Moreover, while performing research on blended learning that included the virtual classrooms for language teaching and learning, the authors have noted difficulties on the part of language

teachers with the management of technology for the effective delivery of the lessons. This study aims to bridge that gap by investigating the specific difficulties encountered by rural digital immigrant language teachers, focusing on the integration of ICT in language teaching, and the barriers to its effective use coupled with the strategies to overcome them. This study sought to derive conclusions from the experiences of the teachers and contribute to the needed canvass from which professional development training and policy proposals of language teachers in rural and disadvantaged areas can be addressed. The main goal was to help digital immigrant teachers enhance their digital skills, bridge the digital separation, and boost their ability to meet the needs of today's technology savvy students.

METHODS

Research Design

This paper uses a phenomenology study design. The approach applied to record true and rich narratives of participants while letting the phenomena articulate themselves. This enables the researcher to gain a more nuanced understanding of the participants' coping strategies and helps shed light on what the language teachers encounter while grappling with the integration of technology in their pedagogy and speaking more of the focal points of language teachers' lived experiences and did not extend beyond the situation under study.

Research Participants and Sampling Procedures

The respondents in this study were the Filipino teachers (n = 6) and English teachers (n = 6) who were digital immigrants from State University in Candijay and Candijay, Anda, Mabini, Alicia, Guindulman (CAMAG) schools under the Subcongressional district of Bohol. All participants used virtual learning as their main method of instruction. They were categorized into two groups: six novice teachers (with three years or less teaching experience) and six experienced teachers (with four years or more teaching experience).

The decision to include novice and experienced language teachers was prompted by a desire to study how different stages of teaching experience affect the problems and coping methods related to ICT integration. This variety permits a fuller understanding of the phenomenon, whereby several perspectives can be considered while concentrating on the common issues of digital competence (Alhazmi and Kaufmann 2022; Kreuder et al. 2024). According to Creswell (2013), evaluating diverse viewpoints enriches our understanding of lived experiences, and grouping participants by experience level is valid as long as they share the fundamental experience under research.

Purposive sampling was used to select participants who could provide rich data to help understand the research problem and central phenomenon of the study (Teddlie and Yu 2007). Using this sampling technique, the participants were selected based on the following criteria: (1) must have taught Filipino or English subjects, (2) must have low or average digital competence, and (3) must use digital learning as an alternative mode of delivery in teaching. A simplified questionnaire based on the UNESCO DigCompEdu Model was used to assess the participants' level of digital competence, which addressed the second inclusion criterion.

Data Collection

The researchers utilized focus groups and interviews to gather the needed information. The researchers developed interview guide questions that represented the focus areas, which looked at teachers' experiences, challenges, and strategies for integrating ICT into virtual language instruction. Key questions included: "Can you describe your experiences using ICT in your teaching?" "In what ways do you use digital tools in your teaching?" and "What challenges do you face when integrating technology into your teaching and how did you overcome them?" The interview questions used in the study were further reviewed by the expert in terms of the clarity, relevance, and alignment of the study's objectives in appropriately addressing the questions asked to the teachers in order to have elaborate responses. Additionally, the guide had undergone trial interview with a group of respondents similar to, but not identical to, the actual study participants. This trial allowed the researchers to refine the questions, ensuring they were clear, unbiased, and effective in capturing meaningful and reliable data. Upon identifying participants, each received a letter requesting permission to conduct interviews. After the participant's consent is acquired, the researchers started the interview, explained the purpose, duration of the study, and the use of interview data. In a qualitative approach, it is important to build trust and openness with participants, and by using this method, it important that participants are willing to undergo an interview (Colaizzi 1978; Creswell 2013).

Subsequently, the in-depth interview took between 45 to 60 minutes. After completing interviews with all of the participants, a focus group discussion (FGD) among colleagues who were experienced in qualitative research was held to validate results. The FGDs lasted 60-90 minutes and had one for each participant, preferably within the same week after the one-on-one interview to refresh the collective discussions. Two fellow workers were requested to participate in the FGDs to make sure that the participants come from the same teaching context and share the same experience. This was to verify the data collected, minimize interview data bias and enhance credibility of the findings.

The involvement of colleagues in the FGD corresponds to the process of peer validation or peer debriefing in qualitative research, which serves to enhance the credibility or trustworthiness of the findings (McLeod 2024). Peers who understand the participants' backgrounds and contexts add supportive or contradictory information to what is found in individual research interviews. As Vivek et al. (2023) point out, data triangulation, which in the present paper involved data and dissenting evidence cross-checking in the focus group discussion, enhanced the credibility and reliability of the study. This process protects the meaning derived from the interviews, in this case the interpretation, from being overly influenced by the researcher's understanding of the phenomenon.

In spite of all of this, inclusion of people associated closely with the participant requires some degree of attention. On the one hand, this introduces some bias relating to the personal ties involved, but on the other hand, it assists in the gaining of insights into the participant's real-world context. Nyumba et al. (2018) illustrates the importance of balancing closeness and familiarity with participants in order to corroborate the data with an unbiased approach, which is commendable. The fact that some colleagues in the FGDs are qualified in qualitative research means that there is not only triangulation but there is also improvement in the discussions and interpretations of the topics in the FGDs (Peters 2022). Gaining direct knowledge after assuming this role and their unique perspective will deepen and broaden the understanding pertaining to the challenges and experiences that the participants encounter. Including these two colleagues addresses potential bias within the exploratory framing of the study because having multiple perspectives can mitigate both researcher and participant biases (Natow 2019). This approached aimed to triangulate data and reinforce the trustworthiness of the conclusions through combining individual interviews with FGDs.

Initially the interviews were crafted in the English language, and the group discussions were held in Filipino, so that the respondents could comprehend what was being asked and could understand. The advantages of having the documents in both English and the native language for the project also include much better clarity and comprehension because of elimination of possible language barriers. In addition, face to face interviews and FGDs as well as the virtual prospects were conducted on google meet with all the safety protocols.

All recordings were transcribed and individually checked to give an accurate target for the phase that would be next in the research. The gained pieces of information through the interviews were all integrated into and analyzed through the systematic framework of Colaizzi (1978) which is known for its rigorous and in depth examination of qualitative studies. Colaizzi's method in this case is on known and consists of 7 steps which all were used in this case study.

Data Analysis

With reference to the steps proposed by Colaizzi (1978) as applied by Morrow et al. (2015), the analysis proceeded to the seventh stage. Analysis of the verbatim transcripts from all of the participants. In the first stage, the transcripts of every participant were systematically analyzed. In the second stage, the analysis turned to the identification of the salient expressions and statements and the extraction of meaning that summarized the phenomenon under study. In the third stage, the vital phrases were organized into clusters of the essences. This was built upon to ascertain focal phenomena as profound in the fourth stage, amplifying the researchers understanding of the phenomenon by revealing synergies in the data through interconnections and associations structured by the themes as detailed. In this cluster, the researchers stretched the meaning of the themes, thus, synthesizing, defined in the fifth step in phenomenological analysis, to advance the meaning of the phenomenon to derive the sophisticated account of the participants' lived experiences.

The structures and emergent themes were verified through member checking in the sixth step. Once the analysis was completed, the researchers involved the participants in a member checking activity, per Guba and Lincoln (1994). After the themes were verified, the researchers conducted thematic cross validation, which included several follow-up FGDs and included colleagues who are seasoned qualitative researchers, which helped to check and validate the synthesized descriptions and the emergent themes identified. Qassimi (2023) states that cross validation increases qualitative research credibility and rigor through the use of different perspectives in finding verification. These colleagues were important as they had a personal relationship with the participants and qualitative research expertise, adding another dimension to the precise findings of the research. The synthesized descriptions, emerging themes, and findings were then taken to the participants' colleagues in the FGDs. The participants were asked to consider the narratives that represented the focus group's lived experiences. Reflections were also solicited regarding the fit of the descriptions with what they understood concerning the participants' perceptions as well as the correspondence of the themes they were identifying with the essences of the lived experiences of the participants. The validation of the descriptions occurred in several iterations of discourse, having peers pose clarifying queries, recommend alterations, or furnish contextual expansion. Ensuring the co-participant validation of the interpretations, the collaborative peer-review cycle offered sufficient opportunity to ascertain that the

interpretations were aligned with the participants' perspectives.

Any time the researchers identified inconsistencies or mistakes concerning particular themes, a recourse to the data was pursued as a first step to a remedy. They revisited the transcripts again to determine if the themes that were confirmed align with the participants' lived experiences. In some cases, it was the specialists themselves who revised these themes. In light of colleagues or informal networks, the constructed or modified themes would be thoroughly refined. It seemed as if there was a cycle, the analysis corresponding to the participants' opinions to a remarkable extent, which helped to authenticate the findings.

Finally, the researchers in the seventh step confirmed the themes after member-check validation as requiring no further changes. This final validation step, corroborated with the participants' feedback, attested to the fact that the meanings and themes were authentic and represented the participants' true lived experiences. This final step confirmed that all seven criteria of Colaizzi's other approach to rigorous and reliable analysis of the data were fulfilled.

RESULTS

Emergent Themes on the Challenges and Coping Strategies of Digital Immigrant Teachers

After several re-readings of the transcripts and extensive data analysis, three (3) themes of challenges emerged from data collected among experienced teachers, while two (2) themes captured the lived experiences of the novice digital immigrant teachers. Table 1 depicts the process of identifying the emerging challenges faced by novice and experienced teachers.

Challenges with Internet Connectivity

Both novice and experienced digital immigrant teachers faced significant challenges due to unstable internet connections, which hindered their ability to integrate ICT tools into virtual classrooms. Teachers in rural areas reported that internet communication and lesson delivery were difficult due to frequent disconnections and signal strength problems.

As shared by one of the experienced English language teachers with over 15 years of teaching experience (P2-E), *"... Kasagaran gayud nga problema ang internet dinhi sa amo. Lisod kaayo ko ug paabot asa makakaplag ug maayo nga signal.* (I always find it difficult to access the internet from our place. It will take me a long time to decide where to find a strong signal.)

Moreover, a similar situation was experienced by a novice Filipino language teacher (P1-N) with 1 year of teaching experience, who shared: *"...dili stable nga internet connection ma'am. Usa jud na sa problema. Pagka-way ayo internet diria. Kanus-a pa kaha ni magtinundo."* (The internet connection is not stable. It is one of the problems here of having extremely slow internet connection. I do not know when it is going right.)

Table 1. Emerging challenges of the novice and experienced teachers

Formulated Meanings	Theme Clusters	Emergent Themes
Experienced Teachers (Four or more years of teaching experience)		
• The participants encountered poor internet service quality, which directly affected their ICT integration.	Having poor and intermittent internet connection	Challenges with Internet Connectivity
• The participants associate their difficulties in integrating ICT tools with language competency due to their lack of digital knowledge and skills.	Having limited knowledge and skills in ICT and language curriculum integration.	Digital Skills and Knowledge Limitations
• The participants think that they are too old to adapt to new language teaching styles and that ICT integration is for young teachers only.	Negative perceptions of ICT integration	Reluctance to Change
Novice Teachers (Three or less years of teaching experience)		
• The participants experienced intermittent internet connection which limit their use of ICT	Having intermittent internet connection	Challenges with Internet Connectivity
• The participants are hesitant to use ICT because of their limited knowledge to technology. • The participants lack the necessary skills and knowledge to use ICT tools.	Having limited knowledge and skills in ICT and language curriculum integration.	Digital Skills and Knowledge Limitations

The responses revealed a growing reliance on technology among digital immigrant language teachers in rural underserved areas. They also indicated that inadequate digital infrastructure limited teachers' ability to sustain productive virtual classroom activities, which consequently reduced instructional efficiency.

Digital Skills and Knowledge Limitations

Both novice and experienced educators reported that their limited ICT knowledge and skills impacted their confidence and ability to integrate technology into instruction. Many sought support from family members or colleagues. This lack of proficiency led to hesitation in incorporating activities into virtual learning environments. Both groups recognized the need for enhanced ICT training to improve their digital skills and pedagogical approaches. The current results emphasized the importance of intergenerational support to overcome digital skills gaps, as well as the ways in which teachers respond to technology problems in the absence of institutional training. It also highlights the pressing need for digital immigrant language teachers to receive formal training, particularly in under-resourced contexts where these are overwhelmingly scarce.

According to the 58-year-old experienced Filipino language teacher (P1-E) with nearly 30 years of teaching experience, *"...dili gayud ko kamao ana inday. Magpatabang pa lagi ko sa akong anak ug mugamit ko. Kanang magbutang ug files ug mga activities sa messenger unya ipada. Kapila nako gitudloan, di man gihapon ko makamao ug ako-ako ra. Wala pod mi tarong training about ani."* (I am not sure Miss. My children helped me whenever I used it, such as putting and sending files in the messenger; they taught me many times, but I still did not know how to do it on my own; we did not have enough training on this.)

Similarly, a 34-year-old novice Filipino language teacher (P4-N) with one year of teaching experience expressed the same problem. *"Kay limitado pa akong hibaw-an niini, kinahanglan ko pa magtuon unsaon kay magduha-duha pa man ko labi na sa akong mga activities oy. Magpangutana pa ko unsaon pagbuhat niini. Pero kon hatagan mi ug training unsaon pag-integrate ani nga mga ICT tools, makasabot ra gayud ko unsaon ni."* (Because I have limited knowledge about it, I still have doubts about its uses, especially when I integrate the activities; I need to ask for help on what to do, but if we are given enough training on how to integrate these ICT tools, I will learn about it without a doubt.)

The findings showed that digital immigrant language teachers expressed a need for professional development tailored to their specific technological and instructional needs. The responses also indicated that training programs providing practical and relevant digital knowledge would be beneficial for their professional growth.

Reluctance to Change

The reluctance of older teachers, termed as digital immigrants, to adapt to modern technology, was explained as a consequence of their advancing age and impending retirement. They suggested that younger teachers are better-suited for incorporating technology. On the contrary, beginner teachers demonstrated a greater propensity for employing ICT, albeit experiencing significant barriers related to inadequate digital competencies and unstable internet connectivity. Their attitude regarding the role of ICT in modern education is a precursor for transformation and learning. This research found a marked intergenerational divide; experienced teachers tended to show resistance to the integration of ICTs citing reasons related to ageing while novice teachers, also faced with similar constraints of technology, showed a readiness and a passion to use digital features. From this angle of ICT teacher adoption, it provides a profound understanding on how teacher's age and career stage impact the propensity of teachers to adopt new technologies. These finding prompts government agencies such as the Department of Education to provide aid or language assistant to the experienced teachers in incorporating ICT.

The experienced Filipino language teacher (P1-E), with nearly 30 years of teaching experience, expressed frustration, saying: *"...Kapoy na gayud kaayo, Inday. Hapit na ko mo retire. Nagsakit na akong mata, dili na nako klaro ang mga gagmay nga letra sa computer. Mas angay na lang gyud sa inyo mga batan-on. Kami mga tigulang na, dili na kinahanglan mag-apil-apil ana. Kanang mobiyahe pa ug lagyo kay mangita ug signal, sometimes mosaka pa ug buntod to get internet connection."* (It is already tiring for me, Miss; I am almost to retire, and I cannot even see those small letters on the computer; that is only good for the young, like you; those of us who are older, like me, should never be involved in that. Such long travel to find strong internet connection. Sometimes, climbing mountains to get internet connection.)

Meanwhile, the experienced English language teacher with 28 years of experience (P4-E) also commented: *"...maolagi langga, taas na ang lubi. Dili nako kugihon. Kamong mga batan-on, sayon ra na ninyo. Mga haniti naman mo ana kami maopay ituon namo. Hingalimtanon na pod samot, maglisud tawon ko lang."* (That's it, dear, the coconut tree is fully grown (idiomatic expression meaning – I am too old to begin). It does not appeal to me. Because you are young, it is simple for you to do. It is extremely difficult for us, who already have a short memory and are just beginning to learn.)

The findings showed that older, more experienced teachers exhibited resistance to change due to age-related perceptions, while younger teachers

demonstrated greater openness to adopting new methods. The results also revealed a significant relationship between teachers' career stages and their use of digital tools.

While digital immigrant language teachers encountered challenges in integrating ICT into their instruction, they also demonstrated the ability to manage these difficulties by employing various coping strategies. Table 2 depicts the process of identifying the emerging coping strategies employed by novice and experienced teachers.

Table 2. Emerging coping strategies of the novice and experienced teachers.

Formulated Meanings	Theme Clusters	Emergent Coping Strategies
Experienced Teachers (Four or more years of teaching experience)		
• The participants took the initiative to find locations with stronger signals to upload digital modules or attend virtual classes. • They sometimes traveled to areas with better connectivity to ensure their responsibilities were met.	Searching locations with stable connections	Initiating Proactive Measures
• The participants relied on colleagues or family members, including their children, for assistance with ICT-related tasks.	Seeking help from social networks	Social Assistance
• The participants viewed ICT integration as part of their professional duties and pushed themselves to meet work requirements, despite hesitations or initial resistance.	Accepting ICT utilization as a job responsibility	Work Compliance
Novice Teachers (Three or less years of teaching experience)		
• The participants look for locations with stronger internet signals to ensure they could upload teaching materials or participate in virtual classes.	Searching locations with stable connection	Initiating Proactive Measures
• The participants relied on colleagues, family members, or community members to assist them with ICT-related challenges.	Seeking help from social networks	Social Assistance

Initiating proactive measures. To cope the challenges with internet connectivity, both novice and experienced language teachers took the initiative to find locations with stable signals. This sometimes involved traveling to areas with better connectivity to ensure they could upload and share digital modules or participate in virtual classes. This coping strategy is described by both novice and experienced digital immigrant teachers in the following excerpts.

The 43-year-old experienced English language teacher with over 15 years of teaching experience (P2-E) mentioned: *"...mangita pa lagi ko ug asa'y kusog nga signal aron ra masend tong akong digital modules sa mga bata."* (I needed to find a location with a strong internet connection so that I could send my digital modules to my students.)

Similarly, a 23-year-old novice Filipino language teacher with almost two years of teaching experience (P3-N) expressed: *"...muadto pa ko sa centro kay kusog man signal adto."* (I had to go to our town center because the internet connection is good there.)

Social Assistance. Due to digital skills and knowledge limitations, both groups sought help from others, including colleagues, children, and community members. This social assistance helped them handled the challenges of using technology in their teaching. Teachers often relied on others to guide them through technical tasks or troubleshoot problems. These statements exemplified how teachers approached this challenge.

As the 58-year-old experienced Filipino language teacher (P1-E) revealed: *"...magpatudlo ko inday, naa si Sir (kauban sa trabaho) sige nako samukon. Maayo man siya ana."* (I let others teach me Miss, (mentioning a colleague's name) I was always bothering him. He is very good at it.)

Comparably, the 42-year-old experienced English language teacher (P4-E) also shared: *"...naa akong mga anak lang maoy akong tig-tudlo. Sigihan nako sila ug pangutana ug di ko kasabot sila akong sugoon."* (I enlist my children's assistance, and I let them do the work if I do not understand.)

Work Compliance. Experienced digital immigrant teachers, especially those reluctant to change, coped by viewing ICT integration as part of their job responsibility. Despite their hesitations, they pushed themselves to meet work requirements, acknowledging that technology is an unavoidable aspect of modern teaching.

As commented by the experienced Filipino language teacher (P1-E) with nearly 30 years of teaching experience: *"…mapugos nalang jod ko ug antos ani kay mao naman jud ni atong trabaho inday."* (I have no choice but to force myself to use ICT; it is already part of our job, Miss.)

DISCUSSION

Challenges with Internet Connectivity

Both novice and experienced digital immigrant teachers teaching in virtual learning environments face challenges due to unstable internet access, impacting their ICT integration and teaching practices. This coincides with Teräs (2022) findings which points out a global issue of inadequate internet connectivity in rural areas. The poor and intermittent internet connections deter experienced digital immigrant teachers from optimizing the use of ICT tools, unfavorably affecting the educational process. Improving digital infrastructure is crucial for teachers to integrate ICT fully into their language teaching instruction.

For experienced teachers, the situation is even worse as they are less confident with technology, intermittent internet further amplifies their reluctance. As stated by Gómez-Fernández and Mediavilla (2022) the ability to connect to the internet has an impact on an instructor's propensity to utilize ICT. Having poor connectivity, teachers do not wish to integrate technology into their instructions because of the likelihood of disruptions due to the technology. This, however, is a challenge that posits some degree of concern. As a result, schools should be partnered with top tier internet providers to improve internet access in the far-flung regions of the country so that teachers concentrate on the real work of teaching. This is an issue that the government and the relevant education authorities need to prioritize with respect to the digital infrastructure gap, specifically the geographical digital divides in the country, and the particular ramifications of this on the teaching of the English language in underserved areas.

Digital Skills and Knowledge Limitations

Inefficient understanding and application of pedagogy ICT integration stands as another predominant obstacle for both novice and experienced educators. For example, many do not know how to embed the language macro skills into digital platforms. Basargekar and Singhavi (2017) found that a lack of ICT knowledge and expertise is seen by instructors as a major hindrance to ICT application. In this study, both novice and experienced teachers recognized that with limited skills it was difficult to learn and use ICT well. This suggests the demand for crafting professional development programs to improve the technical skills and to instill technology adaptability to the digital immigrant educators.

Hennessy et al. (2022) argued that simply providing ICT equipment is no good for teachers if they have no skill in using it effectively. Experienced teachers, in particular, may struggle to shift away from traditional methods, for they may believe that they are not qualified to operate efficiently in a digital environment. On the contrary, novice teachers, while still lacking proficiency, are more motivated to learn. However, they both perceive that the appropriate training on ICT will help improve their competence and confidence in using ICT tools. Schools should provide continuous pedagogical ICT training as well as the development of mentoring and peer support systems to enable knowledge sharing and address the digital divide (Sapad and Caballes 2022). This underscores the necessity of constructing professional development modules that tailor to the needs of the digital immigrant language teachers. With these, useful knowledge is provided in developing training programs that suit the needs of such language teachers.

Reluctance to Change

Change is always met with reluctance, and in the case of educators' resistance is often the highest among the most experienced. A considerable number of these educators view the adoption of any new ICT tool or technique as an attempt to re-learn something, thus perceiving themselves as "too old" or even "set" as they subscribe to the belief that the old ways suffice. The ICT in the classroom practice is influenced in a complex way, as Seifu and Wang (2020) has explained in relation to the attitude or disposition of a teacher towards integrating technology. This reluctance towards change among the more seasoned teachers is rooted in a psychological state of fear and uneasiness towards unfamiliar tools. These observations on ICT integration work provide valid clues to changes in teachers' dispositions. Most teachers reveal ICT access and use barriers, suggesting the need for targeted professional development. Such integration-focused initiatives strengthen the collaboration of the ICT resources.

Conriquez (2020) underscored the importance to know teachers' orientations toward change as their beliefs impact the way they practice in the classroom. Similarly, Dogan et al. (2021), emphasized that the extent to which technology can be successfully integrated depends significantly on teachers' attitudes toward the technologies. Teachers' resistance to change may have the effect of slowing the adoption of technology and affect successful ICT integration into schools (Hamlaoui 2021). Contrarily, novice teachers are more willing to utilize technology albeit that they are hindered by challenges such as lack of skill and connectivity issues. Novice teachers have been reported to be more open to technology for teaching and are more likely to consider technology as an essential for modern teaching (Bice and Tang

2022). This willingness to adopt ICT, even with difficulties, reflects the generation gap amongst teachers when it comes to technology in education, highlighting the need for differentiated strategies to assist digital immigrant teachers at all levels of experience.

In overcoming the persistent challenges associated with the use and integration of digital pedagogies into their curriculum, all educators, irrespective of their digital teaching proficiency face hurdles. This type of situation epitomizes the advancement that educators have made with the paradigm of digital pedagogy, and thus, policy frameworks and support programs should plan integration strategies for technology by considering age and career milestones. In the case of language teachers who are digital immigrants, the ability to systematically use ICT is associated with hurdles of pedagogical ICT usage. This focuses on the ability to overcome ICT usage attitudinal barriers and encourage effective teaching of the complex dynamics of the language system.

Coping Strategies

Initiating proactive measures. Even in the face of multifaceted challenges, both novice and advanced digital immigrant educators have undergone transformative changes in their thinking and behavior concerning the adoption of coping strategies to ICT integration. Seeking improved internet connection and or access is one of the strategies employed. Teachers often relocate to zones within weaker distance to the access point, underscoring their efforts to ensure that their students receive ICT integration material regardless of the distance they have to travel. This is consistent with Munna and Kalam (2021), who noted that teachers enthusiastically combined old and new methods to cope with the challenges ICT integration posed, underscoring their commitment to offering challenges. The immeasurable perseverance and adaptability of teachers, who are classified as digital immigrants, immensely assist the promotion of integrating ICT into the instructional methods and improving the teaching and learning process. These initiatives reflect how language teachers handle such problem on internet connectivity, through proactively seeking out solutions to fulfill their virtual teaching responsibilities.

Social Assistance. Moreover, both teachers indicated the need of social support in order to deal with their inadequacies in ICT skills. Most seek assistance from friends, family, or anyone they perceive to be more tech-savvy. These individuals are willing to help them overcome feelings of low self-esteem and limited ICT skills to access and use digital technologies. This assistance and encouragement from more knowledgeable peers facilitate the use of the Internet with low self-esteem. This is more the case with novice teachers, as they are the ones who are more inclined to adopt such practices. This is consistent with Li et al. (2019) who stated the importance of cooperation and networking support in the context of technology integration. Success in overcoming such challenges is, as Chellammal et al. (2023) points out, predicated on teachers' knowledge, skills and competencies. These findings suggest the need to encourage more formal peer support/mentoring as one approach to closing the digital divide and improving the ICT to productive ratio. Similarly, more collaboration and support networks to address the ICT challenges of remote teaching is what enables language educators to overcome the deficiencies in their digital skills and knowledge.

Work Compliance. An integral part of the profession is adaptability, finding the best creative solutions to challenges that arise. To experienced educators, discomfort with teaching technologies stems from trust in the benefits that thrive ICT facilitates in classrooms. Considering Tortola (2024), there may be a working assumption that seasoned educators are resilient with integrating varied problem-solving approaches harmonious to professional tasks. Advanced competence in teaching and learning requires cognitive and flexible ICT integration. Improving and teaching ICT requires a sustained effort to create a robust teaching workspace and to productively cope with teaching and learning, technology, and personal challenges. Discomfort and reluctance are the limiting variables in bottlenecks and ICT teaching integration. Hence, these interventionist strategies are essential to close teacher's gap in digital and linguistic ICT integration that is expected in 21st educational pedagogy.

By actively embracing these measures, teachers show remarkable resilience and dedication in overcoming the challenges posed by virtual teaching environments. Despite obstacles like limited digital literacy and unreliable internet connectivity, they remain focused on providing their students with a high-quality education.

In conclusion, the use of ICT in remote language teaching requires a balanced multi-faceted approach while understanding the nuances of skill sets, the development of ICT infrastructure, and the relational dynamics while supporting language teachers who are digital immigrants teaching digital natives students. Critical as ICT integration advances in all teaching practices are the facilitators' commitment and vision as they contribute to the phenomenon of digital immigrant educators across diverse educational settings globally. The data speak to the impact of professionally focused school-wide reform initiatives on developing educators' engagement and relational frameworks of adaptability, mentorship, and peer collaboration, which promote information flow and the reduction of the digital divide. The emphasis of the study once again strengthens the argument on the digital desert

phenomenon, particularly the lack of the internet and other digital teaching tools, which remain the most significant barriers to effective ICT-enabled language teaching in a digital society.

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GENERATIVE AI STATEMENT

In creating this work, the researchers utilized ChatGPT for the enhancement of statements and grammar checking. After using the tool/service, the author(s) carefully reviewed and edited the content as necessary and bear(s) full responsibility for the publication's content.

ETHICAL CONSIDERATIONS

The researchers submitted files to their local Research Ethics Committee for validation. After receiving local ethics certification, they informed participants of the strict confidentiality that would be observed throughout the study and asked for their consent to record the interview and focus group discussion sessions. In accordance with Bryman and Bell (2007), the following ten key ethical considerations were central to this study: 1) research participants should not be subjected to harm in any way; 2) respect for the dignity of participants should be prioritized; 3) full consent should be obtained from participants prior to the study; 4) the privacy of research participants must be protected; 5) adequate confidentiality of research data should be ensured; 6) Anonymity of individuals and organizations participating in the research must be maintained; 7) any deception or exaggeration about the aims and objectives of the research must be avoided; 8) affiliations, sources of funding, and potential conflicts of interest must be declared; 9) all communications related to the research must be conducted with honesty and transparency; and 10) it is essential to avoid presenting misleading information or skewing primary data findings in a biased manner.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests to any authors.

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Consumer preference analysis for shrimp characteristics across income groups in Southcentral Mindanao, Philippines

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ABSTRACT

Shrimp is a highly demanded seafood worldwide, valued for its nutritional benefits, versatile culinary applications, and delicious taste. In Southcentral Mindanao, specifically Cotabato province, understanding consumer preference for shrimp quality is critical for enhancing market competitiveness and meeting diverse consumer demands. This study examines shrimp preference patterns by employing a hedonic pricing model to analyze how shrimp attributes and consumer traits influence market price for shrimp. Data collected through face-to-face interviews with 245 respondents across income groups were analyzed using descriptive statistics, ANOVA, and regression analysis. The findings show that size, origin, and freshness significantly affect shrimp prices, with consumers willing to pay premiums for high-quality shrimp. Ocean-sourced, white-colored, fresh shrimp preserved in ice cubes are universally preferred, although low-income consumers tend to opt for freshwater shrimp stored in water, while higher-income groups prioritize ocean-sourced shrimp. Consumer traits, such as education, influence purchasing decisions among low-middle-income groups, but none significantly influence the purchase decisions of those consumers in high-income groups. These findings emphasize the economic value that consumers attribute to shrimp quality and highlight the need for targeted strategies to address diverse preferences. Improving shrimp quality through aquaculture innovations, better preservation methods, and market segmentation, while emphasizing government support to enhance competitiveness, is imperative. Moreover, these insights guide producers and marketers in meeting consumer demands and suggest further research to explore the implicit value of shrimp attributes throughout the supply chain for a more comprehensive understanding of market dynamics.

Keywords: consumer traits, freshness, hedonic pricing model, market segmentation, Mindanao, price sensitivity

INTRODUCTION

Shrimp farming has developed rapidly in the Philippines, driven by potential high profits, the growing demand for high-value seafood products, and the increasing reliance on farmed shrimp due to limitations in supply from capture fisheries (Islam and Yasmin 2017). Additionally, the industry's ability to generate foreign exchanges and provide employment in poor coastal areas has fueled its expansion (BFAR 2020). In the Philippines, it is both a source of livelihood and food for coastal communities. The best-known species for shrimp culture is *Penaeus vannamei* Boone, 1931 (Cuvin-Aralar et al. 2009). The species

has won favor with tropical producers due to properties that include short culture time and fast growth, and it is an entry into the world market (Rosario and Lopez 2005). Its production has tremendously increased over the years, putting on the human menu an important seafood item giving high commercial profit, but the disease has heavily wrecked this (Shinn et al. 2018). While the bulk of shrimp production is in Negros and the Panay Islands, shrimp production has been growing and increasing in other areas, such as in the Southcentral Mindanao regions, Central Luzon and other parts of Central Visayas (PSA 2020a). Globally, shrimp serves as a staple protein source and is one of the most widely consumed seafood products. The increasing demand is fueled by its perceived health benefits, culinary versatility, and the expansion of global seafood markets. However, this growth in consumption has raised concerns about food safety, environmental sustainability, and the socio-economic impacts of shrimp farming practices (Macusi et al. 2022).

Consumer preferences for shrimp are influenced by a range of factors, including sensory attributes, perceived quality, and socioeconomic considerations. Understanding these preferences is crucial for producers and marketers aiming to meet consumer demands effectively. One of the primary sensory attributes influencing consumers' preference is color. Research has shown that consumers strongly associate specific colors with quality. For instance, Parisenti et al. (2011) found that consumers preferred cooked shrimp with a reddish-orange hue, a color linked to freshness and higher quality. Similarly, Zancan et al. (2023) noted that consumers favor lighter-colored raw shrimp and more intensely orange-colored cooked shrimp. These findings underscore the importance of visual appeal in seafood marketing. In addition to color, freshness and perceived quality significantly impact consumer willingness to pay (WTP). According to Hoque et al. (2021), locally farmed shrimp are believed to be fresher and more appealing than imported ones. This belief stems from the influences of culture and national pride, thereby developing such appeal for local products. Nayak and Joshi (2022) also discussed the significant role of appearance, smell, and texture in shaping consumer preference. With this, the value of shrimp produced for the market is contingent upon the sensory and visual preferences that are typical for consumers before making a purchase (Omobepade et al. 2018). Accordingly, Parisenti et al. (2011) revealed that, in terms of size, consumers put a premium on medium-sized shrimp sold in the market. Similarly, bioeconomic modelling of Moreno-Figueroa et al. (2018) showed that the quality and perceived size of the shrimp motivated consumers to increase their satisfaction value. Suthamathy (2012), on the other hand, emphasized that extrinsic characteristics of shrimp, such as carapace size, mass, origin, species, freshness, product form, and preservation method, significantly influence its market price. Hence, these studies emphasize that hedonistic motives for purchasing play a strong role in consumer preference (Sahubawa and Suratno 2023).

Additionally, socioeconomic characteristics influence consumer preference. Studies reveal that educated and affluent households tend to purchase organic or sustainably produced shrimp (Paul and Vogl 2012). While other studies emphasized the personal characteristics of the consumers, Jhan et al. (2023) proved that trust and product features consistently influence consumers' purchase intention on shrimp. For example, Ortega et al. (2014) found that safety-oriented features included in shrimp products are likely to receive higher premiums among consumers. Aside from this, market dynamics influence consumer preferences. Santo and Marques (2022) found the competitive retail pricing present in the e-commerce context led consumers to focus on hedonic benefits in association with perceived value.

Therefore, the price consumers are willing to pay for seafood products, such as shrimp, often depends on quality standards (Suthamathy 2012). While consumers consider various characteristics like nutrition, convenience, food safety, and environmental impact, studies quantifying these preferences remain limited. While product attributes can be observed, they may be subjective. A more objective approach to analyzing quality changes and consumer preference was recommended referring to major hedonic study of Griliches (1961) highlighting the need for further research in this area. Hence, this study attempted to determine the socio-economic characteristic of the consumers, analyze the consumer preference for shrimp characteristics using hedonic price analysis, and determine variations in preference across income groups.

METHODS

Theoretical Framework

The consumer preference analysis is underpinned by the "characteristics" approach to consumer theory developed by Lancaster (1966), which assumed that consumption is an activity in which goods, singly or in combination, are inputs and in which the output is a collection of characteristics. The approach builds upon activity to model the combination of the characteristics that can be achieved given the assumptions on (a) whether the combination of goods is possible or not in the market, (b) whether the combination of goods can be made in a linear way or not, and (c) whether the number of characteristics is smaller or larger than the number of goods containing them (Chumpitaz et al. 2010).

The fundamental theory of hedonic price explains the price (P) of a commodity as a function of

its characteristics. The model assumes that a product is composed of a variety of specific attributes that consumers value independently. For any given commodity, let us consider it characterized by the set of *j*-th specific attributes and are denoted as $Z = (Z_1, Z_2, Z_3)$, and it is assumed that the preference of the economic agents towards the commodity is determined by its corresponding characteristic vector alone. The functional relationship between its price *P* and characteristics vector *Z* is given by Equation 1:

$$P = f(Z) \quad \text{(Eq. 1)}$$

This function specifies the hedonic relationship for a commodity. According to the functional relationship in the Equation 1, the marginal price of the j-th characteristic, say *Zj*, is given by the partial derivative of the hedonic function (Equation 2) with respect to *Z*:

$$\frac{\partial p}{\partial z_j} = \frac{\partial f}{\partial z} \quad \text{(Eq. 2)}$$

The hedonic price $\frac{\partial p}{\partial z_j} = \frac{\partial f}{\partial z}$ indicates how much the price *P* of a good changes if this good is endowed with an additional unit of the characteristics Z_j and all others are constant (Suthamathy 2012).

Respondents of the Study

Shrimp consumers were systematically and randomly selected from the total number of households in the selected market areas - Midsayap, Kabacan, Makilala and city of Kidapawan. These were considered as the major market centers due to its substantial consumer bases. Midsayap has a total population of 165,376; 93,822 for Kabacan; Makilala with a total population of 87,927 and 160,791 people in Kidapawan based on the 2020 Census of Population (PSA 2020b). These consumers were those who bought shrimp from the market. A total of 245 (Midsayap [n = 60]; Kabacan [n = 55]; Makilala [n = 65], and Kidapawan [n = 65]) consumers were considered as the respondents of the study.

Research Instrument

An interview guide was used to gather necessary information for the study. It was composed of two parts. Part I consisted of questions eliciting information about the socio-economic characteristics of the respondents. Consequently, part II was composed of questions pertaining to the quality of shrimp preferred or demanded by the consumer-respondents. The interview guide was validated to ensure applicability and appropriateness.

For the development of the instrument, specifically in part II, the considered shrimp attributes in the study were size, carapace length, species color, freshness, preservation method, product form, and origin. These attributes, except for size and carapace length, were measured using a hedonic scale. For size and carapace length, the researcher brought small weighing scale and ruler for determining the weight (g) and length (mm) of the shrimp and its carapace, respectively.

Data Gathering Procedure

The primary data were elicited through a survey of the consumer-respondents. This was a direct face-to-face interview with the consumer-respondents. According to Owusu and Anifori (2013), face-to-face interviews offered an opportunity to explain some questions to respondents with low literacy levels and did not introduce significant bias to the study. Hence, consumer-respondents were personally interviewed to obtain their socio-economic characteristics as well as their preferences for shrimp characteristics.

The interview was simultaneously done while the consumer-respondents were buying shrimp. This is to ensure that the information on the quality preference is correctly obtained. Further, during the conduct of the interview, the researcher asked for a sample of shrimp to measure and obtain its correct weight and carapace length.

Statistical Analysis

Descriptive statistics. Descriptive statistics such as frequency counts, percentages, and means were computed to describe the socio-economic characteristics of the consumer-respondents. In addition, the key quality attributes preferred by the consumer-respondents were analyzed descriptively. Tabular presentation was used to present the result. Moreover, results were categorized across income groups – low, middle, and high. This categorization was based on the NEDA classification for income groups.

Hedonic regression analysis. The hedonic regression method is used in this study. It recognizes that heterogeneous goods can be described by their attributes or a characteristic, i.e. a good is essentially a bundle of (performance) characteristics (Eurostat et al. 2013) RPPI Handbook 2011). In this method, the regression technique is used to obtain estimates of the willingness to pay for the different characteristics.

The ordinary least square method was used to determine the effect of shrimp qualities on its price. The estimated coefficients were obtained using the STATA 10.0 software. The regression analyses were done separately for different income groups. However, the low- and middle-income groups were pooled together since the sample size for low-income is relatively small with n=24 households. According to Chang et al. (2006), a sample size equal or greater than 30 is considered sufficient for the central limit theorem to hold, hence, can proceed to regression.

Empirically, the hedonic price estimation/analysis is shown in Equation 3:

$$P_s = \alpha_s + \beta_1 S_{(w)} + \beta_2 O + \beta_3 SC + \beta_4 F + \beta_5 PM + \beta_6 PF + \beta_7 Age + \beta_8 Educ + \beta_9 Occup + \beta_{10} Dist + \beta_{11} Gen + \beta_{12} Civil + \varepsilon_s \quad \text{(Eq. 3)}$$

Where:

Ps=actual/prevailing price of shrimp (P/kilogram)

$S_{(w)}$ =Ave. size of shrimp (weight)

O=dummy variable for origin (1 if ocean-sourced, 0 if freshwater)

SC =dummy variable for species color

F =dummy variable for freshness

PM = dummy variable for preservation method

PF= dummy for product form

Age = age of the respondents at the time of study

Educ = education (in years)

Occup = occupation (1 if employed, 0 if unemployed)

Gen = gender

Civil = civil status

Dist = distance from the market

$\propto_S$ = intercept term

$\beta_1 - \beta_7$ = slope of the estimated coefficients

ε_S = error term of the model

Comparison of means. Leven's test was used to determine the homogeneity of variance prior to the use of one-way analysis of variance (ANOVA). The ANOVA was used in determining if there was any significant difference in the consumer traits between income groups in selected large market centers.

The F-distribution was used as a basis of comparison. The hypotheses tested among the means are H_0: $\mu_1 = \mu_2 = \mu_3$ (all means are equal), and the alternative hypothesis (*Ha*): at least two of the means are not equal (Lantican et al. 1996). The condition to reject the null hypothesis is that the F statistic should be greater than the critical value. In this study, Statistical Package for Social Sciences (SPSS) was used.

A post hoc test was done to evaluate the pairwise difference among income groups. In the literature, the most common and recommended post hoc tests used are Tukey's Honestly Significant Difference (HSD) and the Games-Howell multiple comparison. Tukey HSD was used for those variables that do not violate the assumption of homogeneity of variance, and Games-Howell was used if otherwise.

RESULTS

Table 1 shows the socioeconomic characteristics of consumer-respondents across income groups. The average age of respondents across income groups ranges from 36 to 37 years, with no significant variation. Similarly, the average years of education are consistent, spanning 11 to 12 years across all income groups. Gender composition shifts notably between groups. Females predominate in the low-income group (75%), while gender distribution becomes more balanced in the middle-income group (50.9% female and 49.1% male) and slightly favors males in the high-income group (45.1% female and 54.9% male). Civil status shows a clear trend of increasing marriage rates with income level. In the low-income group, 58.3% are married, compared to 70.4% in the middle-income group and 90.3% in the high-income group. Despite these differences, all income groups exhibit a unanimous interest in a health-conscious lifestyle, with 100% of respondents in each category identifying as health-conscious. Moreover, results reveal variations in average household size and distance. While low- and middle-income groups have a similar average household size of 3-4 members, the high-income group shows a slightly larger average of 5 members. The average distance follows a similar pattern, with no significant differences observed across the groups (2.65-3.23). Income characteristics indicate significant disparities among groups. The low-income group has the lowest average monthly income (PHP 3,845.83 or USD 66.17) and annual income (PHP 46,150.00 or USD 794.03), while the high-income group reports the highest values (PHP 13,034.07 or USD 224.26 monthly and PHP 56,408.80 or USD 970.54 annually). The middle-income group falls between these extremes. Per capita income also demonstrates a statistically significant difference ($P < 0.05$), with the high-income group achieving more than double the per capita monthly and annual income compared to the middle-income group and nearly triple compared to the low-income group.

Shrimp Attributes Preferred among Consumer-respondents

As this study examines shrimp attributes preferred by consumer-respondents across income groups, significant differences are observed in price, size, and carapace length (Table 2). The low-income group prefers significantly lower shrimp price (M = PHP 215.83 (USD 3.71), $P < 0.05$) compared to both middle-income group (M = PHP 269.44, $P < 0.05$) and the high-income group (M = PHP 298.14 (USD 5.13), $P < 0.05$). Similarly, the average size of shrimp preferred increases with income, ranging from 28.71 grams for the low-income group to 34.37 g for the high-income group. Carapace length follows the same pattern, with preferences increasing from 31.04 mm in the low-income group to 34.83 mm in the high-income group.

Differences in preference for shrimp origin are notable. The low-income group predominantly favors freshwater shrimp (70.8%), while the high-income group prefers ocean-sourced shrimp (82.4%), with the middle-income group exhibiting a balanced preference. Regarding species color, all respondents in

Table 1. Socioeconomic characteristics of shrimp consumers in selected large market centers by income groups in Southcentral Mindanao. Means followed by the same letter are not significantly different from each other at the 5% level. Post-hoc test: [d]Tukey HSD; [e]Games-Howell. Values inside parenthesis ( ) are percentages of numbers reported. Low: n = 24, Middle: n = 108, and High: n = 113.

Characteristics	Income Groups		
	Low (n=24)	Middle (n = 108)	High (n = 113)
Average Age[d]	36[a]	37[a]	37[a]
Education (ave. in year)[e]	11[a]	12[a]	11[a]
Occupation			
Employed	22(40)	44(67.7)	39(60)
Unemployed	33(60)	21(32.3)	26(40)
Gender			
Female	18(75)	55(50.9)	51(45.1)
Male	6(25)	53(49.1)	62(54.9)
Civil Status			
Married	14(58.3)	76(70.4)	102(90.3)
Single	10(41.7)	32(29.6)	11(9.7)
Lifestyle			
Health Conscious	24(100)	108(100)	113(100)
Ave. Distance to the Market[e]	2.65[a]	3.13[a]	3.23[a]
Ave. Household Size[d]	3[a]	4[a]	5[b]
Ave. Monthly Household Income[e]	3845.83[a]	6449.07[b]	13034.07[c]
Ave. Annual Income[e]	46150[a]	77388.89[b]	156408.8[c]
Per capita average monthly income[d]	1281.94[a]	1612.27[b]	3258.52[c]
Per capita average annual income[d]	15383.33[a]	19347.22[c]	39102.2[b]

Table 2. Quality attributes of shrimp preferred by consumers in selected large market centers by income groups in Southcentral Mindanao. Means followed by the same letter are not significantly different from each other at the 5% level. Post-hoc test: Games-Howell. Values inside parenthesis ( ) are percentages of numbers reported. Low: n = 24, Middle: n = 108, and High: n = 113.

Characteristics	Income Groups		
	Low (n = 24)	Middle (n = 108)	High (n = 113)
Average price per kilogram	215.83[a]	269.44[b]	298.14[c]
Average size (weight in g)	28.71[a]	30.94[b]	34.37[c]
Average carapace length (in mm)	31.04[a]	31.46[c]	34.83[b]
Origin			
Ocean-sourced	7 (29.2)	68 (63)	92 (82.4)
Freshwater	17 (70.8)	40 (37)	21 (18.6)
Species Color			
White	24 (100)	88 (81.5)	86 (76.1)
Black		13 (12)	13 (11.5)
Brown		7 (6.5)	14 (12.4)
Freshness			
Fresh	16 (66.7)	68 (63)	95 (84.1)
Acceptable Fresh	8 (33.3)	40 (37)	18 (15.9)
Preservation Method			
With ice cube	4 (16.7)	58 (53.7)	82 (72.6)
Water only	20 (83.3)	50 (46.3)	31 (37.4)
Product Form			
Whole shrimp	24 (100)	108 (100)	113 (100)

the low-income group prefer white shrimp, while middle- and high-income groups display diversified preferences, with white shrimp being most popular but supplemented by minor preferences for black and brown shrimp. Moreover, the high-income group shows the strongest preference for fresh shrimp (84.1%), followed by the low-income group (66.7%). The preservation method preferences also vary. Low-income groups predominantly favor shrimp preserved in water (83.3%), whereas both middle- and high-income groups show increasing preference for shrimp preserved with ice cubes (53.7% and 72.6%, respectively). Meanwhile, a uniform preference for whole shrimp is observed across all income groups,

with 100% of consumer-respondents choosing this product form.

Hedonic Price Analysis by Income Groups

The result of the hedonic regression analysis shown in Table 3 provides an examination of the factors influencing shrimp prices for low-, middle-, and high-income consumer groups. The use of the low-middle-income group was because the low-income group had fewer respondents (n = 24); hence, it was pooled together with the middle-income consumers. Thus, only two income groups were examined. The results of the hedonic regression analysis provide a detailed examination of the factors influencing shrimp prices for low-, middle-, and high-income consumer groups. The models are robust, as evidenced by the adjusted R^2 values of 0.8929 and 0.8621 for the low-middle and high-income groups, respectively. The adjusted R^2 indicates that the included explanatory variables explain a substantial portion of the variations in shrimp prices (89.29% and 86.21% for low-middle and high-income groups, respectively). Both models are statistically significant, with F-statistics of 85.01 and 54.86 at the 1% level. Using the *ceteris paribus* principle, the significant variables were interpreted to isolate their specific effects on shrimp prices.

For shrimp characteristics, size, origin, and freshness emerge as significant determinants of shrimp prices in both income groups. Shrimp size has a significant positive effect on price in both groups, with coefficients of β = 0.002 (low-middle income) and β= 0.008 (high income), both significant at the 1% level. This indicates that, *ceteris paribus*, a 1-gram increase in shrimp size (measured by its weight in grams) leads to a 0.2% and 0.8% increase in price for low-middle and high-income groups, respectively. The larger coefficient for high-income consumers suggests that this group places a higher premium on shrimp size. Shrimp origin also significantly influences price, with coefficients of β= 0.42 and β= 0.43 for the low- middle and high-income groups, respectively, at the 1% level. This implies that, holding all other factors constant, ocean-sourced shrimp are priced 42% and 43% higher than freshwater shrimp for the respective income groups. Freshness has a positive and significant impact on price, with β = 0.061 for low-middle income and a β = 0.117 for high income, indicating that fresh shrimp, compared to acceptably fresh shrimp, commands a price premium of 6.1% and 11.7% in the two groups, respectively. The stronger effect of freshness among high-income consumers reflects their heightened sensitivity to this quality attribute. In contrast, species color and preservation methods are not significant determinants of shrimp prices for either income group. Hence, these attributes are less critical in consumer preference valuation.

Table 3. Estimated hedonic regression model for the characteristics affecting the price of shrimp by income groups in Southcentral Mindanao. Characteristics followed by letters and not seen in the table are the base dummy variable. Figures in ( ) are t-values. ***, ** - significant at 1% and 5% level; ^{ns} – not significant.

Variables	Income Groups	
Shrimp Characteristics	Low-Middle (n = 132)	High (n = 113)
Size	.002*** (8.01)	.008*** (4.69)
Origin	.42*** (12.79)	.43*** (14.28)
Species Color		
White	a	a
Black	-.012^{ns} (-0.05)	-.015^{ns} (-0.55)
Brown	-.011^{ns} (-0.23)	-.005^{ns} (-0.18)
Freshness	.061** (2.78)	.117*** (4.75)
Preservation Method	-.025^{ns} (-0.87)	-.032^{ns} (-1.46)
Consumer Characteristics		
Age	.001^{ns} (0.98)	-.001^{ns} (-0.73)
Gender	-.015^{ns} (-0.69)	.014^{ns} (0.18)
Civil Status	.017^{ns} (0.54)	-.042^{ns} (-1.26)
Education	.010** (2.08)	-.004^{ns} (-0.74)
Occupation	-.031^{ns} (-1.27)	-.016^{ns} (-0.67)
Distance	-.009^{ns} (-1.20)	-.002^{ns} (-0.31)
Adj. R2	0.8929	0.8621
F	85.01***	54.86***
n	132	113

For consumer characteristics, only education exhibits a significant effect, and this is observed in the low-middle income group (β = 0.010, t = 2.08, P < 0.05). *Ceteris paribus*, a one-year increase in education level leads to a 1% increase in the price low-middle-income consumers are willing to pay for shrimp. This suggests that better-educated consumers in this group are more likely to value quality attributes, potentially due to increased awareness or knowledge. In the high-income group, none of the consumer characteristics significantly influence price, which may reflect their broader financial flexibility and reduced sensitivity to individual demographic factors.

DISCUSSION

Socioeconomic Characteristics of Consumer-respondents

The significant differences in the income of shrimp consumers explain the patterns of shrimp consumption and preference. High-income households tend to have a greater disposable income, allowing them to purchase shrimp more frequently and

in larger quantities compared to low-income households, which may prioritize more affordable protein sources such as finfish (Bashar et al. 2022; Akter and Khan 2021). Studies have shown that low-income consumers switch to less expensive alternatives when shrimp prices rise, indicating a direct correlation between income and shrimp consumption patterns (Akter and Khan 2021). While larger households may have higher gross income, their purchasing decision on shrimp is affected by a lower per capita income. On the contrary, smaller households with higher per capita income may signal a greater willingness to pay for premium shrimp (Nayak and Joshi 2022).

For other demographic factors such as age, gender, civil status, education, and distance to market, research has shown no statistical difference when grouped by income level in the patterns of shrimp consumption. Hoque et al. (2021) argued that shrimp preferences have shown no significant difference in consumption pattern between age or gender. Moreover, distance to market likely does not vary significantly across income level, as all consumers ranked quality and freshness above proximity. Research by Bassett et al. (2021) supported this evidence, demonstrating that marketing structures focused more on product quality rather than demographic segmentation would reach consumer bases more effectively.

Hence, programs that make shrimp more accessible and affordable for low-income households could help improve their protein intake, while promoting sustainable shrimp production for high-income consumers who are willing to pay for premium shrimp products. Moreover, a well-informed and proper market segmentation and marketing plan can be chosen and prepared, respectively.

Shrimp Attributes Preferred among Consumer-respondents

The significant variation in the preference for shrimp attributes such as price, size, and carapace length indicate that low-income consumers tend to look for cheaper alternatives when purchasing shrimp in the market, as they are constrained by their budget. Therefore, premium-quality shrimp motivates consumers to decrease the amount of shrimp consumption. In comparison, consumers in the middle- and high-income brackets are more likely to pay extra for shrimp that has better attributes (Chen et al. 2022). Additionally, evidence suggests that consumers with more income tend to favor large shrimp. These preferences in size are also typically about their belief that larger shrimp has more meat and is of better quality (Sirisha and Subba Rao 2024).

Furthermore, the source of the shrimp, whether it is freshwater or ocean shrimp, changes with income. Low-income groups are more likely to consume freshwater shrimp, which may be more accessible and available in the local markets (Nayak and Joshi 2022). This distinction reflects not only economic factors but also cultural and regional preferences among consumers in different income levels. Freshness, color, and preservation methods further differentiate shrimp preferences among income groups. Low-income consumers may prioritize freshness and affordability, often purchasing shrimp that are locally sourced and less expensive, while high-income consumers are more likely to seek out shrimp that are not only fresh but also exhibit desirable colors and are preserved using advanced methods to ensure quality (Sirisha and Subba Rao 2024). This trend aligns with findings that suggest higher-income households are more inclined to purchase organic and sustainably sourced seafood, reflecting their greater awareness and concern for food quality and environmental sustainability (Paul and Vogl 2012).

Technical improvements, either through genetic engineering, innovative pond management or improve feed formulation can be done since consumers have their own preferred shrimp attributes. Through this, consumer demand can be satisfied at the same time provide profit opportunity for shrimp growers and other actors in the value chain.

Hedonic Price Analysis by Income Groups

The price of shrimp is influenced by several factors, including size, origin, and freshness, which vary significantly between income groups. In general, consumers exhibit a willingness to pay a premium for shrimp that is perceived as fresher, larger, and sourced from reputable origins. Research indicates that size plays a relatively minor role in consumer preferences, particularly among adults, who prioritize factors such as freshness and origin over size (House et al. 2011). In contrast, studies suggest that low-middle-income consumers may be more sensitive to price variations and less likely to prioritize size when making purchasing decisions (Nayak and Joshi 2022). Freshness is a critical determinant of shrimp quality and price. It is widely recognized that consumers are willing to pay more for shrimp that is fresh, as freshness is closely associated with quality and safety (Prema and Visumathi 2023). For instance, Hoque et al. (2021) found that local consumers are willing to pay a premium for both inland and coastal farmed shrimp, driven by perceptions of quality linked to freshness. Additionally, the processing and handling of shrimp significantly affect its freshness, which in turn impacts consumer willingness to pay (Prema and Visumathi 2023). In high-income markets, consumers often demand higher quality and freshness, leading to a greater price premium for shrimp that meets these criteria (Ortega et al. 2014). The origin of shrimp also plays a vital role in pricing dynamics. Whether it is sourced from freshwater or the ocean, it influenced the price of shrimp across income groups. This distinction affects consumer preferences and willingness to pay across different income groups. Freshwater shrimp,

often farmed in controlled environments, tend to be perceived as more sustainable and may command higher prices in certain markets (Paul and Vogl 2012). Conversely, ocean-sourced shrimp, which are often wild-caught, can vary in price based on factors such as fishing regulations, environmental conditions, and market demand (Asche et al. 2011). Research indicates that consumers in high-income groups are more likely to pay a premium for sustainably sourced ocean shrimp, reflecting a growing trend towards environmental consciousness (Paul and Vogl 2012).

In low-middle income groups, the price sensitivity is more pronounced. These consumers often prioritize affordability over origin, leading to a preference for freshwater shrimp, which may be more readily available and less expensive due to lower transportation costs (Thi Phoung Dong et al. 2021). However, as income levels rise, there is a noticeable shift in preferences, with increasing willingness to pay for premium ocean-sourced shrimp, particularly when marketed as organic or sustainably harvested (Soedrijanto and Istiqamah 2016). This trend suggests that education and awareness of environmental issues play a crucial role in shaping consumer behavior in these markets (Paul and Vogl 2012). Education significantly influences the willingness to pay for premium shrimp among low-middle-income groups. Higher levels of education mean increased awareness of food quality and safety, leading to a greater willingness to invest in premium shrimp products (Nayak and Joshi 2022; He et al. 2013). Studies have shown that educated consumers are more likely to prioritize attributes such as freshness and origin, which can justify higher prices (Yang and Yao 2023). This trend is supported by research indicating that consumers with greater knowledge about food safety and quality are more inclined to pay for shrimp that meets higher standards (Ortega et al. 2014).

The present study revealed consumer preferences regarding the physical shrimp attributes across different income groups. However, there is a notable gap in understanding the intrinsic properties of the preferred shrimp, which warrants further investigation. Given the well-established body of literature on the shrimp marketing chain, future research could build upon this foundation by examining both the preferences and the implicit prices assigned by each actor within the chain. This would provide a more comprehensive understanding of the factors influencing shrimp demand and supply dynamics.

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This work has benefited from the use of Generative AI tools (i.e. Quillbot, Grammarly) to assist in the refinement of writing and grammar checking. The authors reviewed, validated, and are fully responsible for the accuracy, interpretation, and conclusions in this paper.

ETHICAL CONSIDERATIONS

Communication letters were sent to different municipalities for the permission to conduct the study. When it was approved, an informed consent was secured from the respondents before the conduct of the study.

DECLARATION OF COMPETING INTEREST

The authors declare no competing interests to any authors.

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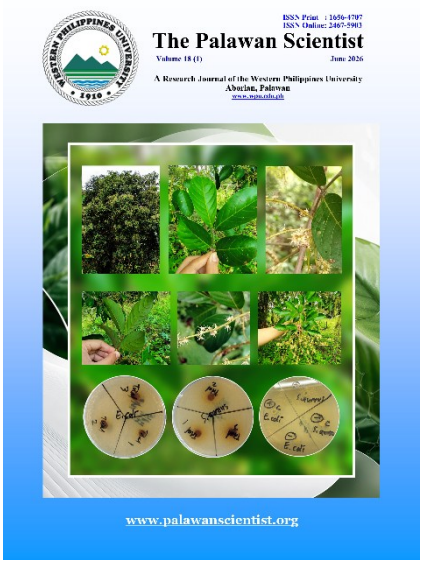

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Agromorphological and phytochemical variations of *Orthosiphon aristatus* (Blume) Miq. morphotypes in the Philippines

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ABSTRACT

Orthosiphon aristatus (Blume) Miq. is a medicinal plant valued for its diuretic properties and potential benefits in treating kidney and urinary diseases in the Philippines. Similar to other plants, intraspecific variation exists in *O. aristatus*, which often leads to ambiguity in the genotype utilized and hinders its effective utilization. Hence, we characterized and evaluated the agromorphological and phytochemical properties of three Philippine accessions of *O. aristatus* with different inflorescence colors through a field trial conducted in a completely randomized design, to select promising accessions that could serve as genetically stable reference materials. Differences were observed in 22 out of 31 quantitative traits, while variation occurred in 12 out of 18 qualitative traits. Phytochemical traits were negatively correlated with biomass. Philippine Biorepository Network (PBN) 2019-119 (white morphotype) exhibited the highest leaf count of 2,001.75 ± 311.40 per plant, shoot number of 53.25 ± 6.65 g/plant, and dry leaf yield of 25.16 ± 3.99 g/plant. However, PBN 2018-073 (purple morphotype) had the highest total flavonoid content of 31.05 ± 1.83 mg QE/g extract and rosmarinic acid content of 2.957 ± 0.22 mg/200 g sample. Based on both agronomic and phytochemical traits, PBN 2019-119 was identified as the most promising genotype, with a high agronomic yield and intermediate phytochemical content. This is the first study to evaluate *O. aristatus* accessions in the Philippines. It highlights the importance of understanding infraspecific variation in *O. aristatus* to improve its utilization and conservation.

Keywords: balbas pusa, Lamiaceae, rosmarinic acid, yield, and yield components

INTRODUCTION

Balbas pusa *Orthosiphon aristatus* (Blume) Miq. is a perennial medicinal herb native to tropical and subtropical Asia and Australia, including the Philippines, in the family Lamiaceae (POWO 2022). An erect, ascending herb that can grow up to 2 m tall, *O. aristatus* generally has 4-angled stems with hairs on younger parts and becomes glabrous when woody. It has narrowly elliptic to ovate leaves, terminal inflorescences up to 25 cm long, and white, pale-lilac, or lilac flowers. Its nutlets are broadly oblong and compressed (Bramley 2019).

The leaves are commonly brewed into a tea remedy for bacterial and urinary tract infections, inflammation, hypertension, rheumatism, and jaundice, due to their diuretic, antioxidant, and anti-inflammatory properties (Hsu et al. 2010; Dayrit and Guidote 2016). Several preclinical studies supporting its use in the traditional medical system have been conducted in Malaysia (Akowuah et al. 2005; Seyedan et al. 2017). These biological activities have been primarily attributed to phenolics and flavonoids, particularly sinensetin and rosmarinic acid (Tezuka et al. 2000; Olah et al. 2003; Akowuah et al. 2005). More than 20 phenolic compounds, including lipophilic flavones, flavonol glycosides, and caffeic acid, have been isolated from this plant. Rosmarinic acid, an ester of caffeic acid present in *O. aristatus*, gained primary interest because of its antioxidant, anti-inflammatory, antimicrobial, anticancer, and antiangiogenic activities (Gamaro et al. 2011). However, many studies mention *O. aristatus* only by its scientific name without properly identifying the genotype or morphotype. This is problematic because *O. aristatus* has synonyms commonly in literature, including *Orthosiphon stamineus* Benth. and *Oryctanthus spicatus* (Jacq.) Eichler. Consequently, literature searches may yield overlapping results under these three scientific names, which refer to the same botanical species. Findings for *O. aristatus* can then be expected from extensive reports under *O. stamineus* and *O. spicatus*.

The *O. aristatus* has two accepted infraspecifics, namely *O. aristatus* var. *aristatus* and *O. aristatus* var. velteri Suddee & A.J.Paton (POWO 2022). The variety *aristatus* has petiolate leaves with a cuneate base, acute bracts, and an acuminate or cuspidate apex (Suddee et al. 2004). Meanwhile, variety *velteri* has sessile or subsessile leaves, usually a truncate base, and bracts with a truncate or apiculate apex. In Indonesia, three morphotypes of *O. aristatus* have been identified based on color of their flowers: white, purple, and intermediate (white-purple) (Faramayuda and Mariani 2022).

In the production of medicinal products, mixtures of different genotypes are commonly practiced due to a lack of reference plant material (DOST-PCHRD 2022). This recurring problem in medicinal plant production, where infraspecific variation is often overlooked, causes ambiguity and misidentification, which could result in poor utilization. Therefore, characterization and evaluation of *O. aristatus* germplasm will elucidate genotypic traits that aid proper identification and ensure appropriate genetic resources are utilized.

The environment, genetic composition, and their interaction influence the quality, yield, and diversity of phytochemicals in medicinal plants (Ncube et al. 2012); thus, it is essential to determine the most suitable types for specific environmental conditions. Lee (2004) reported that purple-flowered *O. aristatus* had more abundant bioactive compounds than the white morphotype. Hence, we hypothesize that the white-flowered and purple-flowered morphotypes of *O. aristatus* in the Philippines differ significantly in their agromorphological and phytochemical traits.

In the Philippines, *O. aristatus* is a commonly used medicinal plant. Its leaves are boiled to treat dysentery and kidney troubles (Dayrit and Guidote 2016). The Food and Drug Administration (FDA)-approved herbal teas and food supplements containing *O. aristatus* are sold in the Philippines (FDA 2020). Despite its wide utilization, morphotype variation also exists in the country. Thus, we aim to elucidate the agromorphological and phytochemical variation among selected Philippine accessions of *O. aristatus* and to identify promising genotypes that could be utilized as genetically stable raw material sources for herbal production and research.

METHODS

Environmental Data During Characterization and Evaluation

Data on the climatic factors in the field were gathered from the Agrometeorological Station University of the Philippines Los Baños (UPLB). At the same time, the soil analysis of the plot was conducted at the College of Agriculture and Food Science-Agricultural System Institute (CAFS-ASI) Analytical Services Laboratory. The soil had medium to high organic matter content, calcium, and cation exchange capacity; medium to low total nitrogen; low phosphorus; and high to medium potassium. The soil was composed of 26-33% sand, 36-42% silt, and 25-39% clay.

Planting Materials and Experimental Design

Herbarium specimens of *O. aristatus* accessions were prepared to verify plant species identity. These exhibited typical characteristics of *O.*

aristatus var. *aristatus* with petiolate leaves, cuneate base, acute bracts, acuminate or cuspidate apex, and exserted stamens extending more than 2 cm from the corolla tube (Suddee et al. 2005; Bramley 2019). The voucher specimens used in the study were deposited at the Philippine Herbarium of Cultivated Plants, Institute of Crop Science, UPLB, under the identification numbers ICROPS-ID-2018-073, ICROPS-ID-2019-119, and ICROPS-ID-2019-571.

Plots for three preselected *O. aristatus* accessions (Table 1) were established at the ICropS field genebank, CAFS, UPLB (N 14 °09'47" E 121 °14'48") from 10 May to 27 September 2024, following a completely randomized design (CRD) with two replicates per accession, which were utilized for agromorphological and phytochemical evaluation. Each replicate was set up in a 3 m × 2 m plot with 0.60 m spacing between plants. Two plants were sampled per replicate. The accessions were propagated from three-node stem cuttings approximately 20 cm in length, initially planted in pots inside a greenhouse and then transplanted to the field after 21 days.

Characterization and Evaluation of Agromorphological Traits

Traits were observed and measured at reproductive maturity, approximately 14 weeks after transplanting, when 80% of the population was flowering. Using a preliminary descriptor list, 29 quantitative and 18 qualitative agromorphological characteristics were recorded (Zainuddin et al. 2023). The Royal Horticultural Society color chart (Royal Horticultural Society 2015) was used to describe the color of plant parts. Quantitative characteristics were reported as mean ± standard error of the mean.

Phytochemical Screening

Phytochemicals were screened from ethanolic extracts. Leaves were dried at 50°C using a forced convection oven. After drying, the leaves were ground with a mortar and pestle and sieved using Mesh #18. For each replicate, 10 g of sample was macerated with 200 mL of 70% ethanol. The mixture was sonicated and filtered. The ethanolic solution was then evaporated at 45°C in a forced-convection oven to obtain a crude extract. These extracts were sealed, labeled, and refrigerated for subsequent analyses.

Detection of flavonoids. A mixture of 2.0 mL of stock extract solution and 1.0 mL of 0.1 N NaOH solution was prepared in a test tube. The development of yellow coloration indicated the presence of flavonoids.

Detection of saponins. A test tube was filled with 5.0 mL of stock extract solution and covered with parafilm. It was then shaken vigorously for about 3 minutes, left to stand for 5 minutes, and observed for frothing. The presence of saponins was confirmed when the froth persisted for 5 to 10 minutes.

Detection of tannins. A test tube containing 2.0 mL of stock extract solution was treated with 5 to 10 drops of 1% $FeCl_3$ solution. The appearance of a blue-to-black color indicated the presence of tannins.

Detection of alkaloids. A mixture of 5.0 mL of stock extract solution and 5.0 mL of 0.1 N HCl solution was prepared in a test tube. The solution was placed in a water bath and agitated for 2 minutes until a color alteration occurred. While still warm, it was filtered using coarse filter paper. The filtrate was collected, and 10 drops of Wagner's reagent were added. The solution was then left to stand for 5 minutes. The formation of a red to brown precipitate indicated the presence of alkaloids.

Total Flavonoid Content (TFC) Determination

Serial dilutions of quercetin were prepared using 70% ethanol at concentrations between 0.96 µg/mL and 0.025 µg/mL. For the standard solution, 0.5 mL of quercetin solution, 1.5 mL of 70% ethanol, 0.1 mL of 10% aluminum chloride, and 0.1 mL of 1M potassium acetate were transferred to a test tube, then brought to volume by adding 2.8 mL of distilled water. The blank contained the same solution as the standard without quercetin. Absorbance values were measured in a 10-mm cuvette using a spectrophotometer (DeNovix® DS-11+). The wavelength with the highest quercetin absorbance was used to plot the calibration curve and obtain the linear equation for quantifying quercetin equivalence (mg QE/g extract) of the *O. aristatus* samples.

High Performance Thin Layer Chromatography (HPTLC) of Rosmarinic Acid

The solvent system consisted of toluene, ethyl acetate, formic acid, and water in a ratio of 3:3:1:0.2. The sample solution was prepared by dissolving 200 mg of the sample in 10 mL of 98% ethanol and sonicated to ensure complete dissolution. A 5 µL of the rosmarinic acid, together with the standards, was spotted onto the HPTLC plate.

Table 1. *Orthosiphon aristatus* accessions used in this study. *Based on inflorescence color (Faramayuda and Mariani 2022).

Accession no.	Morphotype*	Municipality
PBN 2018-073	Purple	Lobo, Batangas
PBN 2019-119	White	Castillejos, Zambales
PBN 2019-571	White	Guindulman, Bohol

Then, dried at 65°C for 30 minutes using a hot plate. The TLC plate was then placed in a developing chamber saturated with the solvent system and dried at 60°C for 5 minutes using a TLC plate heater III. The plate was visualized under UV light at 254 nm and 365 nm using a TLC Visualizer 2, and densitometric scanning was performed at 328 nm. Additionally, the concentration of rosmarinic acid was further analyzed using the Gelanalyzer 23.1.1 by uploading the image of the plate under 365 nm UV light.

Statistical Analyses

To determine differences among accessions, parametric statistical testing via analysis of variance (ANOVA) was conducted. Prior to this, the Shapiro-Wilk test and Levene's test were used to assess normality and homogeneity of variances, respectively. When assumptions were met, ANOVA was followed by pairwise-mean comparison using Tukey's Honest Significant Difference (HSD) test at 5% significance level. The non-parametric Kruskal-Wallis test was used for variables that did not meet the assumptions, followed by pairwise comparison using Dunn's test with Bonferroni correction at the same significance level. Principal component analysis (PCA) using a Pearson correlation matrix was performed to summarize the agromorphological and phytochemical evaluation data, and select the promising genotypes. Analyses were performed using XLSTAT 2016.

RESULTS

Agromorphological Characteristics of *O. aristatus*

Morphological trait differences. Morphological differences were observed among the three accessions (Figure 1, Table 2). The PBN 2018-073 has purple inflorescence, whereas PBN 2019-119 and 2019-571 both exhibit white inflorescences. Additionally, PBN 2018-073 is distinguished by the purple coloration of the flowers, calyces, and leaf veins (Table 3). Another unique trait that differentiates the accessions is leaf shape: PBN 2018-073 and 2019-571 possess rhomboid leaves, while PBN 2019-119 has elliptic leaves. These trait variations between accessions were consistent and observed in their mother plants and asexually propagated individuals in different plots (replicates). Thus, these phenotypic variations are highly stable and indicate genetic differences among the selected accessions.

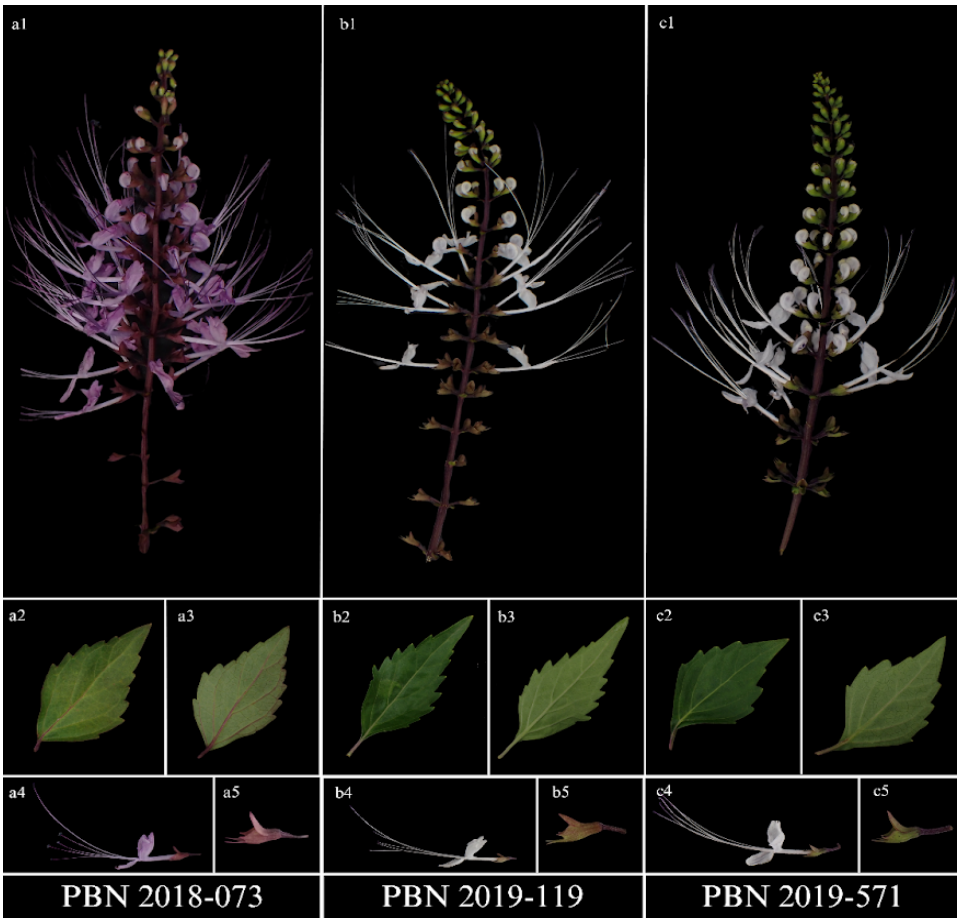

Figure 1. Morphological variations of *Orthosiphon aristatus* accessions.

Table 2. Qualitative morphological characteristics of *Orthosiphon aristatus.*

Character	PBN Accession No.		
	2018-073	**2019-119**	**2019-571**
Growth habit	Erect	Semi-erect	Erect
Stem color	Reddish	Reddish	Reddish
Anthocyanin coloration in stem	Strong	Weak	Weak
Leaf shape	Rhomboid	Elliptic	Rhomboid
Anthocyanin coloration in the leaf	Present	Absent	Absent
Leaf shape base	Oblique	Oblique	Oblique
Leaf apex shape	Acute	Acuminate	Acute
Leaf color	Green	Green	Green
Leaf vein color	Purple	Light green	Light green
Leaf glossiness	Weak	Weak	Weak
Leaf undulation of margin	Very Weak	Very Weak	Very Weak
Corolla color	Purple	White	White
Calyx color	Purple	Light Green	Light Green
Corolla tube hairiness	Dense	Dense	Dense
Upper calyx degree of anthocyanin	Strong	Moderate	Moderate
Filament color	Dark Violate	White	White
Style color	Dark Violate	White	White
Stigma shape	Close	Close	Close

Table 3. Quantitative morphological characteristics of *Orthosiphon aristatus.* Measurements reported as mean ± SEM (n = 10); ^{a,b,c} indicate significant differences in ANOVA; ^{x,y,z} indicate significant differences in Kruskal-Wallis at $\alpha = 0.05$.

Character	PBN Accession No.		
	2018-073	**2019-119**	**2019-571**
Leaf blade length (mm)	29.71 ± 0.69^{x}	56.975 ± 2.35^{y}	49.271 ± 0.92^{y}
Leaf blade width (mm)	17.44 ± 0.65^{b}	25.922 ± 1.20^{a}	28.834 ± 0.98^{a}
Roundness of leaf	0.51 ± 0.01^{x}	0.43^{y}	0.48 ± 0.01^{xy}
Solidity of leaf	0.89 ± 0.01^{x}	0.85^{y}	0.88^{xy}
Petiole length (mm)	3.91 ± 32^{x}	7.097 ± 0.50^{y}	9.809 ± 0.58^{y}
Number of days to flowering	65	77	66
Corolla tube length (mm)	19.66 ± 0.69^{x}	11.828 ± 0.25^{y}	11.705 ± 0.42^{y}
Corolla tube diameter (mm)	1.38 ± 0.06^{b}	1.443 ± 0.07^{b}	1.791 ± 0.06^{a}
Lower lip length (mm)	8.6 ± 0.46^{b}	8.392 ± 0.29^{b}	10.091 ± 0.38^{a}
Lower lip width (mm)	2.61 ± 0.16	2.856 ± 0.28	3.268 ± 0.32
Upper lip length (mm)	8.88 ± 0.18	8.292 ± 0.31	9.161 ± 0.27
Upper lip width (mm)	8.14 ± 0.18	8.142 ± 0.32	8.282 ± 0.59
Calyx length (mm)	6.62 ± 0.17^{b}	7.19 ± 0.17^{a}	6.347 ± 0.30^{b}
Calyx opening width (mm)	4.65 ± 0.14^{b}	5.148 ± 0.19^{a}	4.379 ± 0.16^{b}
Pedicel length (mm)	3.87 ± 0.15	3.955 ± 0.31	4.052 ± 0.16
Stigma length (mm)	57.82 ± 1.62^{y}	57.591 ± 3.16^{y}	46.486 ± 1.24^{x}

Yield performance. There were significant differences in dry leaf yield among the three *O. aristatus* accessions. Specifically, PBN 2019-119 (white-flowered) produced the highest dry leaf yield of 25.16 ± 7.99 g/plant, compared to the purple-flowered PBN 2018-073, which produced only 7.23 ± 2.16 g/plant (Table 4). The PBN 2019-119 also had higher plant height, number of branches, leaf number, and leaf area per plant. However, no significant difference was found in dry leaf yield between the accessions with white inflorescences.

Phytochemical Content of *O. aristatus*

Flavonoids, tannins, saponins, and alkaloids were detected in all accessions (Table 5). Quantitative differences were observed in the TFC and rosmarinic acid levels. The highest TFC was found in PBN 2018-073 (31.05 ± 1.83 mg QE/g extract), while the lowest was observed in PBN 2019-571 (26.06 ± 1.76 mg QE/g extract). Similarly, the highest rosmarinic acid concentration was found in PBN 2018-073 (2.957 ± 0.22 mg/200g sample), whereas the lowest was observed in PBN 2019-571 (1.594 ± 0.09 mg/200g sample).

The HPTLC chromatogram of *O. aristatus* accessions under 365 nm UV light revealed that PBN 2019-119 and 2019-571 contained additional phytochemicals with RF values of 0.664 and 0.708, which were not present in PBN 2018-073 (Figure 2). These consistent variations across replications suggest further phytochemical differentiation between the white and purple morphotypes.

Table 4. Comparison of the agronomic yield of *Orthosiphon aristatus* accessions. Measurements reported as mean ± SEM (n = 4); ^{a,b,c} indicate significant differences in ANOVA; ^{x,y,z} indicate significant differences in Kruskal-Wallis at α = 0.05.

Character	PBN Accession No.		
	2018-073	2019-119	2019-571
Fresh leaf yield (g plant^{-1})	31.36 ± 6.12 ^{x}	106.53 ± 16.46^{y}	41.54 ± 7.71^{xy}
Dry leaf yield (g plant^{-1})	7.23 ± 1.08^{x}	25.16 ± 3.99^{y}	9.49 ± 1.28^{xy}
Stem fresh weight (g plant^{-1})	42.58 ± 4.33	129.92 ± 25.77	59.35 ± 16.99
Stem dry weight (g plant^{-1})	13.12 ± 1.97^{y}	29.69 ± 5.43^{x}	13.44 ± 2.86^{xy}
Root fresh weight (g plant^{-1})	9.66 ± 0.14^{xy}	43.42 ± 16.49^{x}	6.59 ± 1.16^{y}
Root dry weight (g plant^{-1})	6.17 ± 0.23^{xy}	18.25 ± 5.47^{x}	4.30 ± 0.62^{y}
Dry recovery, %	33.17 ± 4.87	26.12 ± 0.56	25.69 ± 3.68
Number of branches	38.75 ± 12.61	53.25 ± 6.65	44.75 ± 1.84
Leaf number (g plant^{-1})	822.25 ± 141.10^{x}	2001.75 ± 311.40^{y}	1144.75 ± 198.28^{xy}
Leaf area (plant-1, cm2)	68828.09 ± 4472.62^{xy}	91265.54 ± 9965.18^{x}	62462.49 ± 4431.77^{y}
Leaf area index	191.19 ± 12.42^{xy}	253.52 ± 27.68^{x}	173.51 ± 12.31^{y}
Diameter of spread (cm)	67.25 ± 3.68^{b}	93.50 ± 7.04^{a}	68.38. ± 4.85^{b}
Plant height (cm)	64.40 ± 1.69	76 ± 5.17	64.33 ± 6.28

Table 5. Descriptive phytochemical detection in *Orthosiphon aristatus* accessions. Indication: + (present), - (absent). Measurement reported as mean ± SEM (n = 4). ^{a,b,c} indicate significant differences in ANOVA. ^{x,y,z} indicate significant difference in Kruskal-Wallis at α = 0.05.

Phytochemical	PBN Accession No.		
	2018 - 073	2019 - 119	2019 - 571
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Alkaloids	+	+	+
TFC (mg QE/g EXTRACT)	31.05 ± 1.83^{a}	26.06 ± 1.76^{b}	20.73 ± 0.56^{c}
RA CONC. (mg/200g SAMPLE)	2.957 ± 0.22^{x}	2.423 ± 0.27^{xy}	1.594 ± 0.09^{y}

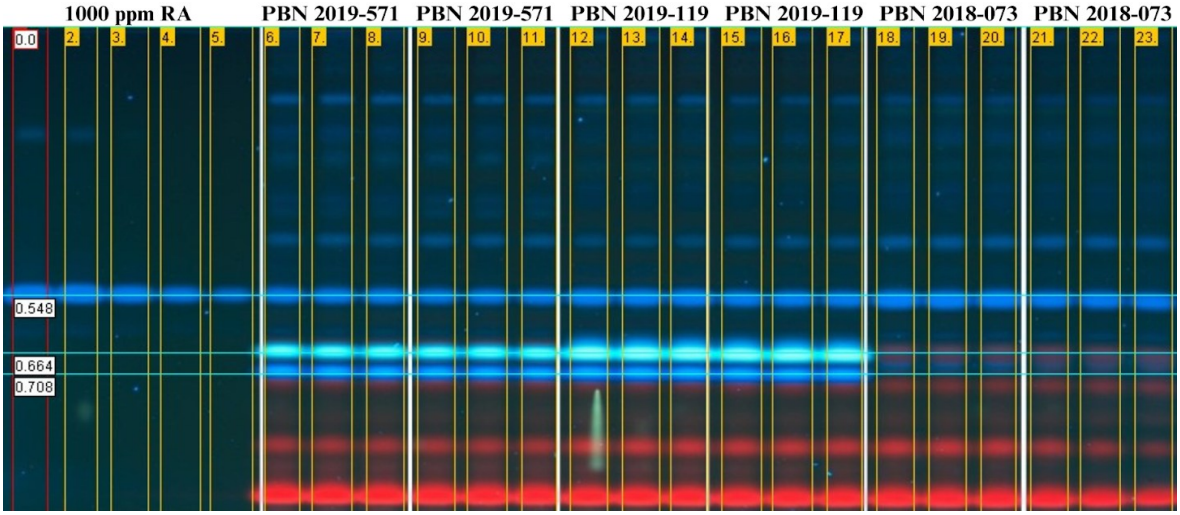

Figure 2. Retention factor (Rf) of potential different phytochemicals present in *Orthosiphon aristatu*s accessions under 365 nm UV light, as viewed using GelAnalyzer.

Promising Genotype of *O. aristatus*

The PCA biplot shows the contribution of 14 quantitative traits of three *O. aristatus* accessions, with a total variance of 100%, indicating that these traits can be summarized into two principal components (Figure 3). Agronomic yield characteristics, such as fresh leaf yield, dry leaf yield, leaf blade width, stem dry weight, and root fresh weight, were negatively correlated with phytochemical characteristics, rosmarinic acid and TFC. This indicates that accessions with superior phytochemical and agronomic properties are difficult to select simultaneously. Although PBN 2018-073 is a good putative source of bioactive ingredients, PBN 2019-119 demonstrated superior characteristics with high biomass, comparable rosmarinic acid content, and an intermediate level of flavonoid content. We also inferred that variations within morphotypes exist. For example, although white morphotypes evaluated generally exhibited higher agronomic yield, PBN 2019-571 and PBN 2019-119 still showed significant variations, such as diameter of spread, leaf area, and root dry weight. These significant variations were also observed in their total flavonoid content.

DISCUSSION

The results demonstrate clear genetic and phenotypic distinctions among the three *O. aristatus* accessions. Dayrit and Guidote (2016) detected the same bioactive compounds in *O. aristatus,* which contribute to its pharmacological properties. The PBN 2018-073 is notable for its anthocyanin-rich purple pigmentation and higher levels of bioactive compounds such as flavonoids and rosmarinic acid. This purple color is strongly associated with the anthocyanins present in PBN 2018-073 (Kalusalingam et al. 2024). The high total flavonoid content of this accession denotes elevated concentrations of important bioactives belonging to the flavonoid class identified in *O. aristatus*, particularly sinensetin, 3′-hydroxy-5,6,7,4′-tetramethoxyflavone (TMF), and eupatorin (Abd Aziz et al. 2021). The difference in rosmarinic acid content, a phenolic compound, also contributes to various beneficial properties, including antioxidant and anti-inflammatory effects (Noor et al. 2022). The values obtained were consistent and within the range of the phytochemical evaluation data recorded by Bovani et al. (2024). These findings indicate that PBN 2018-073 possesses superior phytochemical properties among the accessions evaluated. However, this phytochemical richness appears to trade off with agronomic yield, aligning with the source-sink relationship concept, in which resources are allocated to secondary metabolite production at the expense of biomass (Alem et al. 2021). These differences were previously observed by Bovanni et al. (2024), Faramayuda and Mariani (2022); however, we confirmed these differences through our replicated field experiment. Although our observations were generally consistent with reports in the literature, we recognize that ecological factors can influence the growth of different *O. aristatus* morphotypes. Therefore, we recommend conducting future experiments across multiple geographic locations.

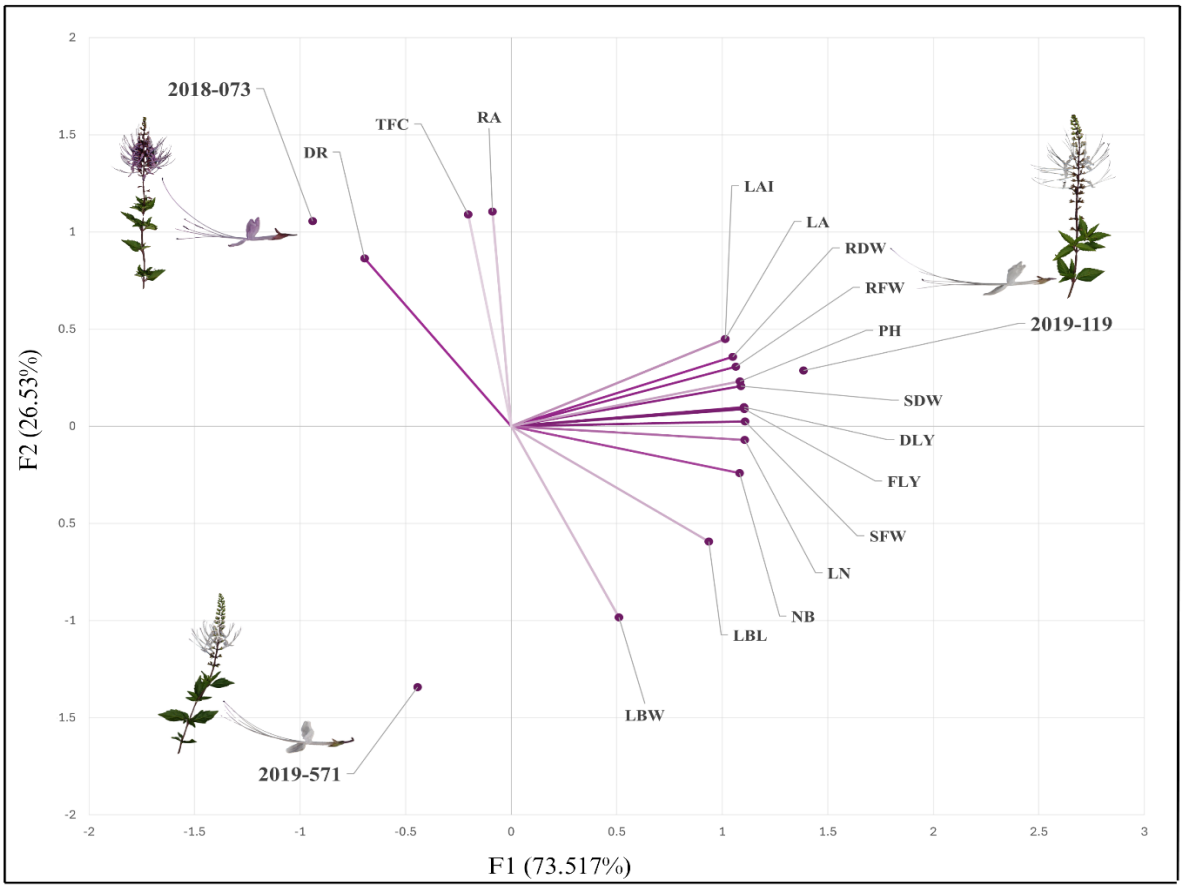

Figure 3. Principal component analysis biplot of *Orthosiphon aristatus* accessions. Lines pointing in similar directions indicate the correlation of traits along the two principal components. Legend: RA - rosmarinic acid; TFC – total flavonoid content; DR – dry recovery; DLY – dry leaf yield; FLY – fresh leaf yield; LA – leaf area; LAI – leaf area index; LBL – leaf blade length; LBW – leaf blade width; LN – leaf number; NB – number of branches; PH – plant height; RDW – root dry weight; RFW – root fresh weight; STW – stem dry weight; and SFW – stem fresh weight.

The PBN 2019-119, on the other hand, produced significantly higher biomass and dry leaf yield. Its intermediate phytochemical content and high yield make it a promising genotype for both medicinal and production purposes. The PCA analysis supports this finding by showing an inverse relationship between yield and phytochemical levels. This is the first report on the yield and yield component evaluation of *O. aristatus* accessions in the Philippines.

Variations even among morphologically similar accessions (e.g., white-flowered types) suggest that selection for both agronomic and phytochemical traits must consider intraspecific variability. This study affirms that phenotypic traits are highly stable and can serve as reliable indicators for genotype selection.

The three accessions of *O. aristatus* in the Philippines were successfully evaluated for their agromorphological and phytochemical performance. Agronomic and phytochemical variations consistently differentiate white and purple inflorescence types. Moreover, phytochemical levels increase as yield decreases, and vice versa. Despite this, we identified PBN 2019-119 as the most promising genotype with high dry leaf yield and intermediate phytochemical content. This is the first report of phytochemical and agronomic evaluation and the selection of promising genotypes of *O. aristatus* in the Philippines. With their distinct, uniform, and stable characteristics observed in the performance trials, these genetic materials can serve as reference accessions, providing well-characterized genetically stable planting materials for herbal production, direct utilization, breeding, and further research.

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GENERATIVE AI STATEMENT

The authors declare that no generative artificial intelligence (AI) tools were used in the writing, data analysis, or preparation of this article. All content, interpretations, and conclusions presented herein are the original work of the authors.

ETHICAL CONSIDERATIONS

This study was conducted in accordance with the ethical guidelines for research involving plants and agricultural resources. The plant materials used in this research were collected and propagated with complete documentation and appropriate permission from relevant authorities. No endangered or protected species were used. All procedures involving field experimentation and data collection were carried out with integrity, transparency, and adherence to local biodiversity regulations. The researchers ensured that data handling, analysis, and reporting were done honestly, without fabrication, falsification, or misrepresentation of results.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no conflict of interest.

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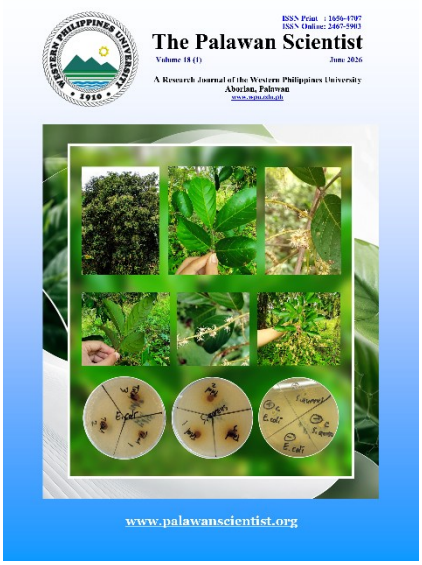

Phytochemical screening and antibacterial properties of "duro" leaves (*Nephelium ramboutan-ake* (Labill.) Leenh. 1986) against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

From the hinterlands of southern Palawan, Philippines, resides one of the oldest tribes in the country, the Palaw'an tribe. As mountain dwellers, they have developed unique healing practices, including the use of the "duro" *Nephelium ramboutan-ake* (Labill.) Leenh. 1986 plant to treat diarrhea. This study aimed to characterize the leaf morphology of "duro," screen for the phytochemicals present, and assess the antibacterial properties of the "duro" leaf extract using the Kirby-Bauer test. Leaves of "duro" were collected from Sitio Sumurum, Brgy. Ransang, Rizal, Palawan. Samples were verified, dried, and extracted before being subjected to phytochemical screening and antibacterial susceptibility test. The morphological characteristics of "duro" leaves include smooth, green-colored, paripinnate compound leaves with an oblanceolate shape arranged oppositely. They have entire margins and pinnate venation, are attached through a petiolate, with the leaf base decurrent at the petiole, and have a mean size of 10.33 cm by 5.77 cm. Only alkaloids, flavonoids, saponins, and tannins were detected among the secondary metabolites tested, while cardiac glycosides and terpenoids were absent. The "duro" leaves extract exhibited moderate antibacterial properties against *Escherichia coli* (Migula, 1895) Castellani and Chalmers, 1919 and *Staphylococcus aureus* Rosenbach, 1884, with mean inhibition zones of 12 mm and 10.67 mm, respectively. The antibacterial properties and presence of phytochemicals suggest the therapeutic potential of "duro" leaves for treating diarrhea. This study addresses knowledge gaps regarding "duro," its medicinal use among the Palaw'an tribe, and promotes further research on indigenous plants.

Keywords: antibacterial susceptibility test, diarrhea treatment, Palaw'an tribe, secondary metabolites

INTRODUCTION

People across the province of Palawan have long used a variety of plants for medicinal purposes, with some of the earliest recorded studies dating back to 1901 (Aguirre et al. 2021; Hirota and Tsuji 2021). Indigenous communities, in particular, have long relied on the local flora and fauna to meet their needs, and this use extends far beyond mere sustenance (Lichtenstein et al. 2017; Menoro and Tablizo 2017). Vegetation plays a key role in enriching their culture, yielding practical and profitable materials, and

providing medicinal resources (Paing et al. 2022). Healing practices are uniquely designed for each group and are rooted in indigenous knowledge passed down through generations (Villanueva 2021).

One of the oldest, yet rarely studied tribes in the Philippines is the Palaw'an tribe (Perez 2014). This tribe, located in Southern Palawan, is composed of excellent farmers and foragers with vast knowledge of wild plants that they use in their daily lives (Bernadas and Peralta 2017). Like other indigenous groups, they rely on their traditional healers who use indigenous plants—wildcrafted plants consumed by native people or introduced to them long ago (Cogill 2015). One of these plants is the pulasan *Nephelium ramboutan-ake* (Labill.) Leenh. 1986 or "duro," as called by the Palaw'an tribe; they utilize it to treat diarrhea by preparing a decoction of its leaves for oral administration.

"Duro" is a tropical plant under the family Sapindaceae that is native to some Southeast Asian countries, including Myanmar, Malaysia, and Thailand, and is rarely cultivated at low elevations in the Philippines (Murugan and Tan 2022). The fruit can be eaten fresh, and its seeds are also edible. The aril has a sweet flavor similar to that of its relative species, *Nephelium lappaceum* L., 1767, commonly known as Rambutan (Djuita et al. 2016). It also contains high levels of antioxidants and helps control blood sugar levels (Hairunisa et al. 2021; Tan et al. 2022). Like many other indigenous plants used for traditional medicine, "duro" is typically overlooked, with only limited studies and factual information available to the public (Maramba-Lazarte 2020; Tan et al. 2022).

Only a few studies on the antioxidant properties of "duro" have been conducted. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging experiment showed that the 80% ethanol extract of its rind has a high antioxidant activity (Sopee et al. 2019). Subsequently, the ethanol extract of "duro" leaves showed very strong antioxidant activity with the value 20.99 µg/mL, while its seeds and peels exhibited no antioxidant activity (Hairunisa et al. 2021).

To learn more about "duro," further studies are needed to address knowledge gaps concerning its bioactivities. This study aimed to characterize the morphology of "duro" leaves and examine their efficacy as a diarrheal treatment through qualitative phytochemical screening of certain secondary metabolites with antidiarrheal properties (alkaloids, cardiac glycosides, flavonoids, saponins, tannins, and terpenoids), as well as an antibacterial susceptibility test against the diarrheagenic bacteria *Escherichia coli* (Migula, 1895) Castellani and Chalmers, 1919 and *Staphylococcus aureus* Rosenbach, 1884. These tests provide a new understanding of the potential medicinal value of the "duro" plant used by the Palaw'an tribe in Rizal, Palawan.

METHODS

Plant Collection, Authentication, and Morphology

A total of 3.5 kg of "duro" leaf samples collected at Sitio Sumurum, Barangay Ransang, Rizal, Palawan were used in the experimental research design. A Wildlife Gratuitous Permit No. 2022-37 from the Palawan Council for Sustainable Development (PCSD) and a letter of request addressed to the Brgy. Captain of Brgy. Ransang, Rizal, Palawan were secured prior to plant collection. The obtained "duro" leaves were verified by a botanist at the Palawan Herbarium of the Palawan State University. Their morphological characteristics were described using the PlantNET (2024). Figure 1 presents the research procedures of the study.

Plant Preparation and Extraction

"Duro" leaves from the middle portion of the stems were used for plant extraction. Following the standard extraction guide by Phaiboon et al. (2019), 1.8 kg of leaves were thoroughly washed with distilled water to remove impurities, air-dried at room temperature for seven days, and oven-dried at 40°C for five hours. The dried samples were pulverized with a laboratory grinder, yielding 793.92 g of "duro" leaf powder. For every 250 mL of 95% ethyl alcohol, 25 g of "duro" leaf powder was soaked at a 1:10 ratio; 12 flask samples were prepared using 300 g of "duro" leaf powder in 3,000 mL of 95% ethyl alcohol. The samples were stored in a fume hood for seven days and filtered using sterile coffee filters to remove solid particles. The residual ethyl alcohol was evaporated using a rotary evaporator at 40°C. The evaporation process was repeated six times for eight hours each, processing 500 mL of sample per batch in the rotary evaporator. Eighteen grams (18 g) of "duro" leaf crude extract were obtained. The percent yield of the extract after evaporation was 6%, computed using the formula by Gonfa et al. (2020):

$$Extraction\ yield\ (\%) = \frac{Weight\ of\ crude\ extract}{Weight\ of\ powdered\ sample}\ x\ 100$$

Qualitative Phytochemical Screening

The standard screening procedures by Bargah (2015), Guevara (2005), Parekh and Chanda (2007), and Raju et al. (2021) were performed for the qualitative phytochemical screening of secondary metabolites such as alkaloids, cardiac glycosides, flavonoids, saponins, tannins, and terpenoids. The volume of the crude extract obtained was used as the stock solution since no additional dilution with any solvent was performed. The aliquot represented the volume of the extract specified for each phytochemical test.

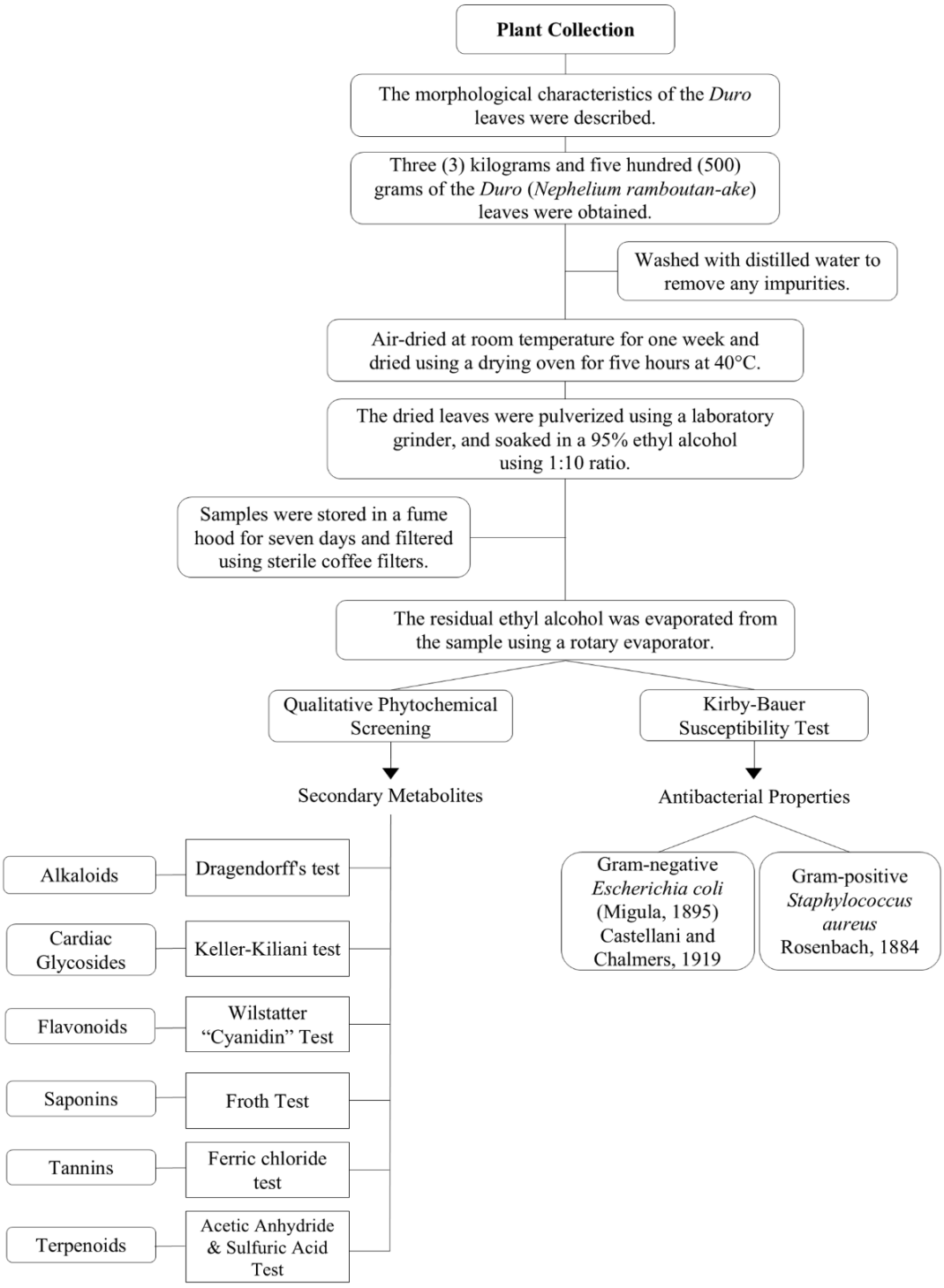

Figure 1. Schematic diagram of the research procedure.

Alkaloids. The Dragendorff's test was carried out, where 2 mL of concentrated hydrochloric acid (HCl) was added to 2 mL filtrate and 1 mL of Dragendorff's reagent. An orange, red, or orange-red precipitate indicated the presence of alkaloids (Raju et al. 2021).

Cardiac Glycosides. The Keller-Kiliani test was performed by adding 1 mL of glacial acetic acid (CH3COOH), 1 mL of ferric chloride (FeCl3), and 1 mL of concentrated sulfuric acid (H2SO4) to 2 mL of filtrate; a green-blue color indicated their presence (Parekh and Chanda 2007).

Flavonoids. The Wilstatter "cyanidin" test was conducted by adding 2 mL of concentrated hydrochloric acid (HCl) to 2 mL of filtrate and adding three to four strips of magnesium turnings or ribbons.

A pink to tomato-red color indicated their presence (Guevara 2005).

Saponins. The Froth test was performed by adding 5 mL of distilled water to 5 mL of filtrate. The solution was agitated for 30 seconds, and the formation of 1.5 cm of stable froth persisting for ten minutes indicated the presence of saponins (Bargah 2015).

Tannins. The Ferric Chloride test was performed by adding 2 mL of ferric chloride (FeCl3) to 2 mL of filtrate, in which the presence of a blue-black precipitate indicates its presence (Parekh and Chanda 2007).

Terpenoids. Two milliliters (2 mL) of acetic anhydride (C4H6O3) were added to 2 mL of filtrate, followed by a few drops of concentrated sulfuric acid (H2SO4). A blue-green ring indicated the presence of terpenoids (Raju et al. 2021).

The presence of secondary metabolites in the "duro" leaf ethanolic extract was classified as high (+++), moderate (++), low (+), or absent (-) (Nadayag et al. 2019).

Kirby-Bauer Susceptibility Test

The diarrheagenic pathogens *E. coli* (BIOTECH 1634) and *S. aureus*–obtained from the University of the Philippines Los Baños, Philippine National Collection of Microorganisms–were used for the Kirby-Bauer susceptibility test (De Las Llagas et al. 2014) at the Clinical Laboratory of Palawan Adventist Hospital (PAH). *Escherichia coli* and *S. aureus* were cultured in Mueller-Hinton broth and adjusted with a densitometer until the turbidity matched the 0.45 sodium chloride (NaCl) turbidity standard. Sterile cotton swabs were used to spread each inoculum on separate Mueller-Hinton (MH) agar plates; triplicates were prepared for both diarrheagenic pathogens. Three sterile paper discs were dipped in the "duro" ethanolic extract and placed on the first MH agar plate inoculated with *E. coli*. Another three sterile paper discs were dipped in the "duro" ethanolic extract and placed on the second MH agar plate inoculated with *S. aureus*. One side of the third MH agar plate was inoculated with *E. coli,* and the other side with *S. aureus*. On the side inoculated with *E. coli*, a sample-free disc for negative control and a 30 mcg ceftriaxone antibiotic disc for positive control were placed, while on the side inoculated with *S. aureus*, a sample-free disc for negative control and a 2 mcg clindamycin antibiotic disc for positive control were placed. All three plates were incubated at 36°C for 24 hours. After incubation, the diameter (or radius, if overlapping) of inhibition zones around the paper discs was measured using a caliper.

The antibacterial properties of the "duro" leaves ethanolic extract were classified as strong (≥ 15 mm), moderate (≥ 7 mm), or inactive (≤ 6 mm) according to the zone of inhibition measurements (Iikasha et al. 2017).

Statistical Analysis

The qualitative phytochemical screening and Kirby-Bauer susceptibility test were conducted in triplicate (n = 3). The mean, standard deviation, and statistical significance between the two bacterial strains in the Kirby-Bauer susceptibility test were analyzed using a paired t-test at $P < 0.05$ with IBM SPSS Statistics version 30.0 (Panpaliya et al. 2019).

RESULTS

Morphological Characteristics

Table 1 and Figure 2 depict the morphological characteristics of the "duro" leaves collected from Sitio Sumurum, Brgy. Ransang, Rizal, Palawan. "Duro" is an evergreen tree that grows upright in tropical forests (Figure 2A). Its green, oblanceolate leaves exhibit pinnate venation and are paripinnate, oppositely arranged leaflets without a terminal leaf, with a mean size of 10.33 cm by 5.77 cm (Figure 2B). Additionally, the smooth-textured leaves possess entire margins and are attached to a petiole, with the leaf base decurrent along the petiole, presenting a lighter green shade and noticeable secondary veins on the abaxial surface (Figures 2C and 2D). Flowers in the budding stage were also documented (Figures 2E and 2F).

Table 1. Morphological characteristics of the "duro" leaves.

Characteristics	Description
Type	Compound
Compound	Paripinnate
Color	Green
Texture	Smooth
Arrangement	Opposite
Margin	Entire
Shape	Oblanceolate
Venation	Pinnate
Attachment	Petiolate
Petiole	Decurrent
Mean Size	10.33 cm by 5.77 cm

Phytochemical Contents

Table 2 shows the results of the qualitative phytochemical screening of the "duro" leaf ethanolic extract. Only alkaloids, flavonoids, saponins, and tannins were detected. Alkaloids and tannins were consistently present in all trials, while flavonoids and saponins were detected in two out of three trials.

Antibacterial Properties

Figure 3 and Table 3 display the inhibition zones of the "duro" leaf ethanolic extract, showing its efficacy in inhibiting the growth of selected

diarrheagenic bacteria *E. coli* (Figure 3A), *S. aureus* (Figure 3B), along with their positive and negative controls (Figure 3C). The test yielded inhibition zones of more than 7 mm for both diarrheagenic bacteria in all trials. However, no significant difference was observed between *E. coli* and *S. aureus* ($P = 0.423$). Furthermore, for *E. coli*, the positive control (ceftriaxone) produced a 20 mm inhibition zone, while the negative control showed no inhibition. Similar results were observed in *S. aureus*, where the positive control (clindamycin) produced a 25 mm inhibition zone, and no inhibition was observed in the negative control.

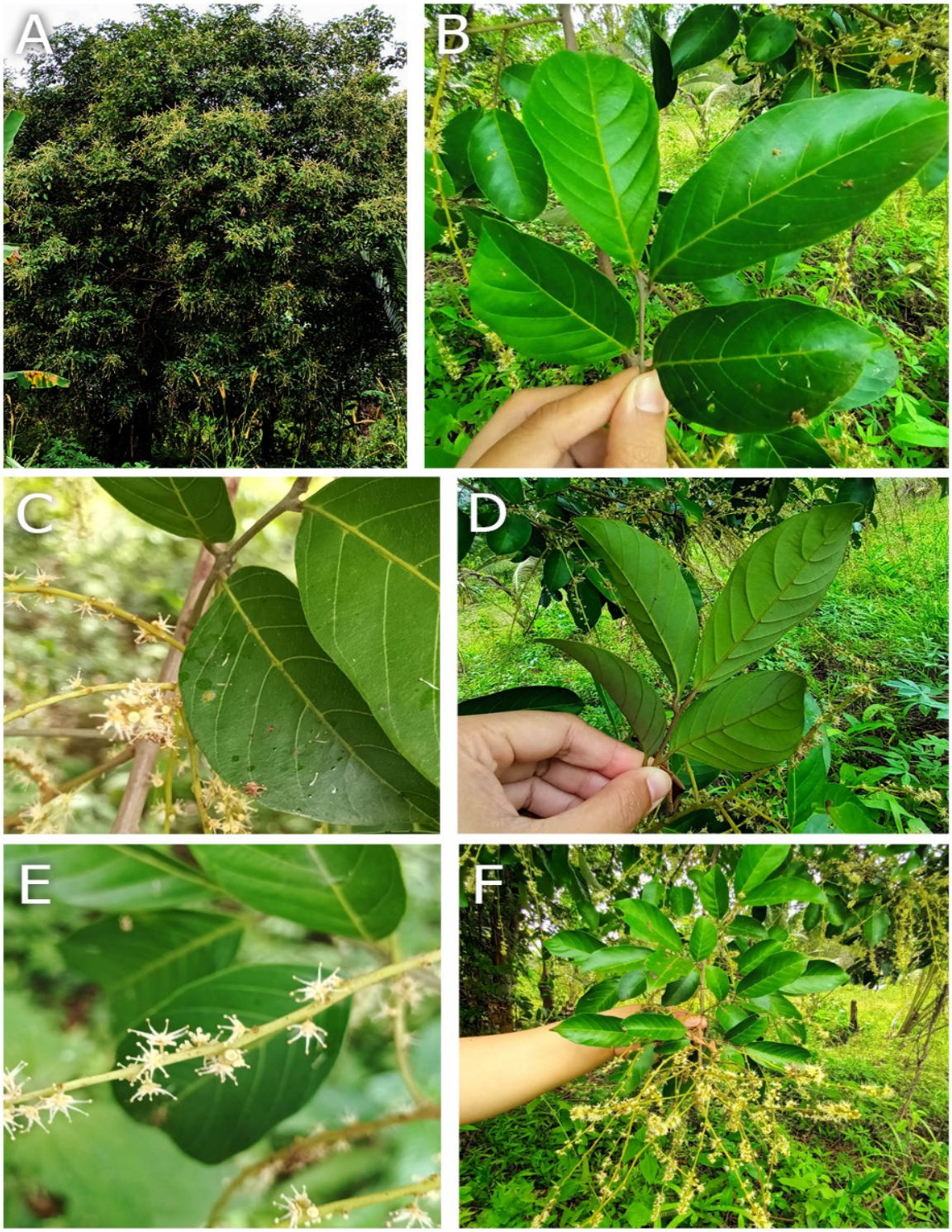

Figure 2. Morphological characteristics of the "duro" leaves.

Table 2. Secondary metabolites identified in "duro" leaves, classified by Nadayag et al. 2019 as highly present (+++), moderately present (++), low (+), and absent (-).

Secondary Metabolites	Methods	Trials*			Interpretation
		1	2	3	
Alkaloids	Dragendorff's Test	+	+	+	Highly Present
Cardiac Glycosides	Keller-Kiliani Test	-	-	-	Absent
Flavonoids	Wilstatter "Cyanidin" Test	+	-	+	Moderately Present
Saponins	Froth Test	+	+	-	Moderately Present
Tannins	Ferric Chloride Test	+	+	+	Highly Present
Terpenoids	Acetic Anhydride and Sulfuric Acid Test	-	-	-	Absent

Figure 3. Susceptibility tests of selected bacteria against "duro" leaves.

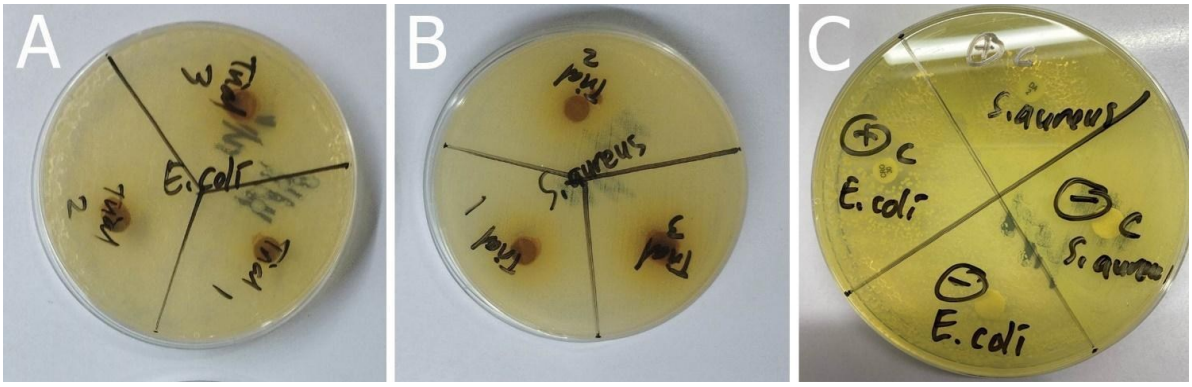

Table 3. Antibacterial activities of the "duro" leaves against *E. coli* and *S. aureus,* classified by Iikasha et al. 2017 as (≥ 15 mm): strong; (≥ 7 mm): moderate; (≤ 6 mm): inactive. (Note: *Mean ± Standard Deviation values represent the three trials, [a]30 mcg of ceftriaxone for positive control of *E. coli,* [b]2 mcg of clindamycin for positive control of *S. aureus,* [c]sample-free discs for negative controls).

Diarrheagenic Bacteria	Zones of Inhibition (mm)					
	Trial 1	Trial 2	Trial 3	Mean ± SD*	Control (+)	Control (-)[c]
Escherichia coli	11	13	12	12.00 ±1.000	20[a]	0
Staphylococcus aureus	11	9	12	10.67 ± 1.528	25[b]	0

DISCUSSION

Morphological Characteristics

The morphological characteristics of the "duro" leaves, as observed in this study, showed both similarities and variations. Most of the traits–such as color, shape, type of compound leaf, arrangement, and texture–closely matched the description provided by Lim (2013) and Morton (1987), which characterized the leaves as green, elliptic to obovate-lanceolate, paripinnate, oppositely arranged, and having a smooth or glabrous texture. However, slight differences were noted in Morton's (1987) observation, which indicated that a terminal leaf may also be present, making the "duro" leaves either paripinnate or imparipinnate (odd-pinnate) compound leaves.

A more contrasting observation was recorded by the National Parks Flora & Fauna Web (2024), which noted that variants exhibit wavy leaf margins, an elliptic shape, spirally arranged leaves, and a leathery to papery texture at maturity. These discrepancies could be attributed to climatic variations, soil conditions, or genetic adaptations across different regions (Everingham et al. 2024).

Phytochemical Contents

The results suggest that the "duro" leaves have antidiarrheal potential due to the high presence of alkaloids and tannins, as well as the moderate presence of flavonoids and saponins. Diarrhea is commonly caused by intestinal irritation and inflammation, which increase intestinal motility and water-electrolyte secretion (Alemu et al. 2022). To inhibit intestinal secretion and peristalsis, alkaloids deactivate the nitric oxide pathway, reducing small intestine volume (Sisay et al. 2017); tannins regulate calcium currents to promote muscle relaxation (Yacob et al. 2016); flavonoids inhibit arachidonic acid secretion to lessen

gut inflammation (Al-Khayri et al. 2022); and saponins suppress spasmogenic activity to prevent excessive muscle contraction (Mishra et al. 2016). The properties of these phytochemicals collectively contribute to the antidiarrheal effects of the "duro" leaves.

A similar finding was reported in the study by Putri et al. (2021), who detected alkaloids, flavonoids, tannins, and saponins in the ethanol extract of "duro" leaves. Other members of the Sapindaceae family, such as Lychee (*Litchi chinensis* Sonn.) and Rambutan, also exhibit similar phytochemicals, including alkaloids, flavonoids, and tannins (Aktar et al. 2022; Perumal et al. 2021).

Antibacterial Properties

Data indicate that the "duro" leaves exhibit moderate antibacterial properties against selected diarrheagenic bacteria (Iikasha et al. 2017). Furthermore, *E. coli* and *S. aureus* showed similar susceptibility, with mean inhibition zones of 12 mm and 10.67 mm, respectively, despite the difference in their Gram properties. However, the difference between the antibacterial properties of the two bacteria was not statistically significant ($P = 0.423$).

In comparison, ethanolic extracts of other "duro" plant parts, such as the peels, demonstrated strong antibacterial properties against *E. coli* and *S. aureus,* with inhibition zones of 29.78 mm and 34.33 mm, respectively, while the seeds exhibited moderate antibacterial properties against the same bacteria, with inhibition zones of 6.11 mm and 8.78 mm, respectively (Fatisa 2013). Similar results were observed in the leaves of a closely related species, Rambutan, which also displayed moderate antibacterial properties against *E. coli* and *S. aureus,* with inhibition zones of 13.3 mm and 12 mm, respectively (Chigurupati et al. 2019). The moderate antibacterial properties of "duro" leaves can be attributed to the presence of secondary metabolites (high alkaloids and tannins, and moderate presence of flavonoids and saponins).

Overall, some inconsistencies were observed in the morphological description of "duro," particularly in its leaf characteristics compared with existing references. The secondary metabolites identified in the "duro" leaves were alkaloids, tannins, flavonoids and saponins. It is notable that while the "duro" leaves showed antibacterial properties, they were less effective than the antibiotics used in the test. Therefore, further studies on the secondary metabolites of "duro" leaves—and their comparison with related species within the Sapindaceae family—should be prioritized, as this could help understand how their shared properties act against diarrhea and potentially aid in developing treatments. Likewise, investigating the leaf morphology and environmental conditions of the "duro" plant from various municipalities of Palawan, as well as in other regions and countries, may provide deeper insights into its ecological adaptations and variations under different environmental conditions.

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GENERATIVE AI STATEMENT

No part of this study was AI-generated.

ETHICAL CONSIDERATIONS

The collection of plant samples in this study was conducted with permission obtained from the Palawan Council for Sustainable Development (Wildlife Gratuitous Permit No. 2022-37) and the Brgy. Captain of Brgy. Ransang, Rizal, Palawan.

DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

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First report of occurrence, abundance, and spatial distribution of *Pyrodinium bahamense* L. Plate, 1906 in Shark Fin Bay, Palawan, Philippines

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ABSTRACT

Harmful Algal Blooms (HABs) pose a significant threat to many coastal communities worldwide, with their spatial distribution steadily increasing over the years. In the Philippines, *Pyrodinium bahamense* L. Plate, 1906 has been identified as the culprit behind paralytic shellfish poisoning. Therefore, it is necessary to monitor *P. bahamense* to ensure public safety during blooms. Hence, we investigated the occurrence, abundance, and spatial distribution of *P. bahamense* in Shark Fin Bay, Palawan, using conventional microscopy techniques. Results revealed the presence of *P. bahamense* in all six sampling stations, with a mean cell density ranging from 15 to 442 cells L^{-1}. In addition, the measured water physicochemical parameters showed no significant correlation ($P > 0.05$) with cell density, suggesting that other factors may potentially influence the abundance and distribution of *P. bahamense* in this area. Thus, we recommend further monitoring efforts to determine the bloom dynamics of the species.

Keywords: dinoflagellate, harmful algal blooms, paralytic shellfish poisoning, red tide

Harmful Algal Blooms (HABs) are recurring events resulting from the proliferation of harmful and toxic microalgae in aquatic environments. In addition, HABs have impacted the tourism industry, food security, livelihood, local economy, and human health of many coastal communities worldwide (Gobler 2020). According to the Intergovernmental Oceanographic Commission of the United Nations Educational, Scientific and Cultural Organization (IOC–UNESCO), there are about 119 species of toxic dinoflagellates (Lundholm et al. 2009 onwards), including *Pyrodinium bahamense* L. Plate,1906. The species is a tropical, euryhaline dinoflagellate that produces saxitoxins and causes paralytic shellfish poisoning (PSP) (Morquecho 2019).

The first occurrence of a toxic bloom involving *P. bahamense* took place in Papua New Guinea in 1972 (Maclean 1989). Following the initial occurrence, blooms of this species were subsequently reported in Southeast Asian countries, particularly in Malaysia and the Philippines (Yñiguez et al. 2021). In the Philippines, the most notable incident caused by *P. bahamense* occurred during red tide events from 21 June to September 1983, resulting in blooms mostly in Maqueda Bay and Villareal Bay, Samar. A total of 278 paralytic shellfish poisoning (PSP) cases were reported, with 21 deaths, primarily due to consumption

of green mussels, *Perna viridis* (Linnaeus, 1758), from the affected bays (Estudillo and Gonzales 1984). Since then, blooms caused by toxic *P. bahamense* have been documented in different parts of the country (Gacutan et al. 1985; Montojo et al. 2006; Siringan et al. 2008; Yap-Dejeto et al. 2018). The expansion of these sites is still increasing, but at a slower pace compared to the 1990's (Yñiguez et al. 2021).

One of the impacted areas is the island of Palawan, where several coastal areas, such as Malampaya Sound, Honda Bay, and Puerto Princesa Bay, are currently being monitored for occurrences of PSP-causing species (BFAR 2024). One area of interest is Shark Fin Bay, located on the east coast of mainland Palawan, within the same municipality where the first case of PSP in the province was recorded (Furio and Gonzales 2002). Studying this area and identifying HAB species within the province are essential not only for the sustainable management of marine ecosystems but also for protecting public health, preserving economic activities, and contributing to global efforts in understanding and addressing the impacts of these blooms. This paper aimed to investigate the occurrence of *P. bahamense* in Shark Fin Bay, Taytay, Palawan. Specifically, it aimed to: (1) measure the physicochemical parameters of the water; (2) determine the presence, density, and spatial distribution of *P. bahamense* in the area; and (3) explore the relationship between water physicochemical parameters and *P. bahamense* cell density.

The sampling stations (Table 1) were established as part of the ongoing research partnership between the Western Philippines University and Sulubaai Environmental Foundation. The Marine Protected Areas comprise Depla, Garden, Pinnacle, and Sandoval, while deeper, non-Marine Protected Areas include Depla North and Macuao (Figure 1).

Table 1. Coordinates of sampling stations in the study area.

Station	Latitude (°N)	Longitude (°E)
Depla	11°5.049'	119°34.130'
Depla North	11°5.890'	119°34.747'
Garden	11°5.416'	119°33.894'
Macuao	11°6.057'	119°33.470'
Pinnacle	11°5.579'	119°33.859'
Sandoval	11°5.506'	119°33.143'

In-situ measurements of water physicochemical parameters and collections of *P. bahamense* were conducted every other month from August to December 2023 and in March 2024, all during the daytime. However, *P. bahamense* cells were only observed in the December samples, which were then subjected to further analysis. Dissolved oxygen (DO) (mg L^{-1}), salinity (ppt), and temperature (°C) were measured using a YSI Pro 2030 (Xylem, USA), while pH was measured using a pH 600 (Milwaukee, USA). Water transparency (m) and depth (m) were measured using a 20 cm diameter Secchi Disk and a portable F12 Echo Sounder (Erchang, China), respectively. *P. bahamense* samples were collected using a plankton net with a 30 cm mouth diameter and 20 µm mesh size. The net was lowered to a depth of 5 m, hauled vertically, and then the collected samples were concentrated to the cod-end using a sprayer. The collected samples were transferred to a pre-labeled plastic bottle and preserved with 5% formaldehyde solution.

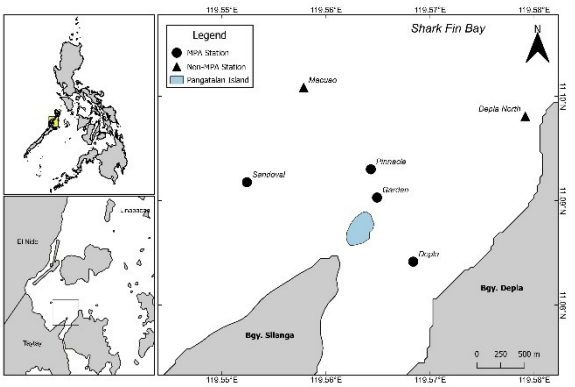

Figure 1. Location of sampling stations in Shark Fin Bay, Taytay, Palawan.

In the laboratory, *P. bahamense* was identified and counted using conventional microscopy techniques. Identifying *P. bahamense* followed references from Tomas (1997) and Omura et al. (2012). Cell counting was performed using a sedgewick rafter counting chamber and viewed under a BA210 light microscope (Motic, Germany). A 1-mL aliquot of the sample was transferred to a sedgewick rafter counting chamber (50 mm x 20 mm grids) in triplicate. Density was computed using the formula used by Relox (2002):

$$\text{Density (expressed in cells L}^{-1}) = \frac{(N \times V_1)}{V_s}$$

Where: N = number of cells in a 1-ml aliquot; V_1 = total plankton sample volume; V_s = volume of the seawater filtered by the plankton net within hauling depth.

To map the spatial distribution, mean density data of *P. bahamense* were plotted using QGIS software version 3.28.14. Moreover, data for correlation analysis were subjected to a normality test using the Shapiro–Wilk test. Since the data did not satisfy the assumption of normality, a non-parametric analysis was performed using Spearman's correlation. The correlation coefficient was calculated using the

'corr' function in R statistics, with the level of significance set at $\alpha = 0.05$, and a correlation matrix was created to visualize the relationships between the data.

The water parameters at Shark Fin Bay showed minimal variations among the sampling stations (Table 2). The water temperature ranged from 29.0 to 29.80°C. The mean salinity was 35.4 ppt, with the lowest recorded in Macuao (34.6 ppt) and the highest in Depla (35.9 ppt). Dissolved oxygen values varied from 4.3 to 5.3 mg L^{-1}, while pH had a mean of 8.5. The highest values for water transparency and depth were recorded in Macuao, measuring 9 m and 23.9 m, respectively.

Pyrodinium bahamense (Figure 2) was observed in water samples collected from Shark Fin Bay only in December 2023, with most cells in chains. At this time, *P. bahamense* was found in all six stations. The cell count ranged from 15 to 442 cells L^{-1}, with the highest density recorded at Garden Station near Pangatalan Island (Figure 3). Lower densities were observed in the eastern stations (Depla and Depla North).

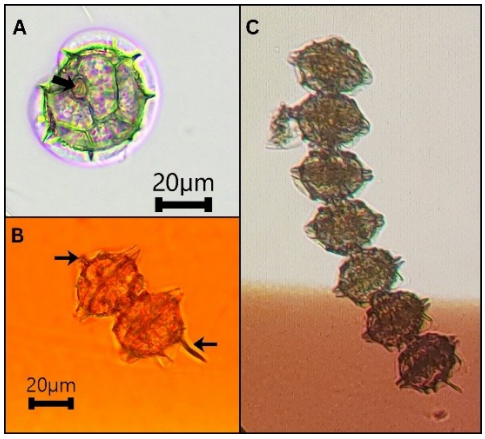

Figure 2. Light microscopy images of *Pyrodinium bahamense* at 100*x* magnification. (A) Apical view showing the plates and the apical attachment pore (arrow); (B) cells showing an apical spine (upper arrow) and an antapical spine (lower arrow); (C) cells in chains.

Table 2. Mean physicochemical parameters at sampling stations in Shark Fin Bay.

Station	Temperature (°C)	Salinity (ppt)	Dissolved Oxygen (mg/L)	pH	Water Transparency (m)	Depth (m)
Depla	29.8	35.9	4.3	8.4	4.6	9.5
Depla North	29	35.2	4.3	8.5	5.2	20.7
Garden	29.7	34.9	5	8.4	4.3	10.6
Macuao	29.2	34.6	5.3	8.5	9	23.9
Pinnacle	29.7	35.8	4.4	8.4	5.3	5.3
Sandoval	29.5	35.8	5.1	8.5	6.5	6.6
Mean	29.5	35.4	4.7	8.5	5.8	12.8

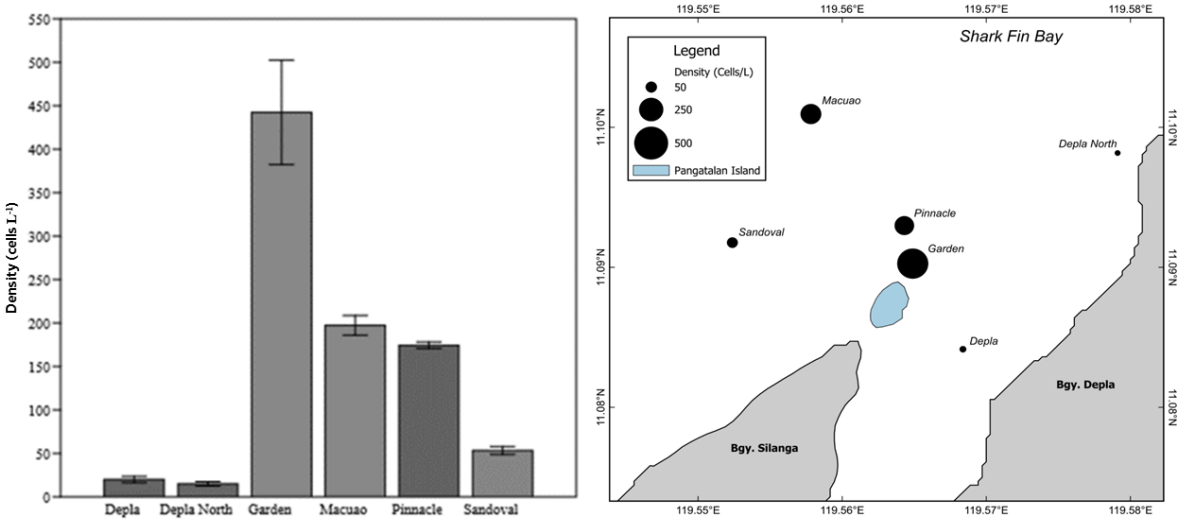

Figure 3. Density and spatial distribution of *Pyrodinium bahamense* in Shark Fin Bay during December 16–17, 2023.

Preliminary analysis of the correlation between *P. bahamense* density and water physicochemical parameters showed a positive correlation for temperature (0.20), DO (0.70), water transparency (0.03), and depth (0.09), while a negative correlation was found for salinity (-0.58) and pH (-0.29), as shown in Figure 4. However, all water parameters showed no significant linear relationship ($P > 0.05$) with cell density due to the limited data. Therefore, a study involving long-term monitoring is needed to address these limitations.

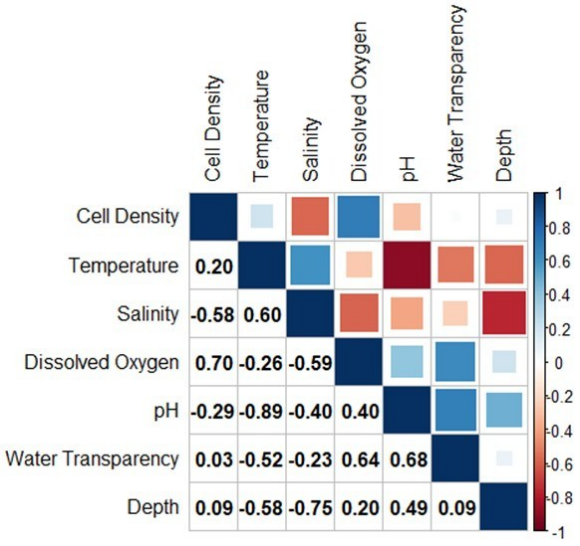

Figure 4. Correlation matrix between water physicochemical parameters and *Pyrodinium bahamense* cell density.

The measured physicochemical parameters were consistent with the optimal environmental conditions for the Philippine *P. bahamense* population, characterized by lower temperature and higher salinity (Gedaria et al. 2007). Notably, the species has also been reported in Eastern Samar, where the temperature ranged from 29 to 32°C and the salinity varied between 27 and 35 ppt (Ravelo et al. 2022). Further, a bloom of the species occurred in the coastal waters off Yemen in the Red Sea, where the water temperature reached 32°C, and the salinity was as high as 37 ppt (Alkawri et al. 2016). Generally, *P. bahamense* blooms occur only in waters with a temperature of more than 20°C and a salinity of 20 ppt or higher (Usup et al. 2012).

To our knowledge, the presence of *P. bahamense* in Shark Fin Bay was the first report of this species in the area. In Palawan, the first report of PSP occurred in 1998 at Malampaya Sound within the municipality of Taytay (Furio and Gonzales 2002). This incident was potentially linked to *P. bahamense*, as resting cysts of this species were documented in the area (Borja et al. 2000). In fact, Sombrito et al. (2004) suggested that *Pyrodinium* cysts may have been present in the sediment before the first recorded toxic algal bloom in the province. In Puerto Princesa Bay, Garcellano et al. (2022) noted the presence of *P. bahamense* in coastal areas inhabited by informal settlers. Furthermore, live cells of *P. bahamense* have been previously isolated, cultured, and characterized from Honda Bay (Onda et al. 2013). Moreover, the Malampaya Sound, Puerto Princesa Bay, and Honda Bay have been listed in the Shellfish Bulletin of the Bureau of Fisheries and Aquatic Resources (BFAR 2024) for PSP monitoring (Figure 5).

The cell density observed in this study was higher than that reported in Puerto Princesa Bay (Garcellano et al. 2022) but did not reach a level indicative of a bloom since there is currently no standardized density threshold for its classification. In contrast, in Sabah, Malaysia, red tide attention is directed to the public when the *P. bahamense* cell density is more than 1,000 cells L^{-1}, while red tide alert is issued when it exceeds 7,000 cells L^{-1} (Jipanin et al. 2019). In the Philippines, PSP monitoring has primarily relied on toxin levels in shellfish rather than phytoplankton cell counts (Arcamo et al. 2014).

The preliminary results from the correlation analysis suggest that other significant environmental factors may be the main contributors to the abundance and distribution of *P. bahamense* in the area. Water dynamics influenced by monsoons and nutrient availability have been vital factors in the blooms of *P. bahamense* in several regions. In Florida, USA, blooms mainly occurred in shallow areas with long water residence times, where abundance was linked to nutrient concentrations (Phlips et al. 2006). In contrast, blooms in Matarinao and Murcielagos Bays in the Philippines did not consistently correlate with the simulated residence time patterns (Lumayno et al. 2021). In Manila Bay, Villanoy et al. (2006) noted higher nutrient concentrations and phytoplankton biomass at depths shallower than 10 m, influenced by the strong vertical mixing of the water column. In addition, they also observed that wind forcing caused the resuspension of *Pyrodinium* cysts, accompanied by an increase in bottom current velocity. Notably, the sampling period in December coincided with the Northeast Monsoon (NEM) in the Philippines, which significantly affects the eastern portion of Palawan. Additionally, there was a tropical depression during the sample collection that strengthened the NEM, contributing to strong water currents in the area. These environmental factors could have contributed to the presence of *Pyrodinium* in Shark Fin Bay during the sampling period.

Given the vast coastal waters of Palawan, the detection of *P. bahamense* in Shark Fin Bay underscores the importance of monitoring, particularly in areas with no prior records of HABs. This study also contributes to the growing knowledge of the species' spatial distribution in Palawan, which is essential for

the effective monitoring and management of PSP during bloom events. Therefore, continued and expanded monitoring is recommended, with the inclusion of other necessary environmental parameters, to generate more substantial evidence on the contributing factors and bloom dynamics in the area. Moreover, the findings of this study may serve as baseline information to support the possible inclusion of Shark Fin Bay as a regular PSP monitoring site by relevant local and national agencies.

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GENERATIVE AI STATEMENT

The OpenAI's ChatGPT was used only to improve grammar, clarity, and formatting references. The authors reviewed and verified all AI-generated content to ensure accuracy and compliance with academic standards.

ETHICAL CONSIDERATIONS

No animals were harmed, and no human subjects were involved during this study.

DECLARATION OF COMPETING INTEREST

The authors declare no competing interests.

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Challenges in agricultural extension: Views of the Ministry of Agriculture, Fisheries, and Agrarian Reform (MAFAR) municipal officers in Basilan Province, Philippines

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ABSTRACT

The Bangsamoro Autonomous Region in Muslim Mindanao (BARMM), located in southwestern Mindanao, comprises five provinces, Lanao del Sur, Maguindanao, Sulu, Tawi-Tawi, and Basilan, relies heavily on agriculture and fisheries as primary sources of livelihood under the Ministry of Agriculture, Fisheries, and Agrarian Reform (MAFAR). However, limited research has examined the specific operational challenges faced by agricultural extension officers in BARMM, particularly in Basilan Province. This study aimed to identify and analyze the challenges encountered by OIC-MAFAR Municipal Officers (OIC-MMOs) to provide contextual insights for improving agricultural extension practices in the region. Using a qualitative phenomenological design, semi-structured interviews were conducted with all twelve OIC-MMOs in Basilan, while data were analyzed using thematic analysis. The study revealed five major challenges: (1) workers' misaligned experience and educational qualification; (2) an undermanned workforce; (3) unresolved conflict with local government units; (4) insufficient salary and logistic support; and (5) lack of facilities and shortage of farm and fishery inputs. These findings emphasize systemic gaps in human resource planning, institutional coordination, and operational support that hinder effective extension delivery. The research contributes to the understanding of field-level realities within BARMM's post-conflict governance structure and underline the need for targeted policy interventions. Strengthening staff training, guaranteeing adequate manpower, improving logistical and financial support, and promoting transparent coordination with local governments are significant for a more efficient and responsive agricultural extension services in municipalities led by OIC-MMOs.

Keywords: capacity building, institutional coordination, local government relations, Mindanao, rural governance

INTRODUCTION

The Bangsamoro Autonomous Region in Muslim Mindanao (BARMM) is located in the southwestern part of Mindanao and comprises five provinces, namely: Lanao del Sur, Maguindanao, Sulu, Tawi-Tawi, and Basilan (ILO 2023). Agriculture and fisheries are the primary sources of livelihood in the

region (World Bank Group 2024). To support rural development, the Ministry of Agriculture, Fisheries, and Agrarian Reform (MAFAR) was established, which merges the former Department of Agriculture and Fisheries (DAF) and the Department of Agrarian Reform (DAR) under the now-defunct Autonomous Region in Muslim Mindanao (ARMM). This institutional restructuring resulted in the creation of the MAFAR Municipal Officer (MMO) position, that combines the roles previously held by the Municipal Agricultural Officer (MAO) and the Municipal Agrarian Reform Officer (MARO).

While this move aimed to streamline local extension services, it also intensified the workload at the municipal level, as one officer is now expected to implement programs across agriculture, fisheries, and agrarian reform. Due to a shortage of qualified applicants for technical positions, MAFAR's regional office designated Agrarian Reform Program Technologists (ARPTs), originally support-level staff, as Officers-in-Charge (OICs) of municipal offices in Basilan. Without adequate staff support, these OIC-MMOs carried out field operations singlehandedly during the early phase of implementation. This situation emphasizes a huge gap between institutional design and actual field capacity, which this study seeks to examine.

Previous literature has identified several challenges in the field of agricultural extension. Key issues in providing extension services include: the absence of a well-developed plan for the agricultural development of the town; a lack of a system for sourcing, archiving, and sharing technology; insufficient support services (particularly low-cost credit); inadequate extension services; and a lack of effective strategies and facilities (Saz 2007). Major challenges also encompass input shortages, low formal market participation, land degradation, limited access to financial resources, a lack of skills, and restricted access to extension services (Mapiye et al. 2021). These problems, although identified in various global contexts, consistently point to a fragmented and under-resourced agricultural extension system. While context-specific, the recurring challenges suggest systemic flaws that transcend national boundaries and thus warrant comparative inquiry.

Under the ministry-based extension model, services are typically free, allowing farmers to visit extension offices at any time for information and advice. However, this approach has consistently faced criticism for its poor performance and the absence of a two-way information flow between extension staff and farmers (Mapiye et al. 2021). General issues with centrally managed and highly bureaucratic extension agencies involve a lack of client accountability, weak linkages to knowledge generation, limited resources for training and operations, and a top-down approach to technology transfer (Azadi and Filson 2009). These limitations suggest that traditional top-down models of extension are outdated and no longer meet the adaptive needs of farmers. However, few studies have explored these criticisms through the direct perspectives of municipal officers, especially in fragile and conflict-affected regions like Basilan in the Philippines.

In India, public extension faces several major challenges, including the burden of non-extension duties, a shortage of qualified public extension professionals, and the reluctance of these professionals to work in remote areas. Furthermore, the focus of public agricultural extension delivery varies depending on state priorities, frequently leading to neglect. The private extension sector in India also encounters numerous issues. A significant concern is that most of India's agricultural extension service providers lack specific standards or quality certification, resulting in unaccountability for any incorrect advice given to farmers, which can lead to losses (Nedumaran and Ravi 2019).

Meanwhile, Malawi's agricultural extension system has faced criticism for failing to significantly contribute to agricultural development and not meeting the needs of smallholder farmers (Magomero and Park 2014). In Ghana, challenges faced by rural farmers, such as limited access to credit, markets, and adequate extension contacts, have been identified. Among these, inadequate extension services are seen as a major barrier to the growth of the agricultural sector and the broader development of rural communities (Danso-Abeam et al. 2018). Despite the diversity of these cases, a common methodological limitation in many of these studies is the focus on either macro-policy analysis or farmer-level surveys. Very few employ qualitative methods such as FGDs with local agricultural officers, those directly responsible for operationalizing extension programs. This creates a gap in understanding how policy is translated into practice on the ground, particularly in vulnerable regions. In many developing countries, governance issues are a critical weakness in public agricultural extension systems. "Governance failures" obstruct the effective performance of public services, including problems like corruption, political misuse, paternalistic approaches, and patronage (Bitzer et al. 2016).

Extension programs have evolved over the years to meet farmers' changing needs and the markets in which they operate. Consequently, a "one-size-fits-all" approach is not recommended for effective service delivery and outcomes due to varying farmer needs influenced by their geographical location, social, and economic structures (Maulu et al. 2021). This reinforces the need to explore localized and contextualized delivery models that reflect specific community constraints and dynamics, as is the case in Basilan province. The Philippines give a specific opportunity to investigate how post-conflict

governance restructuring, through agencies like MAFAR, impacts extension delivery.

Shifting to more effective strategies may be beneficial; these could include training staff in skills for knowledge capture, encoding, sharing, organizing communities of practice, and applying knowledge. Establishing a knowledge repository in the agriculture office is also advisable; knowledge useful for specific farmer groups should be shared with others for trial adoption. Furthermore, staff field visits need to be more systematic and organized. Procuring additional or new computers, with at least one having an internet connection, is essential. The national government, through institutions like the Bureau of Agricultural Research (BAR), Agricultural Training Institute (ATI), and state colleges and universities (SCUs), should assist in linking Local Government Units (LGUs) to information databases and training (Saz 2007).

Diversification is another solution. For many farmers in Ethiopia, integrating crops and livestock is vital for diversifying outputs and due to cultural values. Therefore, aligning agricultural extension services with farmers' demands, knowledge, experiences, and values may enhance the acceptability of the service. As a "public good," agricultural extension should provide inclusive benefits to impoverished and disadvantaged groups, especially in rural areas, to actively promote agricultural transformation in the country (Leta et al. 2017).

The specific formal research-extension linkage mechanisms in various countries include apex management with research, extension, and training within the same institution, matrix management (i.e. research and extension as semi-autonomous bodies under the same ministry), coordinating committees/meetings, communication units or liaison departments, the use of a task force, staff exchanges, and cooperation between university research programs and extension organizations (Agbamu 2000). To improve this situation, it is important to understand why researchers and extension workers operate in their current manner. It is worth considering what incentives they might have to change their behavior, especially if the system of promotions and other rewards remains unchanged. Identifying instances where researchers and extension agents have paid serious attention to the problems of resource-poor farmers and analyzing why this happened is also beneficial (Van Den Ban 1993).

It is suggested that there is a need for the extension system to be reformed to become more cost-effective, smallholder farmer-centered, and pluralistic. The revitalized extension system should incorporate contemporary applications of ICTs in extension processes, emphasize the participation of resource-constrained smallholder farmers, focus on rural women's empowerment, and involve both farmers and extension agents in adaptive research (Mapiye et al. 2021). In sum, although the global literature on agricultural extension points to common constraints such as bureaucracy, poor accountability, lack of innovation, and inadequate support systems, there remains a dearth of grounded empirical studies that explore these issues from the perspective of field-level extension workers in conflict-affected, autonomous regions such as BARMM.

Given the recurring problems in agricultural extension systems identified in various countries, this study focuses on the everyday work of municipal agricultural officers in Basilan under the MAFAR. It examines the specific problems they encounter in relation to governance, resource distribution, and service implementation. The study also identifies current practices used to address these challenges and considers the effects of autonomous governance arrangements on the delivery of extension services in the Bangsamoro region.

Agricultural extension is integral in rural development considering that it facilitates the transfer of knowledge, technologies, and practices from research institutions to farmers. As Anderson and Feder (2007) emphasize, much of agricultural extension carries a public-good character that justifies substantial public investment, especially in developing countries. However, they also stated that the delivery of extension services has been hindered by persistent challenges like administrative and design failures, weak accountability, limited interaction with research systems, fiscal unsustainability, and insufficient political commitment.

The success of extension efforts is closely tied to its policy environment, and difficulties in impact attribution usually weaken domestic political support while encouraging donor dependence. Various models have been implemented over the years (e.g. training and visit programs, decentralized systems, fee-for-service, and farmer field schools), but each presents its own set of trade-offs (Anderson and Feder 2007). These complexities are pronounced in fragile or underserved regions like Basilan, where institutional limitations and resource constraints challenge the responsiveness and reach of agricultural extension systems. Understanding these contextual challenges is essentially important for designing responsive, efficient, and inclusive extension systems.

METHODS

Research Design and Locale of the Study

This study employed a qualitative phenomenological design to explore the lived experiences and perceptions of OIC-MAFAR Municipal Officers (OIC-MMOs) regarding the challenges of delivering agricultural extension services in Basilan Province. Phenomenology is

appropriate for this research as it aims to understand the way individuals make sense of their everyday experiences within a specific social and institutional context (Moustakas 1994). Basilan is one of the five island provinces of the BARMM, characterized by agricultural livelihoods, geographic isolation, and periodic conflict. The province comprises 11 municipalities and one city, making it a strategic site for examining the on-the-ground realities of decentralized agricultural governance under the MAFAR.

Participants and Sampling

The study focused on all 12 OIC-MMOs in Basilan, who serve as the sole MAFAR municipal representatives in their respective localities. A total population sampling strategy was used, as all qualified individuals holding the position were included. Inclusion criteria consisted of active service as an OIC-MMO in Basilan and involvement in field-level agricultural extension activities during the study period. All invited participants agreed to take part in the study.

The ages of the OIC-MMOs range from the mid-twenties to the late thirties, with only two of them beyond their forties. The youngest among them is 25 years old, and the oldest is 45. This age bracket is relatively young for the field of extension, especially compared to LGU-devolved agricultural extension workers, most of whom are nearing retirement age. The OIC-MMOs are notably young and energetic for their age range. In terms of gender, out of the 12 OIC-MMOs, only four are male, with the remaining eight being female.

Regarding marital status, 10 of the 12 OIC-MMOs are married, while two remain single. Most have dependents: one has as many as 10 dependents, and only one has none. Several have between six to eight dependents, a challenging number to support on a salary grade-10, or PhP25,586 (approximately US$430), which is the pay level for OIC-MMOs. This is particularly true considering that most of them live modestly, as evidenced by their reliance on motorcycles as their primary means of transportation.

Data Collection

Data were collected through online semi-structured interviews conducted via Zoom from 30 October to 20 November 2022, each lasting 60 to 90 min. The interview guide was designed to elicit participants' lived experiences, perceptions, and challenges related to their roles as extension officers. Questions were drawn from similar phenomenological studies on agricultural officers (e.g. Ashraf and Hassan 2021) and pilot-tested for clarity. Interviews were conducted by the lead author, a licensed agriculturist and familiar with the regional context. All sessions were audio-recorded with participant consent and transcribed verbatim by a trained assistant. Transcripts in Filipino or local dialects were translated into English and reviewed by the research team for accuracy and consistency.

Data Analysis

Data were analyzed using thematic analysis and was conducted inductively and aimed to capture the essence of the OIC-MMOs' shared experiences. Coding was led by the main author, with other researchers reviewing a sample of transcripts to enhance intercoder consistency. An audit trail and memo-writing ensured analytical rigor. Data saturation was observed by the 10th interview, with no new themes emerging in the final two interviews.

Researcher Reflexivity

One of the authors, a Chief Agriculturist in the area of study, had a better understanding on the research setting, which facilitated rapport and contextual understanding. To address potential bias, data interpretation was done collaboratively with team members external to MAFAR, and reflexive journaling was maintained throughout the research process to monitor subjectivity.

RESULTS

The findings presented below are organized into main themes and sub-themes derived from the participants' narratives. Each theme summarizes participants' shared experiences, challenges, or perceptions, supported by verbatim excerpts. In presenting the results, the most salient statements from the participants were included to support each identified theme. These statements were carefully selected based on their clarity, representativeness, and relevance to the theme being discussed. While numerous excerpts were gathered during the analysis, powerful verbatim quotes were prioritized that best encapsulated the collective sentiments of participants. This approach ensures thematic coherence while maintaining readability and focus on the discussion.

Challenges Encountered in Agricultural Extension

Workers' qualification. Among the OIC-MMOs, only one graduated with a bachelor's degree in agriculture, which is closely related to the tasks of an MMO. Others hold degrees in various fields such as medical courses, education, political sciences, and other areas. According to the qualification standard for a MAFAR Municipal Officer, the most suitable candidates for the position should have agricultural-related qualifications. In fact, the position requires Republic Act 1080 eligibility, meaning an applicant must be a licensed agriculturist under the Philippine Regulation Commission to be qualified.

Regarding their work experience, only one of them had two years of experience in an agriculture-related job and two previously worked in DAR. This indicates that only three OIC-MMOs have relevant work experience, while the rest have backgrounds in diverse fields other than agriculture, fisheries, and agrarian reform. The MAFAR Basilan Provincial Office attempted to address this issue. Over the span of two years, most of them had attended more than 10 training courses designed to enhance their capacities as OIC-MMOs. Through these trainings, they had gained a bit more knowledge and confidence in delivering MAFAR services in their respective municipalities. Only one OIC-MMO is a licensed agriculturist, who is overwhelmed handling the programs of MAFAR's four divisions. Given that the officer's background is solely in agriculture, additional training in the other three divisions was requested. Several OIC-MMOs had to conduct self-research to respond to inquiries from their farmer-beneficiaries. One OIC-MAFAR Municipal Officer mentioned during the interview:

> *"Kalagihan kami subey magmahirin ene magtuun teed duk kami meh MMO hin, kalagihan kami tau magtanem meh tinanem, peggeh dumain kmi tartantu agriculturist. Subey tahati kami bang sumiyan duk kuingge pagtanem sin meh tartantu gantah tinanemin."* (We need training that suits us as MMOs; we need to know how to plant crops, because we are not agriculturists. We need to understand when and how to plant specific crops.)

For more than a year, the MMOs worked alone in their respective municipalities, tackling the challenging tasks of program implementation and Registry System for Basic Sectors in Agriculture (RSBSA) registration of farmers and fisherfolk. It was only last year that a fieldworker for fisheries was hired for each municipality, and in October 2022, twelve fieldworkers for agriculture were recruited to support the OIC-MMOs. A problem with these newly hired fieldworkers is their lack of background in agriculture and fisheries, both in education and experience.

Another issue was that some fieldworkers sometimes act independently of their OIC-MMOs, submitting reports to higher officials in the provincial office without the OIC-MMOs' knowledge or conducting operations without notifying them. This occured because the Division Chiefs in the Provincial Office were the ones signing the daily time records (DTR). The OIC-MMOs had commented that it would be advantageous if the hired fieldworkers had relevant backgrounds and experience and were locals in the municipality, as this could expedite beneficiary identification and program implementation.

Workforce capacity. The main responsibility of the OIC-MMOs is to identify target beneficiaries for all MAFAR programs and projects. These beneficiaries should be considered marginalized farmers and fisherfolk registered in the RSBSA with the assistance of the OIC-MMOs. During the annual planning period, the names of these beneficiaries must be submitted to the regional office along with the proposed projects for the following year. These submitted names are expected to be the recipients of the projects, but the OIC-MMOs have faced several challenges in identifying beneficiaries and registering them in the RSBSA. Additionally, some farmers have had issues with previous extension officers before BARMM, who collected their data and promised government agricultural support that never materialized.

The OIC-MMOs struggled to register farmers due to a lack of support staff. Registering fisherfolk is simpler than farmers because geo-tagging farm locations is time-consuming, whereas this is not required for fisherfolk. It is alarming to think that only one extension worker, the OIC-MMO, handles all these tasks in each municipality.

In 2021, contract service employees were hired for the fisheries division, one per municipality, to assist OIC-MMOs in fisheries program operations. In 2022, another batch of contract employees was hired for RSBSA support in the agriculture division. Before this, OIC-MMOs endured over a year of solitary effort in their respective municipalities. An OIC-MMO recalled during the interview,

> *"Geo-Tagging ngin yene tamanan huletek ne hinang talabey ku. Nyah bulan-bulan gantah subey ubus ku, sah peggeh asal mustahil manamal taubus dendang-dendangan."* (Geo-tagging is the most laborious task I have undertaken. I have monthly targets, but they are almost impossible to accomplish alone.)

Currently, each municipality has three MAFAR employees, which, compared to the original staff plan of six employees per municipality, means that these municipalities are still considered undermanned.

Coordination with other institutions. Aside from the understaffing issue, MMOs were facing challenges with local government officials. Some previous mayors manipulated the list of validated beneficiaries. Although MMOs provided agri-fishery inputs to local executives for ceremonial distribution along with a list of approved and validated target beneficiaries during the planning phase, these mayors decided to select a new set of beneficiaries. They chose individuals who voted for them during the election period, and those farmers and fisherfolk who

did not vote for the mayor, even if they were on the list of target beneficiaries, had their names removed.

Another problem occured at the barangay level, where some barangay captains were reluctant to sign the RSBSA form. For the RSBSA form to be validated, it requires the barangay captain's signature before submission to the regional office for data encoding. An OIC-MMO stated:

> *"Daran ne talabey ku paatag si aku yene meh aahin ganah tarapat de bng gey pili hap saddi barangay kapitan matapit-tapit si siyehin, peggeh barangay kapitan den gey bayah milmahan katas RSBSA den."* (I have experienced instances where beneficiaries were forced to go to neighboring barangays because their barangay captain would not sign RSBSA registration forms.)

Similar to the mayors, some barangay captains tend to favor beneficiaries who voted for them. This highly politicized environment complicates the OIC-MMOs' task of selecting appropriate beneficiaries for various ministry projects. There are many qualified marginalized beneficiaries but were not considered because they are not politically align with the barangay captain. This issue significantly impedes rural poverty reduction interventions. Often, the beneficiaries selected by barangay captains do not belong to the poorer groups in the community.

Undoubtedly, some OIC-MMOs received substantial support from their local governments, particularly from the office of the municipal agriculturist. Several municipal agriculturists, lacking substantial budgets or support from their local government units, rely heavily on the inputs distributed by MAFAR through the OIC-MMOs. These municipal agriculturists and their technicians assist the MMOs in identifying target beneficiaries, conducting site validation, distributing inputs, undertaking farm visitations, and other activities.

However, not all municipal LGUs are supportive of OIC-MMOs. In some towns, OIC-MMOs encountered issues with their mayors, especially during the transition from one administration to another following elections. Newly appointed mayors often failed to recognize the municipal agriculturists installed by the previous administration, instead appointing an acting municipal agriculturist. This situation can cause delays in MAFAR program implementation at the municipal level and may lead to changes in the target and validated beneficiaries. In municipalities with inactive municipal agriculturists, OIC-MMOs sometimes sought assistance at the barangay level through the barangay secretary and the barangay committee on agriculture.

In the fisheries division, one of the most challenging problems faced by OIC-MMOs was political backing of illegal activities. In the province of Basilan, there had been reports of ongoing illegal fishing. Large fishing boats were prohibited from operating in municipal waters, which were reserved for local small fishermen, to protect their livelihoods. However, some mayors permitted these boats to fish in their waters in exchange for under-the-table payments. The OIC-MMOs, afraid to report such fraudulent activities to higher authorities, find themselves in a difficult position. One of their mandates under the fisheries division is to reduce illegal fishing activities through reporting, but this becomes complicated when such illegal activities are supported by politicians.

Logistical support. A few OIC-MMOs interviewed expressed their passion for their work, citing the satisfaction of helping people through the distribution of agri-fishery inputs. However, most OIC-MMOs feel overburdened by their responsibilities. They frequently compare their situation to the defunct ARMM, where the Municipal Agrarian Reform Officer (MARO) and the Municipal Agricultural Officer (MAO) were separate roles, each with a salary grade 20, or PhP62,867 (approximately US$1,055) and a monthly Representation and Travel Allowance (RATA).

In contrast, OIC-MMOs are at a salary grade 10 level and do not receive RATA. The actual plantilla position of OIC-MMOs is Agrarian Reform Program Technologist (SG-10), the only permanent MAFAR employees at the municipal level, that is why they are designated as OIC-MMOs. Additionally, one OIC-MMO is a contract-service, non-permanent employee, facing issues like delayed salaries, a common problem for contract workers. Imagine the operational challenges faced by an OIC-MMO with delayed pay. One OIC-MMO even hired a geo-tagger and paid the wages out of her own pocket to meet the monthly target for RSBSA farmer registrations. Another mentioned:

> *"Asal kahunitan ku teed bilang OIC-MMO, yene tabettad ku hap munisipal si puluh, yene piye hadja puluh sakup ne. Palintas ku amban dambuwah puluh pi si dambuwah puluh asal jari-jari ne gastukun, duk kamuwe ilih gastukun ineddoh weh ku amban gadji-gadji ku."* (It is very challenging for me as an OIC-MMO, being assigned to an island municipality composed of several islands. Hopping from one island to another is quite costly, and I have been using a substantial amount of my personal salary for this.)

Since OIC-MMOs were not permitted to receive RATA or Maintenance and Other Operating

Expenses (MOOE), the provincial office decided to provide them with fuel to support their municipal-level operations. However, the amount of fuel provided was barely sufficient for their needs and often did not last even a week. Although the provincial office has an adequate fuel supply, the OIC-MMOs are advocating for a more strategic fuel distribution and are requesting an increase in their monthly fuel allotment.

Most OIC-MMOs do not have offices in their assigned municipalities. Some store their agri-fishery inputs in the houses of trusted farmers, which then serve as their temporary offices. Without tables, chairs, or office supplies, many OIC-MMOs have opted to report to the provincial office instead. This situation makes them less effective, as being in their respective municipalities would allow for easier communication with farmers.

In addition to poor working conditions, all OIC-MMOs report that the agri-fishery inputs received from the region are insufficient. They lack enough input for walk-in clients, particularly goats and chickens, which were very limited in number. Furthermore, there are municipalities where the peace and order situation are not conducive to extension work, with insurgencies and killings being rampant. For those in island municipalities, weather conditions usually pose significant challenges to regular operations. Typhoons and huge waves are common obstacles when traveling from the mainland to the islands.

Suggestions from the OIC-MMOs

All the OIC-MMOs requested additional training to enhance their capacity as MAFAR heads in their respective municipalities. They need 'training-of-trainers'-type sessions covering all areas of their responsibility, such as agriculture, fisheries, and agrarian reform. As only one of them is an agriculturist, eleven out of twelve have requested crop production training, and all have requested training in fisheries technologies and agrarian reform methodologies. Two OIC-MMOs have requested a supervisory development course to advance their careers.

Besides training, the OIC-MMOs suggest they should have authority in identifying beneficiaries, as they conduct validations at the grassroots level. They argue that higher-ups should limit their influence in selecting beneficiaries, as OIC-MMOs, spending most of their time in the field, are better positioned to know which farmers are more deserving of support. Given the limited number of agri-fishery inputs, they believe that the most marginalized farmers should be prioritized, and only OIC-MMOs truly know who these farmers are.

Considering their workload is disproportionate to their salaries, and receiving RATA is not an option, most OIC-MMOs are requesting MOOE (Maintenance and Other Operating Expenses) at the municipal level. This budget allocation would cover logistical support such as fuel expenses and office supplies. Officer-In-Charge MAFAR Municipal Officers in island municipalities are requesting a monthly MOOE of PHP 10,000 (or approximately USD 170) while others suggest PHP 5,000 (or approximately USD 85) monthly. Some are also advocating for monthly incentives and a communication allowance.

DISCUSSION

Workers' Qualification

The majority of OIC-MMOs are not expert on their field assigned because their educational backgrounds are unrelated. Agricultural extension effectiveness is often limited by insufficient technical expertise among personnel, especially when they lack formal education in agriculture or fisheries (Makapela 2015). They usually resort to conducting personal research or watching online videos to enhance their knowledge, but this self-research approach is inadequate. Braun et al. (2006) suggest that while digital learning resources provide supplementary information, they are not a substitute for structured field training and hands-on experience in agricultural extension. Farmers frequently have many questions that OIC-MMOs are unable to answer. The lack of technical competency in extension officers has been shown to reduce farmers' trust in advisory services and hinder the adoption of modern agricultural techniques (Suresh et al. 2024). To minimize this issue, MAFAR should develop a comprehensive training program tailored to the needs of OIC-MMOs. Exposure to all the banner programs of each division is crucial, and relevant topics should be covered in depth.

Workforce Capacity

The central issue for the OIC-MMOs is the lack of support staff. Managing farmers' registration, geo-tagging, distributing inputs, and other extension activities is overwhelming for just one to three employees at the municipal level. This aligns with Davis et al. (2010) stating that insufficient agricultural extension personnel hinder effective service delivery, especially in decentralized agricultural systems. Although the two additional contract fieldworkers relatively eased the workload, the staffing level is still considered inadequate. According to Binswanger-Mkhize and Zhou (2012), a well-staffed extension service greatly improves agricultural productivity and technology adoption among farmers, which emphasizes the necessity of sufficient personnel in MAFAR offices. The MAFAR Municipal Office, covering agriculture, fisheries, and agrarian reform at the municipal level, requires a sufficient number of

extension workers to achieve the expected output. The plantilla positions remain vacant due to a lack of qualified applicants. This situation mirrors national and global trends where agricultural extension services struggle to attract and retain qualified personnel, usually due to low salaries and limited career incentives (Swanson 2008). However, MAFAR's top management could potentially resolve this by hiring more qualified and experienced contract fieldworkers per municipality. Considering there are six plantilla positions in a municipality, including the MMO, hiring five additional fieldworkers to support the OIC-MMO would be beneficial.

The MAFAR-Basilan Provincial Office hired two contract fieldworkers per municipality to assist the OIC-MMOs, one specializing in fisheries and the other in agriculture. Regrettably, these fieldworkers lack experience in both fields and do not possess degrees in any related or allied courses in agriculture and fisheries. With eleven out of twelve OIC-MMOs not holding degrees in agriculture and fisheries, the effectiveness of the extension service would be greatly enhanced if the hired fieldworkers had knowledge in agri-fishery technologies. Extension agents with formal agricultural education and continuous training play a large role in increasing farmers' adoption of new technologies and improving farm productivity (Arowosegbe et al. 2024). Additionally, employing local individuals as fieldworkers in their respective municipalities could significantly improve operations. Localized hiring strategies have been found to strengthen agricultural extension efforts by leveraging local knowledge and promoting stronger engagement between extension workers and farmers (Ferris et al. 2014). For successful field operations at the municipal level, MAFAR-Basilan Provincial Office should rigorously hire qualified fieldworkers and select applicants best suited for the role. Furthermore, fieldworkers should be directly supervised by OIC-MMOs, with their Daily Time Records (DTRs) signed by the OIC-MMOs rather than the Division Chiefs in the provincial office. This change could prevent the trespassing issues experienced by OIC-MMOs.

Coordination with Other Institutions

Another major challenge for OIC-MMOs is the influence of Municipal and Barangay Officials in their areas of responsibility, which significantly impacts the selection of target and validated beneficiaries. This issue is well-documented in agricultural and social protection programs, where political interference can distort the intended distribution of resources and services, frequently favoring non-marginalized groups (Norman 2017). To navigate this situation, OIC-MMOs usually feel compelled to accommodate these officials' requests to include their chosen beneficiaries, who are often not marginalized farmers and fisherfolk. Political patronage in rural development programs can reduce effectiveness, limiting support to genuinely disadvantaged communities and reinforcing systemic inequalities (Brinkerhoff and Goldsmith 2002). This concession is made to facilitate their work within municipal and barangay areas, addressing a social issue that is difficult to resolve. A pragmatic approach for OIC-MMOs is to allocate a portion of the beneficiary quota to mayors and barangay captains. While such compromises may guarantee smoother program implementation, research suggests that stronger institutional safeguards, such as independent oversight and transparency measures, mitigate undue political influence in agricultural extension and development initiatives (Brinkerhoff 2001). Ideally, excluding local officials from the selection and distribution of beneficiaries would be more effective, but this may not always be feasible.

Logistical Support

Apart from this, most OIC-MMOs do not have offices in their areas of responsibility. When agri-fishery inputs are delivered, they commonly store them in the homes of trusted farmers. This is consistent with Odongo et al. (2023) that presents the importance of physical infrastructure in agricultural extension services, where the absence of formal offices leads to inefficiencies in service delivery and weak farmer-extension linkages. This lack of formal office space reduces the OIC-MMOs' effectiveness, as farmers are unsure where to go for consultations. Accessible and well-equipped extension offices boost farmers' participation in agricultural programs and improve knowledge transfer (Sahoo et al. 2024). MAFAR should prioritize establishing municipal stations or offices for OIC-MMOs, as these would serve as vital centers for the extension system at the municipal level. One approach to address this issue is to allocate a budget for renting office space for OIC-MMOs. The budget should also cover necessary office equipment, such as tables, chairs, computers, and other office supplies.

Compounding this problem, OIC-MMOs often face budget shortfalls during their daily field operations, leading them to use a significant portion of their salary for regular activities. Just like international challenges in agricultural extension services, inadequate operational funding forces extension officers to cover work-related expenses out of pocket, ultimately reducing job satisfaction and program effectiveness (Davis et al. 2010). This situation is particularly challenging, considering they receive only the minimum salary of an ordinary employee despite working as heads of office at the municipal level. Research have shown that insufficient financial support for extension workers leads to lower motivation, decreased field presence, and limited farmer outreach (Ragasa et al. 2016). While MMOs

are at salary grade 20, which disqualifies them from receiving Representation and Travel Allowance (RATA), MAFAR should consider providing OIC-MMOs with Maintenance and Other Operating Expenses (MOOE), ranging from PHP 5,000 to PHP 10,000 (or approximately USD 85 to USD 170). Allocating dedicated funds for operational expenses has been found to significantly improve the effectiveness of extension programs by ensuring continuous field activities and minimizing financial burdens on personnel (Anderson and Feder 2007). This financial support would greatly facilitate the smooth implementation of programs at the municipal level.

This study provides insights into the operational challenges encountered by OIC-MMOs under the MAFAR in Basilan Province, BARMM. It traces the impact of institutional restructuring, logistical constraints, and political interference on the implementation of agricultural extension programs within fragile and decentralized governance contexts.

The findings indicate that the OIC-MMOs are burdened by mismatched qualifications, inadequate staffing, political interventions in beneficiary selection, lack of field offices, insufficient logistical support, and the absence of operational funding. These systemic issues hinder the delivery of effective extension services, particularly in conflict-prone areas. The paper identifies the dissonance between policy design and field realities, a concern that has remained largely unaddressed in previous extension literature, especially in post-conflict and autonomous regions.

Given these results, the following prioritized actions are recommended: 1) comprehensive, modular training programs for OIC-MMOs that cover agriculture, fisheries, and agrarian reform; 2) hiring of qualified and locally based contract fieldworkers to support the municipal offices, with clear lines of supervision under OIC-MMOs; 3) provision of MOOE at the municipal level to ensure continuous operations; 4) installation of basic office infrastructure in all municipalities to improve accessibility and visibility; 5) implementation of transparency mechanisms to safeguard beneficiary selection from political manipulation; and 6) development of supervisory and career advancement pathways for OIC-MMOs to boost retention and morale.

Future research could undertake mixed-method studies across other BARMM provinces to assess whether similar patterns persist in varied contexts. Comparative analyses can help identify scalable reforms and context-sensitive adjustments. Moreover, studies focusing on the perspectives of farmer-beneficiaries and LGU counterparts could give a fuller picture of the implementation landscape and help triangulate data from the extension workforce.

Empowering field-level officers through targeted support and structural reform remains central to advancing BARMM's macro-level goals of rural development and peacebuilding. Informed by the lived experiences of OIC-MMOs, this research lays the groundwork for reimagining the delivery of agricultural extension in post-conflict autonomous regions such as BARMM.

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GENERATIVE AI STATEMENT

The authors used OpenAI's ChatGPT (version GPT-4, July 2025) to assist in the revision and refinement of the manuscript. The tool was used primarily for editing the grammar and readability of the manuscript. All substantive content, analysis, and interpretations were developed by the authors. The final manuscript was reviewed and approved in its entirety by the authors to guarantee accuracy, originality, and scholarly integrity.

ETHICAL CONSIDERATIONS

Oral informed consent was obtained from all participants prior to the interviews. They were clearly informed about the study's purpose, their right to voluntary participation, the option to withdraw at any time, and the confidentiality of their responses. Given the localized and remote context of Basilan, where access to formal Institutional Review Boards (IRBs) is limited, this research did not undergo formal IRB review. However, the study strictly followed ethical protocols aligned with national guidelines for social research in the Philippines. Personal identifiers were excluded from transcripts and findings to ensure confidentiality and protect participants' anonymity.

DECLARATION OF COMPETING INTEREST

The authors declare no financial or commercial competing interests. The professional role of one of the authors is acknowledged as a potential source of contextual advantage, but it has been managed to ensure the study's integrity, impartiality, and adherence to ethical standards.

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Blue swimming crab (*Portunus pelagicus* Linnaeus, 1758) spawner's survival and egg hatchability in net cages

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ABSTRACT

The declining population of the blue swimming crab (BSC) (*Portunus pelagicus* Linnaeus, 1758) necessitates effective resource management and hatchery development for crablet production, which is vital for reseeding and aquaculture. This study aimed to determine the water quality and evaluate the survival rate (SR) of spawners and the hatching rate (HR) relationships to the spawners' carapace width (CW) and body weight (BW) using a net cage hatchery system in the coastal waters of Bocana, Ilog, Negros Occidental, Philippines. Berried BSC were individually stocked in net cages and grouped into five size categories based on CW: S1 (100.00-104.99 mm), S2 (105.00-109.99 mm), S3 (110.00-114.99 mm), S4 (115.00-119.99 mm), and S5 (120.00-124.99 mm). The findings indicated that the physicochemical parameters in the coastal waters of Bocana were suitable for BSC spawning. Larger CWs were associated with higher SR (mean overall 80 ± 40.82%). Spawners with a CW ranging from 110 to 124.99 mm exhibited higher HRs compared to those ranging from 100 to 109.99 mm. The mean (±SD) hatching rate (51.89 ± 29.67%) was comparable to previous studies with highly controlled environmental conditions. The BSC spawners' CW ($r(23) = 0.614$, $P < 0.001$) and BW ($r(23) = 0.563$, $P < 0.003$) were significantly correlated with their HR. The correlation between HR vs. CW and HR vs. BW was significant. The net cage system offers a method that can contribute to a sustainable blue swimming crab fishery industry and serves as a potential model for community-based aquaculture initiatives.

Keywords: berried crabs, body weight, carapace width, fecundity, net cage hatchery system

INTRODUCTION

Worldwide, excessive fishing has resulted in a considerable proportion of marine fish populations being harvested at biologically unsustainable rates. This trend indicates a troubling escalation, with 33.1% of evaluated fish stocks classified as overfished by 2015, a significant increase from 10% in 1974 (FAO 2018). These declines significantly affect both coastal and inland fisheries, which are frequently vital for the sustenance and economic stability of underprivileged communities (Lynch et al. 2017). The consequences of

these declines raise serious concerns regarding global fish availability, especially in developing nations that depend heavily on marine resources. Effective management and sustainable production strategies are essential due to the ongoing demand for marine products and the significant role of fisheries in addressing poverty and ensuring food security (FAO 2018; Mesa et al. 2018).

Among these fishery resources is the blue swimming crab (BSC) (*Portunus pelagicus* Linnaeus, 1758), an economically significant species prevalent throughout the Indo-Pacific, particularly in Southeast Asia. Its high commercial value has led to increasing exploitation (Andrés et al. 2010; Baylon 2010; Quinitio et al. 2011; Azra and Ikhwanuddin 2015), which has led to a decline in natural stocks in regions like the Philippines (FAO 2018; BFAR 2018; BFAR 2020; Gascon et al. 2023). In eastern Visayas (de la Cruz et al. 2015; FAO 2018; Mesa et al. 2018; Doronila 2019) in particular, this scarcity of BSC stocks, coupled with the global understanding that reliance on finite natural resources is unsustainable, creates a strong imperative for the aquaculture of BSC. Hatchery production, particularly for commercial-scale operations, emerges as the most promising strategy for seed supply. Recent research in BSC culture has primarily focused on refining larval rearing procedures in tanks to enhance survival and growth (Azra and Ikhwanuddin 2015). However, this method is expensive to maintain in the Philippines.

To address the decline in commercial and municipal fisheries, this study examines the survival and hatching rates of blue swimming crab spawners using net cages to increase the BSC stocks for reseeding and aquaculture. Specifically, this study determined the water characteristics of the area, assessed the survival rate (SR) and the hatching rate (HR) of BSC spawners within the net cage system, and examined the relationships between the hatching rate (HR) and the spawners' carapace width (CW) and body weight (BW). The results of this study will be beneficial for the sustainability of the BSC fishery and aquaculture.

METHODS

Research Design

This study on the spawner's survival and hatching rate of blue swimming crabs in a net cage under natural conditions utilized an observation-correlational research method.

Time and Location of the Study

The research was carried out from 05 December 2020 to 27 March 2021 in the coastal waters of Barangay Bocana, Ilog, Negros Occidental, with the coordinates 10°0'43.859"N and 122°42'29.113"E (Figure 1). It is 873 m from the shoreline, with a water depth of 80 cm during the lowest low tide and 230 cm during the highest high tide based on a zero datum mean lowest low water reference. The study site is situated in the neritic zone, a nearshore marine environment distinguished by adequate light penetration that facilitates active photosynthesis. This area generally features well-oxygenated waters, consistent temperatures, and reduced hydrostatic pressure in comparison to deeper zones, frequently composed of fine sediments like silt. The neritic zone exhibits high biological productivity, fostering a variety of communities such as phytoplankton, zooplankton, protists, small fishes, and shrimp, which collectively establish a crucial foundation for a significant segment of global fisheries (Garrison 2017; Castro and Huber 2019).

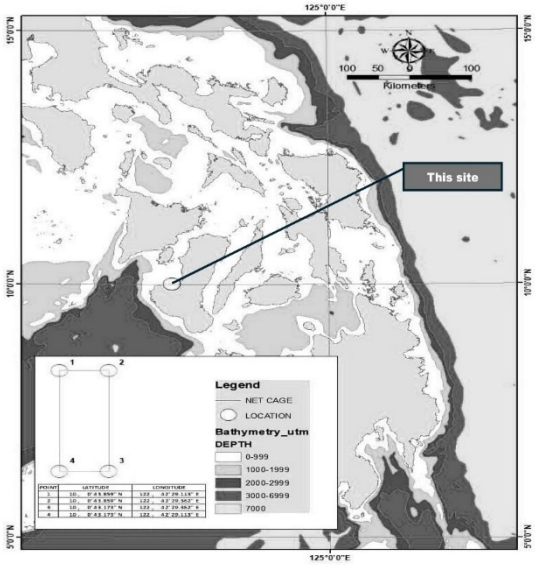

Figure 1. The study site in Barangay Bocana, Ilog, Negros Occidental, Philippines.

Field Layout

The study utilized a complete randomized design (CRD) in a floating cage area of 108 m^2 (Figure 2). The CRD was applied to maintain experimental rigor and ensure unbiased distribution of individuals across the different size groups (Deaton and Cartwright 2018). The floating net cage system measures 7 m in width and 21 m in length. The system contains 25 net cages designed to house the BSC spawners, each measuring 1 m by 1 m with a height of 0.5 m. Each net cage is 1 m apart to ensure adequate dissolved oxygen distribution. To examine the

relationships between spawner size and reproductive success, five distinct groups of berried BSC were established. These classifications were precisely delineated by CW, ranging from Group 1 (S1), encompassing individuals measuring 100.00 to 104.99 mm, through successive increments to Group 5 (S5), which included crabs between 120.00 and 124.99 mm. This structured grouping allowed for the rigorous assessment of how SR and HR correlate with these key morphological dimensions (Table 1). The sizes are based on the minimum legal size and the BSC female CW range at average size of maturity (Mesa et al. 2018; Abrenica et al. 2021; Gascon et al. 2023; Picoy-Gonzales et al. 2023).

Source and Collection of Spawners

The 25 stage-1 live BSC spawners were gathered within the coastal area of Barangay Bocana to ensure their viability for the study. Sourcing live, stage-1 berried spawners (Figure 3) directly from experienced local fishers ensured access to naturally gravid and healthy individuals, reflecting the typical broodstock acquisition method for local aquaculture efforts (Quinitio et al. 2011). The crabs were collected within one week to ensure minimal variances during the spawning time (Oniam et al. 2021). The collected crabs were stored in the net cages at the study site.

Preparation and Installation of Floating Net Cages

The openings of the blue High-Density Polyethylene (HDPE) plastic drum floats were first sealed using marine epoxy, then tied to bamboo frames using a plastic strap. The floats were secured to the bamboo cage frames at intervals of 7 m (Figure 4). The HDPE drums and polyvinyl chloride (PVC) pipes were chosen for their durability, buoyancy, and resilience in marine environments, to ensure the lasting integrity of the floating system (Beveridge 2004).

Each net cage was constructed using a fine-meshed nylon screen (24 mesh, 0.71 mm) sewn to dimensions of 90 cm in width and 90 cm in length, with a height of 40 cm, which is smaller than the cage frame to ensure a secure attachment. The fine-meshed nylon screen played a crucial role in containing newly hatched zoea larvae, facilitating adequate water exchange while effectively preventing predator intrusion (Cabacaba and Salamida 2015). The cage frames were made using a one-half-size blue PVC pipe, connected using the "L" and "T" connectors, and fitted with PVC solvent (Figure 5). The cages were installed three days before stocking to ensure their durability and fittings.

S2	S1	S5	S4	S1	S3	S5	S2	S5
S4	S3	S1	S2		S2	S3	S4	S3
S5	S1	S4	S3		S1	S4	S2	S5

Figure 2. Field layout of the study showing the S1 to S5, wherein S1 (100.00-104.99 mm; S2 (105.00-109.99 mm; S3 (110.00-114.99 mm; S4 (115.00-119.99 mm), and S5 (120.00-124.99 mm) are categorized as Groups 1, 2, 3, 4, and 5, respectively, and replicated 5 times in a complete randomized design within the floating cage area.

Table 1. Treatments, replications, and blue swimming crab characteristics.

Treatments (size class) (mm)	Replications/ Number of study cases	Stocking Density (m^2)	Average (±SD) Size Range Carapace Width (mm)	Average (±SD) Incubation Duration (days)
S1 100.00-104.99	5	1	101.32 ± 0.87	10.00 ± 0.00
S2 105.00-109.99	5	1	108.32 ± 0.84	10.00 ± 1.00
S3 110.00-114.99	5	1	113.18 ± 1.61	10.60 ± 0.89
S4 115.00-119.99	5	1	118.44 ± 1.25	9.50 ± 0.58
S5 120.00-124.99	5	1	123.56 ± 2.02	10.60 ± 0.55
Total	25		112.97 ± 8.00	10.20 ± 0.77

Figure 3. Stage 1 blue swimming crab spawner (*Portunus pelagicus*) used in the study, caught in the coastal area of Barangay Bocana, Ilog, Negros Occidental, Philippines.

Figure 4. The net cage system with a floating bamboo frame, where the polyvinyl chloride cage frame holds the net cage for spawning of the blue swimming crab.

Figure 5. The net cage is tied to the polyvinyl chloride pipe frame for individual blue swimming crab spawners, demonstrating the construction for isolated rearing.

Stocking of Spawners in the Net Cages

At 1800 h, when the temperature was desirable, collected live berried females (spawners) were stocked into their corresponding net cages. Stocking at this hour coincides with cooler ambient temperatures and reduced light intensity, minimizing handling stress on the crabs during transfer (Quinitio et al. 2011). The stocking distribution followed a CRD (Figure 6), with placement determined explicitly by their sizes.

Figure 6. Stocking of blue swimming crab spawners with a stocking density of one crab per 0.5 m^3 net cage.

Feeding and Monitoring of the Spawners

The BSC spawners were fed daily using green mussel (*Perna viridis* Linnaeus, 1758) meat at a ration of 10% of the crab's body weight per variate after shelling and weighing (Parado-Estepa et al. 2002; Azra and Ikhwanuddin 2015). The remaining feed was frozen in preparation for the next feeding. Feeding was done once daily from 1800 to 1900 h.

The development of crab eggs was monitored daily at 0600 h. The color of each egg was visually inspected and recorded to properly detect the day of possible spawning (Ikhwanuddin et al. 2012; Soundarapandian et al. 2013).

Data Gathering

Carapace width and body weight. Before stocking the BSC spawners in their corresponding cages, the CW was measured using an electronic digital vernier caliper to the nearest hundredth from the tip-to-tip of its carapace spine. The BW of individual crabs was measured using a digital laboratory scale (SARTORIUS Practum 6101-1S). Two data points were taken for the BW: the total BW, and the BW without the eggs on its pleopods.

Water physicochemical parameters. A 200 mL bottle was submerged at approximately 50 cm deep in a bottom-up position (90º) perpendicular to the seafloor. It was then gradually tilted to 180º going upward, reaching the water column in three random points outside the individual net cages but within the study area. To ensure consistency, water samples from inside the net cages were obtained via the same method, targeting three randomly chosen cages across all experimental groups. Water physicochemical parameters were measured using a turbidity sensor (PASCO PS-3215), an optical dissolved oxygen (DO) sensor (PASCO PS-2196), a digital temperature sensor (PASCO PS-3201), a refractometer (Magnum Media Salinity-ATC technology with Automatic Temperature Compensation) for salinity, and a wireless pH sensor (PASCO PS-3204). All physicochemical parameters were measured twice daily, from 0600 h – 0700 h when the temperature was at its minimum and from 1400 h - 1500 h when the temperature was at its maximum.

Spawners' survival rate. Crabs were monitored daily at 0600 h from day one, or the day after stocking, up to day 12 when all BSC spawned and were removed from the net cages. The survival rate of the spawners was gathered per treatment and calculated using the formula:

$$Survival\ Rate = \frac{Number\ of\ crabs\ in\ net\ cage}{Live\ crab\ after\ spawning} x100.$$

Estimation of egg mass and batch fecundity. Batch fecundity, defined as the quantity of eggs produced by a female in a single batch, and the overall egg mass attached to the pleopods during the embryonic stage were determined (Soundarapandian 2009). Embryonic stages were visually categorized by egg color (Figure 7; Ikhwanuddin et al. 2012; Soundarapandian et al. 2013): stage one (yellow or orange-yellowish), stage two (brown), and stage three (black-grey or black).

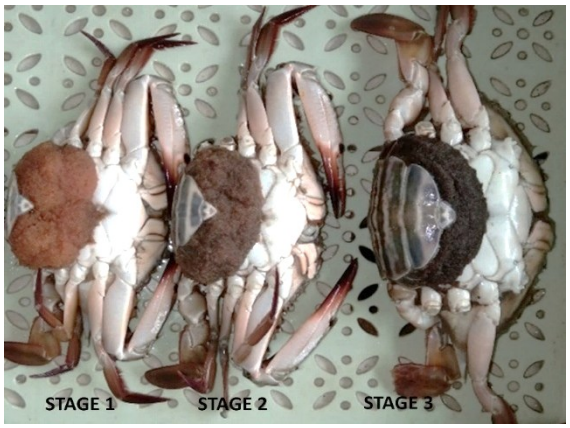

Figure 7. Egg development stages of the blue swimming crab spawners, visually categorized by color: Stage 1 (yellow or orange-yellowish), Stage 2 (brown), and Stage 3 (black-grey or black).

For the determination of fecundity from the 25 collected BSC spawners, the procedure outlined by Ikhwanuddin et al (2016) was adhered to. From each wet egg mass, three replicate subsamples were randomly collected. Eggs were then counted under an

electronic binocular light microscope (Motic®-BA210) until a count of 300 eggs was reached in each subsample. Subsequently, the mean weight of eggs per unit subsample was precisely measured using an electronic analytical balance (Sartorius BSA124S-CW Max 120 g d = 0.1 mg). Fecundity for each spawner was calculated using the following formula, adapted from Ikhwanuddin et al (2016):

$$Fecundity = \frac{Total\ egg\ mass}{Mass\ of\ individual\ egg}.$$

Determination of HR per group. The spent stage occurs when the crab has released all eggs from its pleopods (Mesa et al. 2018). The spawned crab was removed from the net cage, then the net cage was removed from its PVC frame and placed in a 40 L linear low-density polyethylene (LLDPE) plastic basin. A half-filled plastic basin containing hatched larvae (zoea) was softly stirred to ensure an even distribution of zoea larvae in the water. One liter (1 L) sample was taken at random points from the plastic basin. The zoea larvae were counted using volumetric sample analysis. To quantify larval density, ten 10-mL replicate subsamples were collected from each 1 L water sample taken from the plastic basin. Larvae within each 10-mL subsample were counted, and the mean density was calculated. Subsequently, the estimated number of hatched larvae in 1 L was extrapolated to the total volume of the plastic basin to determine the total number of larvae produced. The hatching rate was then determined using the formula provided by Ikhwanuddin et al (2016):

$$Hatching\ Rate = \frac{Number\ of\ larvae\ hatch}{Fecundity} x100.$$

Data Analysis

All statistical analyses were performed using SPSS Statistics (IBM Corp., Version 26.0). The mean and standard deviation were used to determine the water characteristics, the spawners' survival, and the hatching rate. Pearson's correlation was used to determine the relationship between the spawner's body size (CW and BW) and their HR. The assumptions of normality and homogeneity of variance were verified prior to conducting parametric analyses. A one-way Analysis of Variance (ANOVA) was conducted to evaluate significant differences across the five size groups concerning CW and BW at a 95% significance level. To mitigate Type I error in multiple comparisons, a post-hoc analysis employing Tukey's Honest Significant Difference (HSD) test was performed to determine which specific groups exhibited statistically significant differences. The choice of these tests was carefully considered for their effectiveness in examining relationships and group differences within a normal dataset (Field 2024).

RESULTS

Coastal Water Characteristics in Bocana

The results of the water physicochemical analysis in the study area, which is located in the neritic zone of the coast of Barangay Bocana, showed the following levels: DO, 5.71 ± 0.564 mg L^{-1}; temperature, 29.18 ± 0.14°C; pH, 8.30 ± 0.12; salinity, 27.88 ± 0.39 ppt, turbidity, 130.10 ± 57.12 NTU (Table 2).

Survival Rate and HR of Blue Swimming Crab Spawners

Survival rates were highest in groups S3 and S5, both achieving 100 ± 0%. These were followed by S4 with 80 ± 44.72%. Groups S1 and S2 exhibited lower SRs at 60 ± 54.77%. The overall mean SR for BSC spawners was 80 ± 40.82%.

The HR of S1 was the lowest among the groups (26.64 ± 25.36%), while S5 had the highest among the groups (77.54 ± 1.91%). The overall HR ranged from 0.00 to 80.17% (51.89 ± 29.67%) (Table 3). Significant differences were observed between the groups, as established by one-way ANOVA (F(4,20)

Table 2. Water physicochemical analysis within the incubation period in the coastal waters of Barangay Bocana, Ilog, Negros Occidental, Philippines (PPT - parts per thousand, NTU - Nephelometric Turbidity Unit).

Physicochemical parameters	AM	PM	MEAN
	min, max (Mean ± SD)	min, max (Mean ± SD)	min, max (Mean ± SD)
DO (mg L^{-1})	4.79, 6.71 (5.42 ± 0.53)	5.21, 7.05 (6.15 ± 0.67)	4.96, 6.73 (5.71 ± 0.56)
Temperature (°C)	28.02, 29.10 (28.10 ± 0.06)	29.60, 30.67 (30.34 ± 0.30)	28.89, 29.40 (29.18 ± 0.14)
pH	8.05, 8.41 (8.31 ± 0.13)	8.05, 8.53 (8.28 ± 0.17)	8.16, 8.49 (8.30 ± 0.12)
Salinity (ppt)	26.67, 28.10 (27.82 ± 0.57)	27.50, 28.67 (28.13 ± 0.38)	27.07, 28.40 (27.88 ± 0.39)
Turbidity (NTU)	29.35, 218.83 (135.26 ± 61.04)	34.67, 209.00 (125.46 ± 54.25)	34.41, 211.20 (130.10 ± 57.12)

Table 3. Survival rate and hatching rate of blue swimming crab spawners in each 5 mm carapace width size group in the net cage in the coastal water of Barangay Bocana, Ilog, Negros Occidental, Philippines.

Treatments (size class) (mm)	Survival Rate min, max (Mean ± SD)	Hatching Rate min, max (Mean ± SD)
S1 - 100.00-104.99	0.00, 100.00 (60.00 ± 54.77)	0.00, 56.02 (26.64 ± 25.36)
S2 - 105.00-109.99	0.00, 100.00 (60.00 ± 54.77)	0.00, 55.84 (27.27 ± 25.69)
S3 - 110.00-114.99	100.00, 100.00 (100.00 ± 0.00)	60.06, 74.81 (67.83 ± 6.43)
S4 - 115.00-119.99	0.00, 100.00 (80.00 ± 44.72)	0.00, 78.09 (60.2 ± 33.92)
S5 - 120.00-124.99	100.00, 100.00 (100.00 ± 0.00)	74.85, 80.17 (77.54 ± 1.91)
Total	0.00, 100.00 (80.00 ± 40.82)	0.00, 80.17 (51.89 ± 29.67)

Table 4. Comparison of the mean hatching rate of blue swimming crab spawners based on their carapace width.

Treatments (size class) (mm)	n	Mean	Std. Deviation
S1 - 100.00-104.99	5	26.64[b]	25.36
S2 - 105.00-109.99	5	27.27[b]	25.69
S3 - 110.00-114.99	5	67.83[a]	6.43
S4 - 115.00-119.99	5	60.20[a]	33.92
S5 - 120.00-124.99	5	77.54[a]	1.91
Total	25	51.89	29.67

Table 5. Correlations of hatching rate, carapace width and body weight of blue swimming crab spawners (**. Correlation is significant at the 0.01 level, 2-tailed)

		Hatching Rate
Hatching Rate	Pearson Correlation	1
	Sig. (2-tailed)	
	N	25
Carapace Width	Pearson Correlation	0.614**
	Sig. (2-tailed)	0.001
	N	25
Body Weight	Pearson Correlation	0.563**
	Sig. (2-tailed)	0.003
	N	25

= 5.566, $P = 0.004$). The Tukey's HSD post-hoc test revealed that the HR's between groups were significantly different, with S1 (26.64±25.359) and S2 (27.27 ± 25.692) showing significantly lower HRs compared to S3 (67.83 ± 6.434), S4 (60.2 ± 33.917), and S5 (77.54 ± 1.912) (Table 4). This indicates that the HRs of the larger BSC spawners with CW ranging from 110.00 mm to 124.99 mm are significantly higher than those of the smaller spawners with CWs ranging from 100.00 mm to 109.99 mm.

Correlation Between Hatching Rate and Carapace Width and Body Weight

In the current study, the HR and the CW of the BSC spawners were found to have a moderate positive correlation, $r(23) = 0.614$, $P < 0.001$. The HR and the BW were also found to have a moderate positive correlation, $r(23) = 0.563$, $P < 0.003$. These results show that the CW and BW of the BSC spawners are significantly correlated with their HRs (Table 5). This suggests that a higher CW results in a higher HR (Figure 8), and a similar trend is observed between BW and HR (Figure 9; Figure 10).

DISCUSSION

Water Quality Conditions and Suitability

The water quality in the coastal waters of Barangay Bocana is suitable as the spawning ground for BSC. The water characteristics are considered optimum for the reproductive performance of the BSC spawners, aligning with conditions known to support crustacean reproduction, except for salinity, which is lower than that reported in previous studies (Charmantier and Charmantier-Daures 1995; Arshad et al. 2006). The optimum salinity and temperature for the hatchability of BSC are 30-32 ppt and 28-30°C,

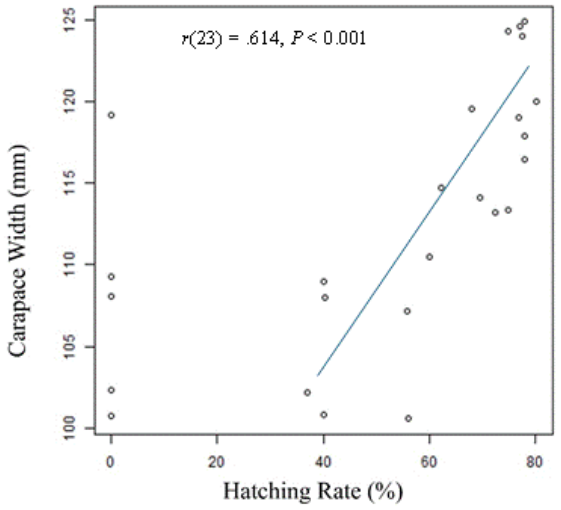

Figure 8. A scatter plot illustrates the relationship between blue swimming crab spawners' hatching rate (%) and carapace width (mm). A significant positive correlation was observed ($r(23) = 0.614$, $P < 0.001$), indicating that hatching rate tends to increase with larger carapace width. The line represents the linear regression trend.

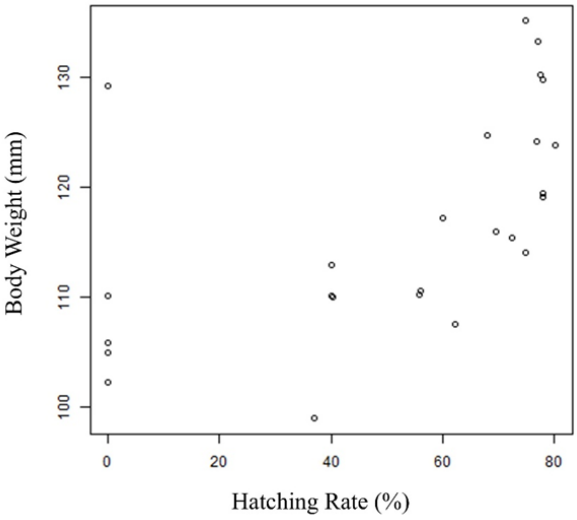

Figure 9. A scatter plot illustrates the relationship between blue swimming crab spawners' hatching rate (%) and body weight (g). A significant positive correlation was observed ($r(23) = 0.563$, $P < 0.003$), indicating that hatching rate tends to increase with larger body weight. The line represents the linear regression trend.

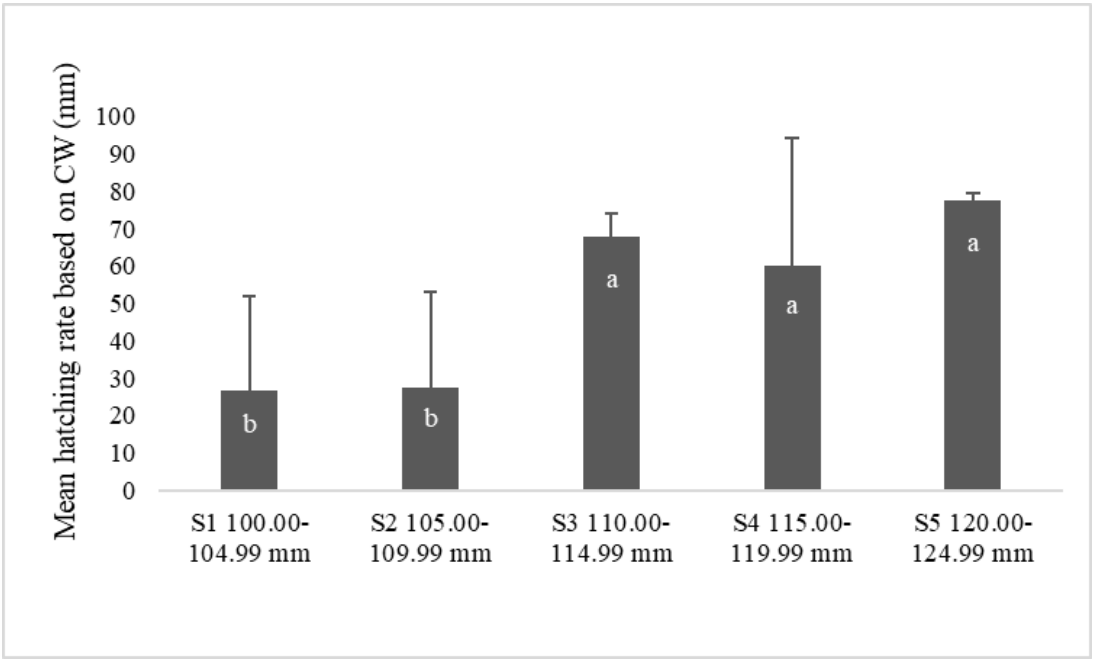

Figure 10. Comparison of the mean (±SD) hatching rate of blue swimming crab spawners based on their carapace width in Bocana, Ilog, Negros Occidental.

respectively (Arshad et al. 2006; Kunsook et al. 2014). Studies on the reproductive performance of BSC using a net cage in natural waters are limited. Nevertheless, it was reported that the gonad index did not significantly correlate with water transparency, dissolved oxygen, or temperature (Batoy et al. 1988). It was also noted that the reproductive cycle of the species is significantly influenced by salinity, with higher salinities being more conducive to breeding (Batoy et al. 1988). Salinity and temperature were identified as the major ecological factors affecting the distribution of the crab (Kunsook et al. 2014). Although specific studies suggest that optimal hatching occurs at 30-32 ppt (Arshad et al. 2006), the findings in Bocana showed that the best hatching percentages occurred within the 25-30 ppt salinity range. This finding may be linked to local adaptation, given that all spawners in our study were obtained from the coastal waters of Bocana, likely demonstrating physiological acclimation to the existing local conditions (Ikhwanuddin et al. 2016). A prior investigation indicated elevated spawning percentages for *P. pelagicus* in their natural environment at salinities ranging from 30 to 35 ppt, implying a general inclination for populations to adjust to their surrounding environmental conditions (Ikhwanuddin et al. 2016).

In addition, the levels of water physicochemical in the current study did not affect the reproductive performance of BSC spawners (Lignot et al. 2000; Arshad et al 2006; Maheswarudu et al. 2008). The difficulty of seed production and water quality management in hatchery facilities in the tank needs technical expertise and state-of-the-art facilities (Cabacaba and Salamida 2015). The hatchery technique in this study, combined with a favorable coastal water site, may help simplify seed production in the country.

Influence of Spawner Size on Survival and Reproductive Output

The SR appears promising; however, the differences noted among groups necessitate additional investigations. Identifying the precise reasons for these differences necessitates a more thorough examination of the specific conditions affecting individual spawners, as the current study's framework did not facilitate a detailed analysis of mortality factors. Further extensive research is warranted to investigate the relationship between CW and SR. The encouraging SRs obtained from this innovative net cage system provide important preliminary data for the expanding field of BSC aquaculture technology and seed production. The findings indicate that the smaller size groups (S1 and S2) demonstrated lower responses in both spawner SR and HR, especially in comparison to the larger size groups (S3, S4, and S5). The lower SRs observed in the smaller BSC spawners could be linked to physiological stress experienced during the collection and initial handling processes (Wells 2009; Oniam et al. 2021). Consequently, the reduced HR in groups S1 and S2 is directly linked to their lower SR, where a complete lack of hatching success was noted in some individuals within these groups. The variations in HRs among different size classes are probably influenced by a complex interaction of biological and environmental factors. Particularly among these are nutritional status and energy allocation toward reproduction (Litulo 2004; Leme 2006; Quinitio and Parado-Estepa 2008). Access to adequate and nutritious food is essential, as it directly influences processes like oocyte development and yolk formation. The effective distribution of energy for gonadic growth, mainly in the period leading up to the peak spawning season, plays a vital role in determining both the quantity and quality of eggs produced, which in turn affects their eventual hatchability (Soundarapandian et al. 2013). Additionally, the reproductive history of the spawners, along with the possibility of multiple spawning events during the year, could influence the variations observed in their current reproductive performance (Dickinson et al. 2006; Darnell et al. 2009; Oniam and Arkronrat 2012).

The correlation between spawner size and reproductive output identified in this study aligns with observations made in other populations of *P. pelagicus*. For example, researchers have observed that female crabs with CW ranging from 91.0 to 95.9 mm displayed the lowest levels of fecundity and reproductive potential. In contrast, the largest females (>156.0 mm CW) showed the highest fecundity and productivity (Zairion et al. 2015). They also identified the 126.0-130.9 mm CW range as containing the most fecund individuals (Zairion et al. 2015). This is consistent with the principle that larger, more mature females generally exhibit enhanced reproductive capacity (De Lestang et al. 2003; Johnson et al. 2010).

The HR recorded in this study (51.89 ± 29.67%) aligns with findings from tank-based hatchery systems reported in existing literature. An HR of 54.01 ± 24.47% has been documented for pond-reared BSC spawners that were spawned in fiber tanks (Oniam et al. 2012). Comparable findings were observed with an average HR of 56.90 ± 22.60% in a tank hatchery that employed pond-reared BSC (Oniam and Taparhudee 2010). The significance of this comparability lies in the suggestion that the net cage system, even under natural conditions with possibly less control than indoor tank facilities, can attain

comparable levels of reproductive performance. The observed hatching response is linked to reduced salinity throughout the study (Ates et al. 2011; Ikhwanuddin et al. 2016). It may also be attributed to the high turbidity of water within the site (130.10 ± 57.12 NTU) (Table 2), which is caused by the presence of non-settling suspended matters consisting of planktonic organisms and coarsely distributed non-living substances with specific gravity lower than that of water, and exceedingly finely distributed non-living materials and minute organisms such as nannoplankton (Kutty 2020). Furthermore, female BSC tend to migrate to deeper, clearer water under fair-weather conditions (Mesa et al. 2018). Turbid waters can affect species composition (Lunt and Smee 2020) and the abundance of milkfish fry (Villalva and Dolorosa 2014). Hence, to potentially enhance the reproductive performance and HR of BSC in the net cage system, there is a need to implement measures to achieve lower turbidity in the area.

Spawner Size and Reproductive Output Correlation

Although considerable research has concentrated on the reproductive performance of BSC, especially concerning fecundity or batch fecundity in relation to their morphology (CW and BW), there is a lack of literature that examines hatching performance in natural water conditions. This research gap highlights the unique contribution of the current study. In their study, Oniam and Taparhudee (2010) utilized a fiber tank with regulated water quality and discovered that CW and BW were associated solely with fecundity, rather than HR. In contrast, the current study observed moderately positive correlations between HR and both BSC spawner CW and BW (Figures 8 and 9, with $r(23) = 0.614$, $P < 0.001$ for CW and $r(23) = 0.563$, $P < 0.003$ for BW). The findings of the current study demonstrate that larger BSC spawners consistently yield a higher number of zoea larvae. This result aligns with previous studies indicating that larger BSC spawners, possessing greater CW and BW, typically produce a greater quantity of eggs compared to smaller individuals (De Lestang et al. 2003; Kumar et al. 2003; Arshad et al. 2006; Hamasaki et al. 2006; Johnson et al. 2010; Oniam et al. 2012; Soundarapandian et al. 2013; Zairion et al. 2015).

An overall spawner survival rate of 80 ± 40.82% was recorded. The results demonstrate a distinct correlation between the size of spawners and their reproductive success: larger BSC spawners, especially those with CWs between 110 and 124.99 mm, consistently exhibited significantly higher HRs than their smaller counterparts (100 to 109.99 mm). The mean HR of 51.89 ± 29.67% achieved in this system is comparable to results from tank-based hatchery operations, which typically demand high technical skills and specialized infrastructure. Moreover, the linear and statistically significant correlations observed between the spawners' CW and BW and their HRs highlight that larger BSC spawners reliably produce higher hatching responses. The findings underscore the net cage system as a viable and practical method for improving BSC seed production.

Though this study provides valuable insights, it is important to acknowledge certain limitations. The sample size of n=5 per group is small, which may affect the statistical power of the ANOVA results. Although a robust post-hoc test (Tukey's HSD) was used to mitigate this, future studies should consider using a larger sample size to confirm these findings with greater statistical confidence. Furthermore, the study was conducted in a single location with specific water quality parameters, which may limit the generalizability of the results to other coastal areas. Future research should focus on replicating this study in different coastal areas and under varying environmental conditions to validate the effectiveness of the net cage hatchery system in diverse settings.

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GENERATIVE AI STATEMENT

The authors declare that NO generative AI technologies, such as Large Language Models, were used during the writing or editing of this manuscript.

ETHICAL CONSIDERATIONS

The experiment was conducted in strict compliance with national laws and institutional guidelines for animal care and use. The study protocol was reviewed and approved by the Central Philippines State University Research Ethics Review Committee.

DECLARATION OF COMPETING INTEREST

The authors assert that they have no identifiable competing financial interests or personal

affiliations that may have obstructed the work presented in this study.

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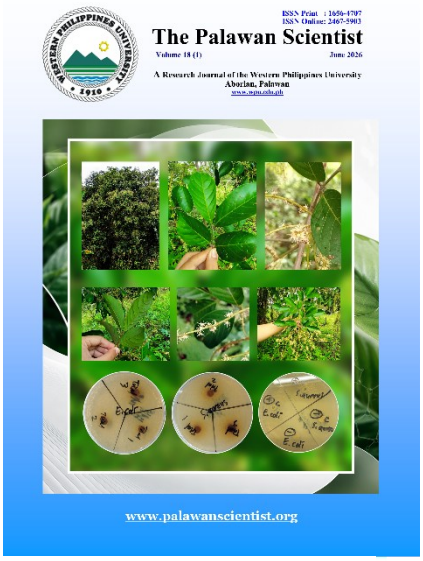

Notes on stranded dugong in Pavillion Island, Taytay, Palawan, Philippines

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ABSTRACT

A live stranding of a female dugong *Dugong dugon* (Müller, 1776) Palmer, 1895 occurred in Pavillion Island, Taytay Bay, Palawan on 11 July 2024. However, the weak animal died about two hours after it was spotted. It measured 2.75 m (straight total length) and weighed approximately 300 kg. Other than minor scratches on the skin, no signs of physical injury or evidence of fishery interaction were observed. During necropsy, nylon strands were found in the stomach extending to the small intestine. Although the cause of death remained inconclusive, this incident reflects the threats of discarded fishing nets and plastic pollution to large marine mammals like the dugong in Palawan waters.

Keywords: discarded fishing nets, marine mammal, plastic pollution, threatened species

On 11 July 2024, at around 0815 h, the Municipal Agriculture Office (MAO) received a report from one of its fish wardens in Pavillion Island (10°53'39.81"N, 119°37'8.73"E), Taytay Bay (Figure 1) about a stranded female dugong (*Dugong dugon*). The MAO immediately reported the incident to the Community Environment and Natural Resources Office (CENRO). Upon receiving the report, the CENRO team, together with representatives from the MAO, the Palawan Council for Sustainable Development (PCSD), and Community-Centred Conservation (C3) Philippines, responded to the incident two hours after the report.

Upon arrival at the stranding site at around 1000 h, the warden reported that the dugong was found alive at around 0600 h but showed signs of reduced energy and was too lethargic to escape from approaching threats. They found the animal swimming in the shallow sandy waters of the island, and they thought that it was drowning since it was not even responding when they were approaching. Thus, they decided to ask for intervention from the team of CENRO, PCSD and C3 Philippines, and before the team could intervene, the animal stopped breathing. The dugong was an adult female, measuring 2.75 m in straight total length and weighing approximately 300 kg (Table 1). Upon thorough external examination, a few minor scratches were found on the skin of the dugong's belly and flippers. There were no signs of lacerations, entanglement, or other injuries (Figure 2).

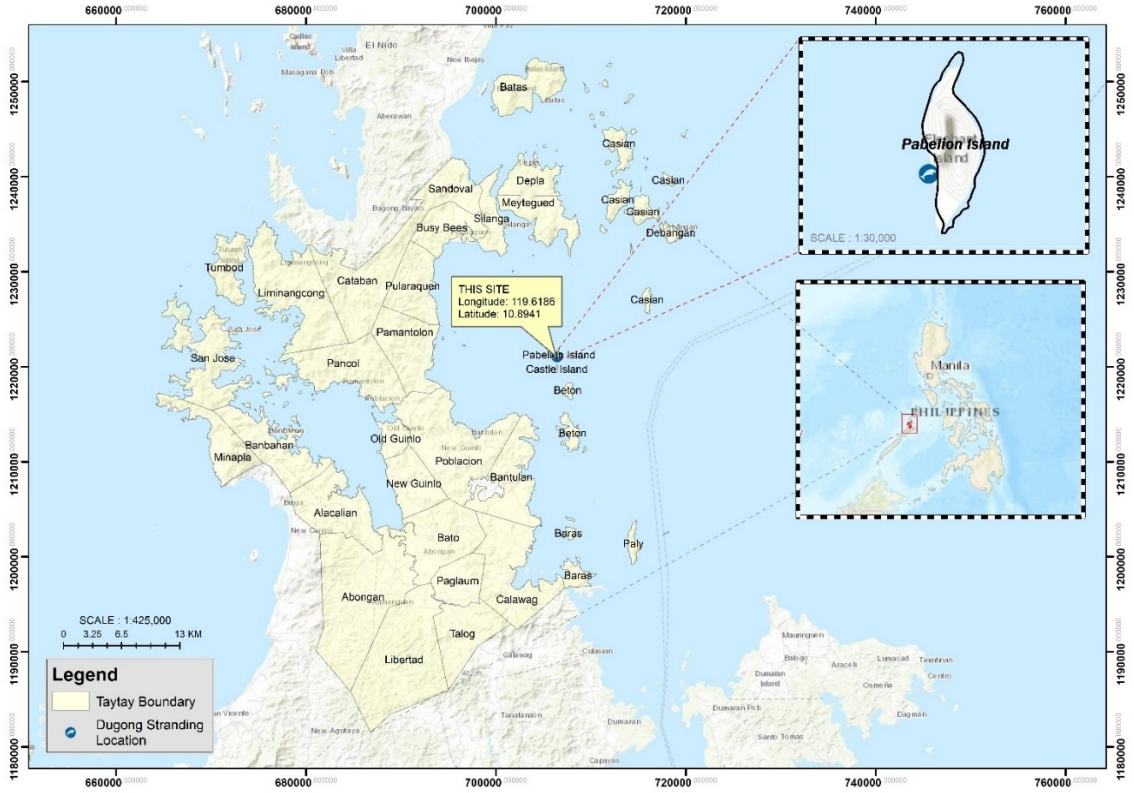

Figure 1. The location of the stranded dugong in Pavillion Island, Taytay, Palawan, Philippines.

Table 1. Some measurements of the stranded dugong in Pavillion Island, Taytay, Palawan, Philippines.

Measurement	
Straight length	275 cm
Weight	~300 kg
Tip of snout to tip of fluke	284 cm
Tip of snout to center of anus	184 cm
Tip of snout to center of genital aperture	174 cm
Tip of snout to center of umbilicus	120 cm
Tip of snout to anterior end of flipper	54 cm
Tip of snout to center of eye	23 cm
Tip of snout to external ear	41 cm
Center of eye to ear	26 cm
Distance between centers of eyes	31 cm
Center of eye to center of nostril	19 cm
Flipper length, anterior to tip	38 cm
Flipper length, axilla to tip	69 cm

A joint team led by representatives from MAO, C3 Philippines, PCSD, the Philippine Coast Guard (PCG), Large Marine Vertebrates (LAMAVE), and the CENRO – Taytay conducted a necropsy on 12 July 2024. The team found 0.50 mm nylon strands (Figure 3) in its stomach up to the small intestine. Observations of the internal lining of the stomach and small intestine showed an accumulation of nylon strands about 3 m long in the small intestine, causing rupture and inflammation, which probably contributed to the animal's system failure, weakening, and stranding.

Figure 2. The stranded dugong in Pavillion Island, Taytay, Palawan. A) Some minor scratches on the ventral side of the carcass; B) The dugong carcass being moved to the beach, C) Orientation and necropsy of the dugong carcass, D) Dugong head, side view.

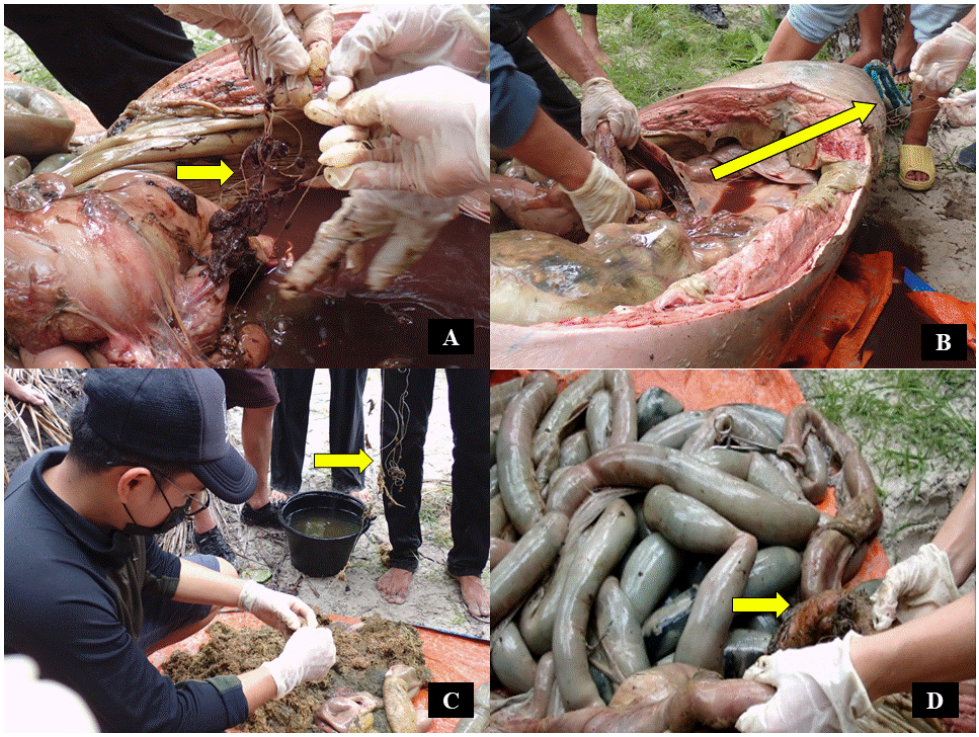

Figure 3. Nylon strands (A, B and C) and rupture (D) were found in the stomach and small intestines of the dugong.

The nylon strands could have been part of discarded fishing nets, such as those used in hook-and-line fishing, commonly used in the area to catch fish such as groupers. This can also be part of the ghost fishing gears left in the area that could have been discarded and had settled in a seagrass bed. As such, these nylon strands were then taken up by the dugong with the seagrass while foraging.

Discarded fishing nets are a common and increasingly serious problem in Palawan (Sajorne et al. 2021) and other parts of the world (Chellia et al. 2024), a leading cause of entanglement of large marine species globally (Perroca et al. 2024). More documentation of such incidents, not only with dugongs but with other marine wildlife, is badly needed to assess the effects and severity of this threat in the Philippines.

This incident is clear evidence that ingestion of plastics, particularly those from fishing gear, occurs in dugongs in their seagrass habitat. The consequences of such ingestion could be fatal to marine wildlife and will remain a threat if not addressed. More dugongs and other large marine species' lives remain at stake unless fishers discard their nets responsibly. Continued efforts are needed to address marine pollution to protect large marine species, including the dugong.

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This was conducted in line with the duty of the authors who are affiliated with their respective organizations.

GENERATIVE AI STATEMENT

Grammarly was employed solely to assist with grammar checking, language refinement, and the improvement of the manuscript's flow and readability.

ETHICAL CONSIDERATIONS

The intention was to rescue the dugong and return it safely to its natural habitat, but the dugong died before the team could take action.

CONFLICT OF INTEREST

No conflict of interest among authors.

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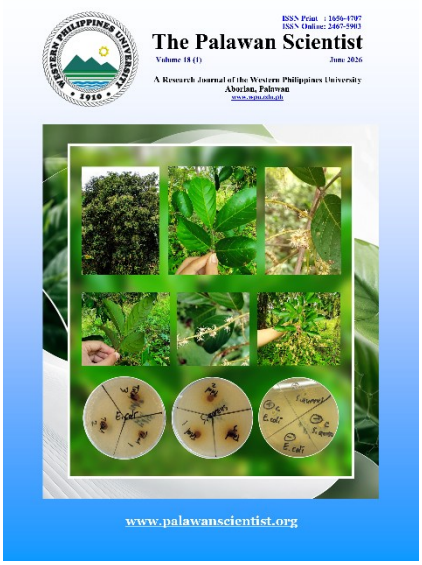

Abundance and diversity of gastropods in replanted and natural mangrove sites in Guang-guang, Dahican, Mati City, Philippines

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ABSTRACT

This study compared gastropod abundance and biodiversity in naturally grown and replanted mangroves in Guang-guang, Dahican, Mati, Philippines. To do this, a total of 12 transects (50 m) were laid perpendicular to the shore, and vertical quadrats were used for mangrove trees assessed every 5 m along the line transects. Gastropods were identified and counted on the mangrove tree leaves, trunks, and roots found in the natural and replanted sites. A total of 16 gastropod species from four families (Littorinidae, Neritidae, Trochidae, and Siphonariidae) were found. All 16 species were found in the natural sites, whereas four species—*Nerita albicilla* Linnaeus, 1758, *Nerita histrio* Linnaeus, 1758, *Siphonaria sirius* Pilsbry, 1894, and *Siphonaria atra* Quoy & Gaimard, 1833 —were absent from the replanted sites. Overall, more gastropods found in the replanted sites compared with the naturally grown sites (*t*-tests, df = 166, mean difference = -0.463, *t*-value = -7.42, *P* = 0.000) although the natural sites hosted more diverse species (*H'* = 2.41 vs 2.39). The results suggest the need to improve mangrove conservation, including planting more mangrove species suitable for these habitats as they act as refugia for gastropods, other invertebrates and marine organisms.

Keywords: arboreal gastropods, diversity, Guang-guang, mangrove fauna, Mati City

The mangrove ecosystem is highly valuable both for conservation and for providing ecological services (Bindiya et al. 2023; Galon et al. 2021). Mangroves can protect shorelines from tsunamis and tidal flooding, support the juveniles of invertebrates and finfish by providing habitat space and facilitate nutrient absorption and sediment filtration (Macusi and Tipudan 2021; Nallos and Macusi 2023). Moreover, the mangrove forest in Pujada Bay has been a site for human activities such as exploitation, trampling, fishing, educational field trips, seaside strolling, photographing, and field studies, which can disturb the habitat and reduce the population of finfish and gastropods of commercial importance (Deepananda and Macusi 2012; Onyena and Sam 2020). Mangrove-associated fauna are highly diverse and include gastropods, bivalves, crabs, finfish, reptiles and birds including seagrasses and various seaweed species (Albarico et al. 2020; Macusi et al. 2023; Seniel et al. 2024). In addition, gastropods play a vital ecological and economic role, providing essential ecosystem services and contributing to tourism and trade (Dewi et al. 2023). They serve as food for fish, birds, and humans and are valued in

export and domestic markets for products like crafts, pearls, and dyes (Alves et al. 2018). In India, they are used for ornamentation, medicine, and lime production (Ramachandra et al. 2012). Ecologically, gastropods act as filter feeders, helping to purify marine water and they also serve as biological indicators, detecting environmental stress and assessing habitat quality (El-Gendy et al. 2021). Their presence or absence can signal changes in aquatic ecosystems.

Gastropods can be abundant due to their high fecundity, reproductive capacities, and planktonic larvae, but they can also be affected by high gleaning pressure, especially among commercially harvested species (Maynawang et al. 2024). In a previous paper (Maynawang et al. 2024), the low diversity index (0.99) of gastropods in Guangguang indicated a possible overexploitation of gastropods in the area. Despite these estimates, accurately determining species richness remains a challenge due to factors such as habitat variability, sampling limitations, and taxonomic identification uncertainties. This study, therefore, aimed to identify, compare, and record the abundance of arboreal gastropods found in the roots, trunks and leaves of mangrove trees found in natural and replanted sites. The natural sites are composed of a mix of various Rhizophoraceae species (*Bruguiera cylindrica* (L.) Blume*, Rhizophora apiculata* Blume*, Rhizophora mucronata* Poir*, Rhizophora stylosa* Griff.) and Lythraceae (*Sonneratia alba* Sm.), with both young and older trees and lower density, while the replanted sites are mostly composed of Rhizophoraceae (*B. cylindrica, R. apiculata, R. mucronata, R. stylosa*), younger trees, and higher density. To do this, six transects were laid in the naturally grown sites, and another six transects were laid in the replanted sites, for a total of 12 transects measuring 50 m each, laid perpendicular to the coastline in Guang-guang, Barangay Dahican, Mati, Davao Oriental (see Figure 1). There were 30 m intervals between each transect, and individual trees found within 5 m intervals along the transect were included in the survey; once the tree was identified and measured, a vertical quadrat (2 m × 1 m) was used to sample gastropod species found in the roots, trunks, and leaves of the tree. The Guang-guang mangrove area is considered part of the National Integrated Protected Areas System (NIPAS), part of the Pujada Bay Protected Landscape/Seascape under Proclamation No. 451 dated 31 July 1994, issued by the Philippine government with an approximate area of 168 km^2 (Abreo et al. 2020).

In this study, only arboreal gastropods were counted and handpicked from leaves, trunks, and roots of mangrove trees in naturally grown and replanted mangrove sites sites during low tide. Representatives of different gastropods were preserved and placed in sample jars for later identification. We used the following references for assistance in field identification: Venkatesan et al. (2010); Khade and Mane (2012); Irma and Karina (2012); Picardal and Dolorosa (2014); and MolluscaBase, to guide our decisions in making correct identifications (MolluscaBase 2025). All count data from the field were first checked for their descriptive statistics (mean, median, mode) and graphical analyses of normality and variance before statistical analysis took place. We then used one-way ANOVA and a *t*-test to compare the count data from the two sites. Density was calculated using the common density formula ($D=n_i/A$) where n_i is the total number of individual species sampled and A is the total area of all transects sampled or area. For diversity calculation, we used the Shannon-Wiener diversity index.

In this study, eight littorinid species were identified in the mangrove forests, for example, on mangrove leaves (Figure 2 A, B, C, D). Littorinidae, a diverse family of intertidal gastropods, comprises small air-breathing snails that graze on algae and bacterial films in mangrove ecosystems (Sanpanich et al. 2004; Cob et al. 2012). Neritidae is another group of herbivorous gastropods that inhabit the intertidal zone and often form dense aggregations. Three *Nerita* species were found and recorded during the study (see Figure 2); they are known to be intertidal. Trochidae was represented by *Monodonta canalifera* Lamarck, 1816 and *Trochus maculatus* Linnaeus, 1758 (see Figure 2). These species are known to be frequently associated with mangrove and estuarine habitats, displaying diverse shell colors and morphological variations (Afsar et al. 2013; Masagca et al. 2010). Lastly, Siphonariidae, a group of air-breathing marine pulmonates, included *Siphonaria javanica* (Lamarck, 1819), *Siphonaria sirius* Pilsbry, 1894*,* and *Siphonaria atra* Quoy & Gaimard, 1833 (Figure 2). These species ascend mangrove roots at high tide, presumably to avoid submersion and predation by fish or crabs (Maia and Tanaka 2007; Reid 2001).

Table 1 shows the density and relative abundances of gastropods found in the replanted and naturally grown sites. Overall, *Littoraria ardouiniana* (Heude, 1885) (14.88%) had the highest relative abundance, followed by *Littoraria bengalensis* D. Reid, 2001 (12.72%) and *Littoraria pallescens* (R. A. Philippi, 1846) (10.57%) in the natural sites. In contrast, in the replanted sites, *Nerita undata* Linnaeus, 1758 (14.94%), *L. pallescens* (13.33%), and *L. bengalensis* (12.74%) were the most abundant. In addition, these three species were also found to have the highest density overall: *N. undata* (59 ind m^2), *L. pallescens* (52 ind m^2), and *L. bengalensis* (50 ind m^2). Their abundances in terms of location on tree leaves, trunks, and roots were also compared but showed no significant differences (df = 2, MS = 0.07987, $F = 0.30$, $P = 0.745$).

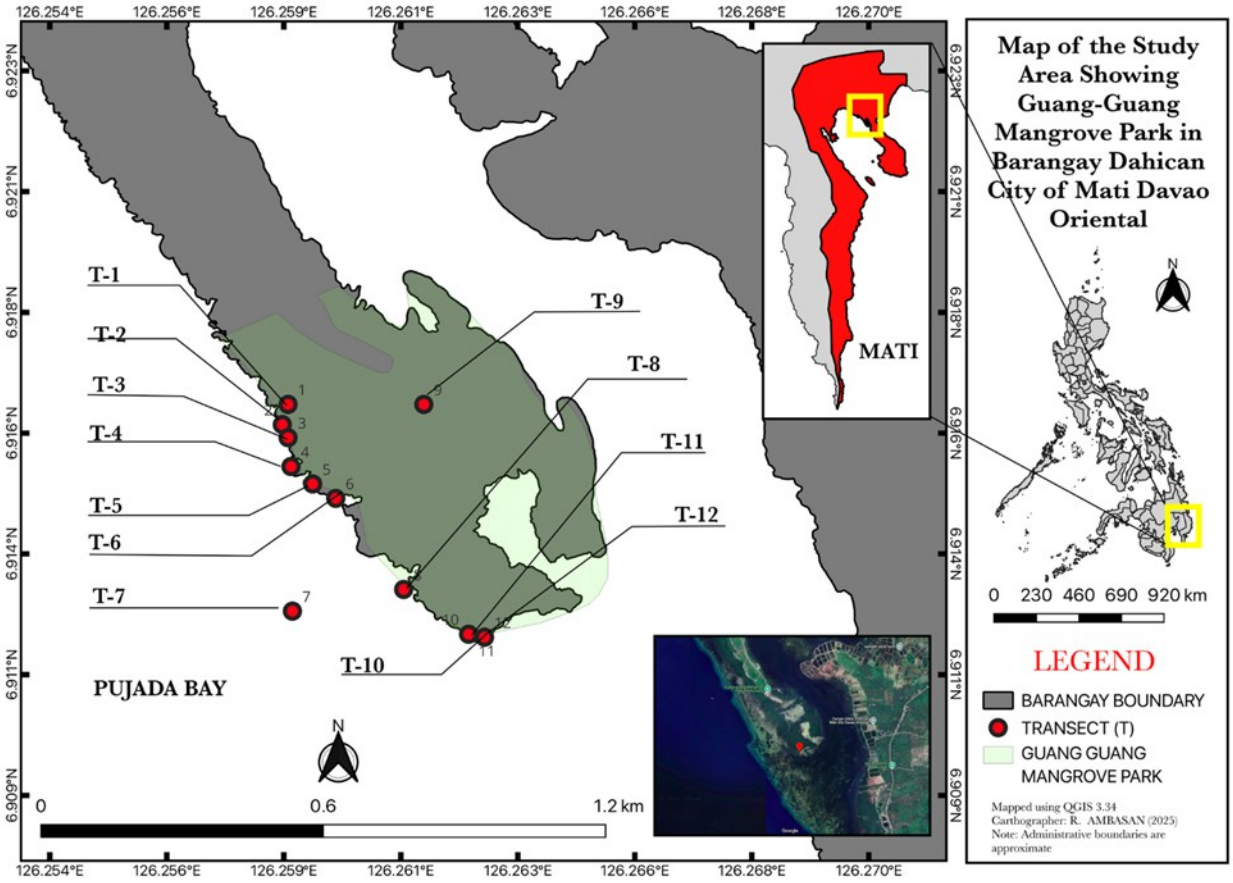

Figure 1. Map of the study area (T1 to T6 represent natural sites, while T7 to T12 represent the mangrove replanted sites) in Sitio Guangguang, Barangay Dahican, Mati City, Davao Oriental.

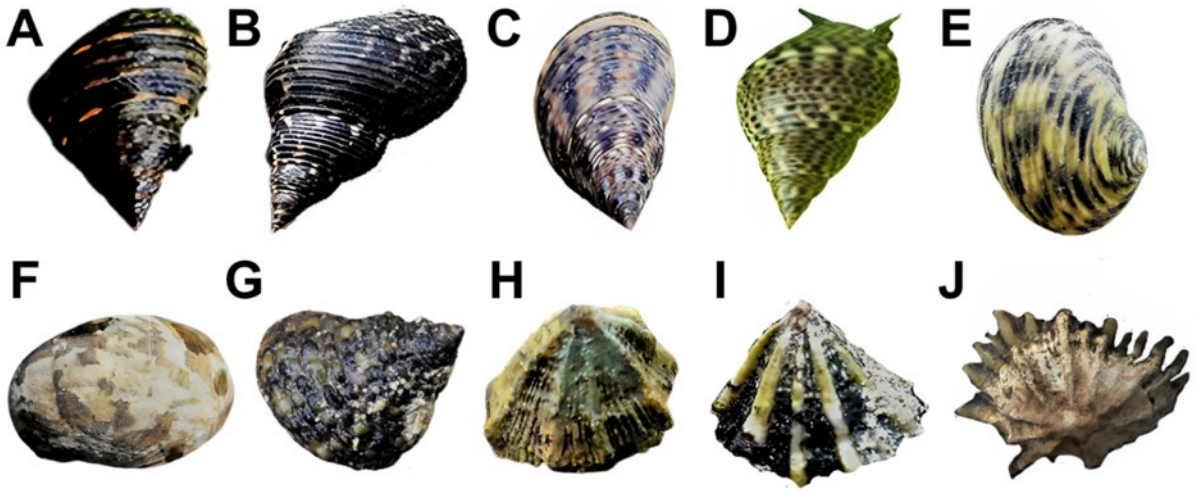

Figure 2. The various species found in the mangroves: *Littoraria bengalensis* (A), *Littoraria scabra* (B), *Littoraria carinefera* (C), and *Littoraria intermedia* (D), *Nerita undata* (E), *Nerita albicilla* (F), *Monodonta canalifera* (G), *Trochus maculatus* (H), *Siphonaria javanica* (I) and *Siphonaria sirius* (J).

A comparison of the different transects found in the replanted and naturally grown sites showed significant differences in the abundances of gastropods (df = 11, MS = 1.0154, $F = 6.52$, $P = 0.000$). The gastropod counts in the replanted sites were significantly higher compared to those in the other transects. In addition, a two-sample *t-test* of the sampling sites also showed highly significant differences between the replanted and natural sites (df = 166, mean difference = -0.463, *t*-value = -7.42, $P = 0.000$). The replanted sites had higher gastropod counts, 40 vs 18 in the natural sites, although the natural sites had higher biodiversity (H'=2.41 vs 2.39). Our results support the literature showing that mangrove ecosystems support diverse gastropod communities, which play a crucial role in nutrient cycling, organic matter decomposition, and energy transfer (Lopez and Levinton 2011). The abundance of gastropods found in the replanted sites indicates ecological adaptation and is probably attributable to a more favorable habitat structure for these gastropods, such as habitat complexity and heterogeneity provided by the root systems of *Rhizophora* sp., which protect them from predators. Although the natural stand had a lower number, the higher biodiversity can be attributed to the presence of more mangrove species located in the natural site. These differences may be due to vegetation composition, as Rhizophoraceae species dominate replanted areas and are more suitable for certain gastropod species, but other gastropods may find more favorable microhabitats in other mangrove species (Chen et al. 2021). Statistical analysis showed no significant difference in gastropod abundance among mangrove parts (leaves, trunks, and roots), suggesting uniform resource utilization. Overall, the findings of this study highlight the ecological importance of mangrove-associated gastropods and the need for conservation strategies to mitigate mangrove ecosystem disturbances.

Table 1. Density and relative abundance of gastropods found in naturally grown and replanted mangrove trees.

Species	Replanted		Natural	
	Density (ind $10m^2$)	Relative Abundance (%)	Density (ind $10m^2$)	Relative Abundance (%)
Littoraria ardouiniana (Heude, 1885)	20	5.20	31	14.88
Littoraria articulata (R. A. Philippi, 1846)	30	7.73	11	5.406
Littoraria bengalensis D. Reid, 2001	50	12.74	26	12.72
Littoraria carinifera (Menke, 1830)	30	7.65	8	3.54
Littoraria intermedia (R. A. Philippi, 1846)	36	9.18	15	7.24
Littoraria filosa (G. B. Sowerby I, 1832)	34	8.64	9	4.16
Littoraria pallescens (R. A. Philippi, 1846)	52	13.33	22	10.57
Littoraria scabra (Linnaeus, 1758)	15	3.82	19	8.89
Nerita undata Linnaeus, 1758	59	14.94	15	7.27
Monodonta canalifera Lamarck, 1816	33	8.39	14	6.84
Siphonaria javanica (Lamarck, 1819)	13	3.26	7	3.49
Trochus maculatus Linnaeus, 1758	20	5.10	16	7.75
Nerita histrio Linnaeus, 1758			7	3.40
Nerita albicilla Linnaeus, 1758			3	1.24
Siphonaria sirius Pilsbry, 1894			3	1.34
Siphonaria atra Quoy & Gaimard, 1833			3	1.24

FUNDING

This research did not receive any funding.

GENERATIVE AI STATEMENT

This study did not use any generative AI for its photos or editing.

ETHICAL CONSIDERATIONS

Permission for this research was obtained from the City Environment and Natural Resources Office (CENRO) of Mati City prior to the conduct of the study to ensure compliance with local regulations governing protected areas. The study involved only field observation and limited collection of gastropod

species for taxonomic identification, with no endangered or threatened species harmed.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests among the authors.

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Reading profiles revealed: Exploring the complexities of pre-service elementary education teachers' reading competencies

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ABSTRACT

This study explored the reading profiles and competencies of pre-service elementary education teachers, a topic that remains underexplored in the context of teacher education institutions in Palawan. Using a descriptive quantitative approach, 38 purposively sampled Bachelor of Elementary Education students participated. Most are female, aged 21–22, financially supported by their parents, and the majority belonged to the Tagalog and Cuyonon ethnic affiliations. Data were collected through surveys and reading assessments evaluating reading experience, attitude, efficacy, strategy, habits, challenges, reading rate, oral reading level, and comprehension. Findings revealed that participants' early reading experiences were grounded in their mother tongue, facilitated by family encouragement, despite the limited availability of formal materials in local languages. They also expressed positive attitudes toward reading. While they reported using effective strategies like re-reading and vocabulary lookup, their reading self-efficacy varied. Although many read for pleasure and engaged in writing activities, challenges such as poor concentration, limited summarization skills, and difficulties in connecting ideas persisted. Competency assessments showed a below-average reading rate (121–181 words per minute), high oral reading fluency, but notably low reading comprehension—71.05% of respondents did not meet expectations. This highlights a paradox: fluency does not equate to comprehension. In conclusion, there is an urgent need to strengthen pre-service teachers' reading comprehension through targeted instruction. Recommendations include enhancing reading self-efficacy, implementing reading strategy training, promoting consistent reading habits, and designing supportive interventions focused on fluency and deep understanding. Addressing these areas will better equip future educators to model and teach effective reading practices, ultimately improving literacy outcomes in their future classrooms.

Keywords: below-average reading rate, high oral reading skills, reading attitude, reading challenges, reading comprehension, pre-service teachers

INTRODUCTION

The Philippines struggles with low reading proficiency, ranking significantly below the global average in the Program for International Student Assessment (OECD 2019). This persistent underperformance highlights the need for comprehensive interventions to address the underlying factors contributing to low reading proficiency. Teacher training is a crucial area for improvement, as

teachers play a pivotal role in fostering literacy development (International Literacy Association 2025; NAEYC 2025; NCTE 2007). However, research indicates that inadequate teacher training in effective reading instruction methods is a major contributing factor to the issue (Concepcion 2024). Teachers often lack the necessary skills to diagnose and address reading difficulties effectively, leading to undetected reading problems among students (Concepcion 2024). Furthermore, research emphasizes the importance of teacher motivation and the use of diverse reading strategies to bridge learning gaps (Rosales 2024). Teachers significantly impact student reading performance by delivering high-quality, differentiated instruction tailored to individual needs (Neuman and Cunningham 2009). Personalized guidance and adaptive strategies empower students to assess their own learning, improving both their self-regulation and enthusiasm for academic growth (Van Der Boom and Jang 2018). Proficient instruction not only reduces reading struggles but also supports struggling readers, demanding that teachers possess extensive expertise in language, literacy development, and instructional strategies to meet the needs of all students (Moats 2009).

Pre-service teacher training in the Philippines emphasizes the development of effective reading instruction skills, crucial for promoting literacy among students. The programs vary, covering diverse teacher preparation aspects like practical experiences and theoretical knowledge. Pre-service teachers are taught about the reading process and strategies to help struggling readers, enhancing their teaching practices through reflection and adaptation (Liu et al. 2016). Some pre-service teachers initially have negative reading attitudes that can impede their progress, but some show improvements in attitudes and abilities over time (Vansteelandt et al. 2022). Structured training, including reading methods courses, significantly shapes pre-service teachers' understanding of reading, enhancing their instructional capabilities (Shaw 1994). Despite the focus on equipping pre-service teachers with essential reading skills, challenges such as varying attitudes towards reading and resource limitations persist. Addressing these issues is crucial for enhancing literacy outcomes in the classroom.

However, research on pre-service teachers' reading performance is limited, highlighting a need to improve their training to address the low reading performance of students (Pace Miles and Fletcher 2023; Concepcion 2024). Teachers often lack the necessary skills to diagnose and address reading difficulties effectively, leading to undetected reading problems among students (Concepcion 2024). While existing literature points out systemic issues such as poor national reading proficiency, inadequate teacher training, and variable pre-service education programs, there is limited research that directly examines and documents the reading competencies and challenges of pre-service teachers themselves—the very individuals expected to become literacy facilitators in classrooms.

This study is grounded in constructivist and sociocognitive theories of reading development, which view literacy as a product of individual experiences, cognitive engagement, and social context (Mol and Bus 2011; Price and Kalil 2019). Bandura's concept of self-efficacy further informs the analysis, particularly in exploring how beliefs about reading ability influence reading behaviors and outcomes. Reading self-efficacy refers to an individual's belief in their ability to perform reading-related tasks successfully, such as understanding, interpreting, and engaging with texts (Ciampa and Gallagher 2018). Reader-response theory also underpins the investigation of students' reading attitudes and habits, highlighting the importance of personal and cultural engagement with text. These perspectives guide the interpretation of data on reading attitudes, strategies, challenges, and competencies among future educators.

Therefore, this study aimed to identify the reading profile of elementary education pre-service teachers. The study sought to answer the following research: 1) What is the reading profile of the respondents in terms of reading experience, reading attitude, reading self-efficacy, reading strategy, reading habits, and reading challenges? 2) What is the reading competency of elementary education students in terms of reading rate, oral reading level, and reading comprehension? This study also highlighted the importance of early literacy development and the need for targeted interventions to improve reading outcomes for Filipino students. The ultimate goal was to foster a culture that values and prioritizes literacy development among pre-service teachers and, consequently, improved reading outcomes for students.

METHODS

Research Design

The researcher employed a descriptive quantitative approach to describe the reading profile of pre-service elementary education teachers at one of the universities in Palawan, Philippines that offers elementary education program.

Research Participants and Sampling Technique

There were 38 pre-service teachers specializing in elementary education who served as respondents for this study. Pre-service teachers in the Philippines were enrolled in teacher education programs, preparing to enter the teaching profession. Purposive convenience sampling was utilized to select these 38 pre-service teachers. This technique

facilitated participant selection due to limited availability, time constraints, and resource limitations. While this non-probability technique limits generalizability, it enabled the inclusion of a diverse cross-section of students from various academic levels within the research locale. The selected sample represented a substantial proportion of the pre-service teacher population enrolled in the university during the study period. Although the number of participants may not reflect the entire population of pre-service teachers in Palawan, the sample was adequate for the study's objective of conducting an initial exploration of reading competencies—a topic with limited prior research in the region. This limitation was acknowledged, and future research is recommended to include a larger and more randomized sample to strengthen external validity and broader applicability.

Table 1 shows the demographic profile of the respondents in terms of their age, sex, ethnic affiliation, and financial support. The respondents' age distribution predominantly was within the 21-22 years bracket (57.89%), aligning with typical tertiary education age ranges. Only a small percentage are aged 27-28 years (2.63%). Female made up the majority at 89.47%, while males constitute 10.53%. The largest ethnic group was Tagalog (36.84%), followed by Cuyonon (28.95%), and then Bisaya and Hiligaynon at 7.89% each. Financial support mainly came from parents (57.89%), with working students and scholarship recipients, each at 13.16%. Siblings and partners contribute (7.89%). Overall, the profile depicts predominantly female students in their early twenties, mostly supported by their parents, with Tagalog being the largest ethnic representation.

Table 1. Demographic profile of the respondents.

Profile	Frequency (f) N=38	Percentage (%)
Age		
19-20	9	23.68
21-22	**22**	**57.89**
23-24	3	7.89
25-26	3	7.89
27-28	1	2.63
Mean age	21	
Sex		
Male	4	10.53
Female	**34**	**89.47**
Ethnic Affiliation		
Bisaya	3	7.89
Cagayanen	2	5.26
Cuyonon	**11**	**28.95**
Hiligaynon	4	10.53
Muslim	4	10.53
Tagalog	**14**	**36.84**
Financial support		
working student	5	13.16
Scholarship	5	13.16
supported by parents	22	57.89
Siblings	3	7.89
husband/partner	3	7.89

Data Gathering Procedures

A survey method was employed, encompassing Likert scale reading profile covering reading experience, attitude, reading self-efficacy, strategies, habits, and challenges (adopted from Boakye 2017) and reading competency assessment (reading rate, oral reading level, and reading comprehension). The reading assessments used in this study were adapted from the Philippine Informal Reading Inventory Manual (DepEd 2018) for measuring oral reading fluency and comprehension, and from Custureri (2014) for reading rate classification. While these tools are guided by national benchmarks, they are not standardized tests but rather locally adapted diagnostic instruments widely used in Philippine basic education settings. The comprehension questions were constructed based on DepEd-aligned criteria to assess mastery levels. As such, these assessments served as diagnostic tools reflective of academic expectations rather than norm-referenced standardized measures.

To evaluate reading speed, fluency, and comprehension, three expository (non-fiction) passages were used, each ranging from 250 to 450 words. The texts were selected for their grade-level appropriateness and academic relevance, covering familiar topics such as environmental awareness, history, and basic science. These selections aimed to minimize content bias and support equitable assessment across participants.

During the oral reading assessment, participants read the passages aloud while the researcher recorded the time using a stopwatch and documented any miscues. This process enabled simultaneous measurement of both reading rate and oral fluency. For the comprehension assessment, the same passages were reread silently by the respondents, after which they answered multiple-choice questions designed to measure literal, inferential, and evaluative comprehension skills. Reading rate was computed using Custureri's (2014) formula, and comprehension scores were interpreted according to DepEd mastery standards. The overall data collection followed a systematic procedure using established tools and protocols to ensure consistency and reliability.

Data Analysis

Descriptive statistics including frequency counts, percentages, and means were applied with classifications ranging from Strongly Agree to Strongly Disagree. Computing oral reading scores involved a formula based on words read and miscues, and reading rate calculations used a formula integrating words read per minute. Comprehension scores were determined using a formula factoring correct answers as a percentage of total test items.

To analyze the reading competency level of the respondents, the researcher used three reading pieces that contained 250 to 450 words. To compute the oral reading score of the pre-service teachers, the researcher used the oral reading score formula (DepEd 2018):

$$\frac{Number\ of\ words\ -\ Number\ of\ Miscues}{Number\ of\ Words}\ \text{x}\ 100$$

The researcher used the reading speed classification to interpret the reading rate. With the formula:

$$\frac{Number\ of\ words\ read}{Number\ of\ Seconds\ Spent\ to\ Read}\ \text{x}\ 60\ (minute)$$

On the other hand, the student's comprehension scores were classified with the formula:

$$\frac{Number\ of\ Correct\ Answers}{Number\ of\ Test\ Items}\ \text{x}\ 100$$

RESULTS

Reading Profile of the Respondents

The reading profile that includes the reading experience, reading attitude, reading self-efficacy, reading strategy, reading habits, and reading challenges shapes the reading skills of elementary education students.

The respondents' reading experiences revealed a strong consensus on certain key aspects (Table 2) They strongly agree on developing foundational reading skills in their mother tongue, likely referring to informal literacy experiences at home rather than engagement with formal printed texts (4.21) and receiving encouragement from family members to read books (4.16). Additionally, reading for enjoyment during childhood also garnered agreement (4.03). However, there was disagreement regarding the teacher's emphasis on reading for knowledge (2.5). Respondents were uncertain about whether high school teachers encouraged reading novels (3.08) or promoted frequent visits to the library (3.13). Overall, the respondents positively evaluated their reading experiences, with a weighted mean of 3.64.

Table 2. Reading experiences of pre-service teachers.

No.	Statement	Mean	Description
1	I read books for enjoyment during my childhood.	4.03	Agree
2	I started reading between the ages of 5-6.	3.92	Agree
3	Before I could read for myself, members of my family used to read to me first.	4.03	Agree
4	I started reading in my mother tongue.	4.21	Strongly Agree
5	My family members used to encourage me to read a lot of books.	4.16	Agree
6	When I was a child, I always visited the library to read or to borrow books to read at home.	3.13	Uncertain
7	I used to read fictional stories per week when I was in high school.	3.87	Agree
8	My teachers in high school encourage me to read novels.	3.08	Uncertain
9	There are many books in our house.	3.47	Agree
10	My teacher emphasized that reading helps us to be more knowledgeable.	2.5	Disagree
Weighted Mean		3.64	Agree

The attitudes of respondents toward reading generally reflect a positive outlook, as shown in Table 3. There was strong agreement on the benefits of reading (4.76) and a high level of interest in engaging with compelling topics (4.39). However, some uncertainty was observed regarding their enjoyment of complex texts (3.13) and academic literature (3.03). Overall, the respondents demonstrated a favorable attitude toward reading, although there was some hesitancy when it comes to handling difficult texts and academic materials.

Regarding reading self-efficacy, Table 4 displays the respondents' confidence and belief in their reading abilities, particularly in sharing insights (3.95), completing reading tasks (3.61), and comprehending texts (3.55). However, there was uncertainty regarding answering questions (3.13) and summarizing key points (3.18). The overall weighted mean of 3.31 suggests respondents feel uncertain about their reading efficacy.

When it comes to reading strategies (Table 5), respondents place a strong emphasis on seeking the meanings of unfamiliar words (4.21) and frequently re-reading when facing difficulties in understanding (4.13). Additionally, they engage in strategies such as previewing content pages before reading (4.0), mentally summarizing key points (3.87), and utilizing tutorials for comprehension (3.84). These varied strategies yield an overall weighted mean of 3.79, reflecting the respondents' adaptive approach to reading challenges.

In Table 6, the reading habits of respondents show agreement in reading for pleasure (3.63), meeting academic requirements (3.47), and engaging in writing activities after reading (3.42). However, there was uncertainty regarding the frequency of book reading (2.53), novel consumption (2.79), interest in historical books (3.03), and visits to the library for course-related information (3.21). The weighted mean of 3.19 indicates an overall uncertainty in the respondents' reading habits.

Table 3. Attitude towards reading of pre-service teachers.

No.	Statement	Mean	Description
1	I always believed that reading was a good thing to do.	4.76	Strongly Agree
2	I have favorite subjects that I read about.	4.11	Agree
3	I enjoy reading when I know the plot.	3.92	Agree
4	Reading different books well will help me with my studies.	3.39	Agree
5	I do not enjoy reading if the story has difficult texts.	3.13	Uncertain
6	I feel there is too much to read in the literature course.	3.76	Agree
7	I find academic books difficult to read.	3.03	Uncertain
8	Reading helps me understand difficult topics.	4.45	Strongly Agree
9	If the assignment is interesting, I can read difficult book material.	3.82	Agree
10	If my instructor discusses something interesting that catches my attention, I will read more about it.	4.39	Strongly Agree
Weighted Mean		3.88	Agree

Table 4. Reading efficacy of pre-service teachers.

No.	Statement	Mean	Description
1	I think I can read well with understanding.	3.55	Agree
2	I read slowly and I have problems with understanding.	3.24	Uncertain
3	I can easily complete the reading assignments given to me.	3.61	Agree
4	I read slowly so it makes me tired and bored.	2.74	Uncertain
5	I have difficulty understanding words and sentences (50% or more) in my reading assignments.	3.16	Uncertain
6	I have often translated what I read into my dictionary before I understand.	3.5	Agree
7	I struggle to comprehend university-level texts, especially when they contain unfamiliar vocabulary.	3.03	Uncertain
8	I have difficulty extracting the main points from what I read.	3.18	Uncertain
9	When I read, I always share and explain what I understood with my friends and classmates.	3.95	Agree
10	I always find it difficult to answer questions based on readings assigned by teachers.	3.13	Uncertain
Weighted Mean		3.31	Uncertain

Table 5. Reading strategies of pre-service teachers.

No.	Statement	Mean	Description
1	I use a different reading approach when I read a novel compared to other types of texts.	3.39	Agree
2	Before I read a textbook, I look at its contents page.	4	Agree
3	The first thing I do when I see an unknown word is to look up the meaning.	4.21	Strongly Agree
4	I record new words and try to memorize them with their meanings.	3.61	Agree
5	I always take notes on important things in reading pieces.	3.61	Agree
6	When I am reading, I always underline or highlight parts that I think are important.	3.55	Agree
7	I use questions like why, what, and how to help me understand what I am reading better.	3.68	Agree
8	I always re-read the piece when I do not understand what I am reading.	4.13	Agree
9	I summarize the main ideas in my head as I read.	3.87	Agree
10	The tutorials helped me understand difficult concepts.	3.84	Agree
Weighted Mean		3.79	Agree

Table 6. Reading habits of pre-service teachers.

No.	Statement	Mean	Description
1	I read for pleasure.	3.63	Agree
2	I still read magazines and or newspapers.	3.24	Uncertain
3	I read at least one novel every month.	2.79	Uncertain
4	I always go to the university library to look for information related to my courses.	3.21	Uncertain
5	I always read my lecture notes.	3.37	Uncertain
6	I only read academic articles when I have an assignment.	3.47	Agree
7	I strictly follow my schedule in studying and reading.	3.24	Uncertain
8	I read a lot of historical books.	3.03	Uncertain
9	After reading, I started to write my story or reading piece.	3.42	Agree
10	I read 2-3 books a week.	2.53	Disagree
Weighted Mean		3.19	Uncertain

Finally, the respondents' reading challenges reveal both areas of concern and uncertainty, as shown in Table 7. They acknowledge difficulties with concentration (3.79), remembering or summarizing information (3.66), phonemic awareness, defined as the ability to recognize and manipulate individual sounds (phonemes) in spoken words (3.55), and connecting ideas within passages (3.47). On the other hand, uncertainties emerge in relation to punctuation (2.87), responding to questions (2.84), and applying textual content to personal experiences (2.79). The overall weighted mean of 3.36 signifies uncertainty in the respondents' reading challenges

Table 7. Reading challenges of pre-service teachers.

No.	Statement	Mean	Description
1	I have trouble sounding out words.	3.45	Agree
2	I have experienced confusion between letters and the sounds they represent.	3.55	Agree
3	I sometimes ignore punctuation and comma while reading.	2.87	Uncertain
4	I experience confusion about the meaning of words in sentences while reading.	3.71	Agree
5	I sometimes experience a lack of concentration during reading.	3.79	Agree
6	I have experienced the inability to connect ideas in a passage.	3.47	Agree
7	I have trouble remembering or summarizing what I have read.	3.66	Agree
8	I experience difficulty in connecting what I have read to my prior knowledge.	3.45	Agree
9	I have difficulty applying the content of a text to a personal experience.	2.79	Uncertain
10	I have difficulty answering questions after reading.	2.84	Uncertain
Weighted Mean		3.36	Uncertain

Reading Competency of the Respondents

The results of the reading rate assessment conducted among the respondents after providing ample time to read the materials are shown in Table 8. All participants were instructed to read the passages aloud during the reading rate assessment, while the researcher timed and recorded their reading durations. This consistent oral reading approach ensured uniform measurement across respondents, although it is acknowledged that reading aloud may result in slower reading rates compared to silent reading. The findings revealed that the majority of respondents (92.11%) demonstrated a reading rate ranging from 121 to 181 words per minute, categorizing them as below-average readers on Custureri's (2014) reading speed benchmarks. In contrast, a small percentage (7.89%) displayed a reading rate of 120 words per minute or less, indicating significantly reduced reading fluency, which may reflect difficulties in automatic word recognition or processing speed. The mean reading rate of the respondents was calculated at 139.36, indicating an overall below-average reading rate among the participants.

The oral reading level of students revealed that the majority (33 or 86.84%) were independent with 97-100 reading scores, while only 5 (13.16%) have a word reading score of 90-96, which was described as instructional level (Table 9). The mean score was 97.59 described as independent.

The reading comprehension assessment results for pre-service teachers (Table 10) focused on their grasp of the reading materials. Among the 38 participants, there was a varied distribution of scores: one achieved an Outstanding score (2.63%), while another attained a Very Satisfactory rating (2.63%). Additionally, four participants were classified as Satisfactory, and five as Fairly Satisfactory. However, the majority (71.05%) did not meet the expectations, indicating room for improvement. With an average score of 63.42, the group collectively fell below the anticipated comprehension level, pointing to the need for additional support or interventions to enhance their reading skills.

Table 8. Reading rate of pre-service teachers (Custureri 2014).

Words per minute	Frequency (f) N=38	Percentage (%)	Interpretation
350 to 500 wpm	-	-	Above Average
240 to 350 wpm	-	-	College Level
181 to 240 wpm	-	-	Average
121 to 181 wpm	35	92.11	Below Average
120 and below	3	7.89	Poor; Slow
Mean	**139.36**		**Below Average**

Table 9. Oral reading level of pre-service teachers (DepEd 2018).

Word Reading Score	Frequency (f) N=38	Percentage (%)	Interpretation
97-100	33	86.84	Independent
90-96	5	13.16	Instructional
89 and below			Frustration
Mean	**97.59**		**Independent**

Table 10. Reading comprehension level of pre-service teachers.

Comprehension Score	Frequency (f) N=38	Percentage (%)	Interpretation
90-100	1	2.63	Outstanding
85-89	1	2.63	Very Satisfactory
80-84	4	10.53	Satisfactory
75-79	5	13.16	Fairly Satisfactory
Below 79	27	71.05	Did Not Meet Expectations
Mean	**63.42**		**Did Not Meet Expectations**

DISCUSSION

Reading Profile of the Respondents

Reading experiences. This study revealed a positive outlook on childhood reading experiences among the respondents, emphasizing the importance of parental support and mother tongue instruction. Respondents frequently mentioned being encouraged by their families and having access to diverse reading materials such as storybooks, comic books, magazines, religious texts, and school textbooks, which contributed to the development of foundational

literacy skills. These findings are aligned with prior studies that underscore the importance of early exposure to reading in predicting academic achievement (Mol and Bus 2011; Price and Kalil 2019). Furthermore, access to school libraries—equipped with books, digital media, and educational technologies—plays a vital role in enhancing literacy skills, fostering information literacy, and promoting lifelong learning (Johnston-Rodriguez and Henning 2019; Ernst 2023).

Despite the positive experiences at home, the study revealed a tendency for students to underutilize library resources, which may hinder their readiness for academic-level reading in higher education. This underutilization may be attributed to limited library access, lack of awareness of available materials, or a preference for digital sources and mobile devices over traditional print resources. This points to a need for reimagined library services that reflect students' evolving interests and support the development of academic reading habits (Jayakumar and Tamilselvan 2016; Rahmi and Elviana 2022). Additionally, the discrepancy in teacher emphasis on the importance of reading for knowledge emphasizes the need for supportive pedagogical approaches to motivate students effectively (Brandt et al. 2021). Students engagement in activating prior knowledge before, during, and after reading—combined with collaborative learning—can enhance comprehension and deepen their ability to recognize connections between concepts (Hattan 2024).

These findings underscore the need to integrate early reading experiences into pre-service teacher education programs. Institutions must help future educators understand how personal reading backgrounds shape their teaching. At the same time, schools and libraries should design culturally responsive, engaging reading environments that bridge home and academic literacy. Strengthening these connections may empower pre-service teachers to sustain and model effective reading behaviors, ultimately fostering a deeper culture of literacy in classrooms.

Reading attitude. The study revealed pre-service elementary education teachers' positive attitude towards reading, emphasizing its importance and benefits. Their willingness to explore diverse topics and recognize reading as a tool for understanding complex subjects demonstrates a favorable disposition (Lim et al. 2015; Osisanwo 2016; Purnamasari et al. 2022). While they exhibit openness and a willingness to engage with various texts, reservations about challenging and academic materials suggest a potential lack of confidence in handling complex content (Fielding-Barnsley and Purdie 2005; Rahim 2013; Dania and Adha 2021).

Cultivating a positive attitude towards challenging texts can enhance critical thinking, comprehension, and academic performance among students. Educators can facilitate this by incorporating engaging reading materials and activities to foster a view of reading as an enjoyable and rewarding experience. Encouraging a growth mindset around reading—where struggle is viewed as part of learning—can encourage students to persist and thrive in more rigorous reading situations.

The findings highlighted the importance of developing instructional approaches that build confidence and resilience in reading academically complex texts. Teacher education programs should provide pre-service teachers with structured opportunities to engage deeply with scholarly materials, alongside guided strategies that demystify academic language and enhance reading self-efficacy. By empowering pre-service teachers to approach difficult texts with curiosity rather than apprehension, such programs can enable them to model perseverance, cultivate critical reading habits, and promote deeper literacy engagement in their future classrooms.

Reading self-efficacy. Pre-service elementary education teachers exhibited varying levels of reading self-efficacy, which reflect their confidence in performing reading tasks. This variation likely stems from a combination of factors, including prior reading experiences, academic backgrounds, and individual learning styles. Their beliefs about their reading abilities may also evolve as they progress through coursework and gain practical field experience (Ciampa and Gallagher 2018). The findings suggest a need for targeted instructional support to address the specific areas where confidence is low, particularly in handling complex academic texts and responding to comprehension questions.

Although many respondents expressed confidence in collaborative learning settings and completing assignments, they struggled with advanced comprehension and fluency—skills essential for academic success and effective teaching (Fang 2016; Willegems et al. 2017; Amendum et al. 2018). This calls for greater emphasis on building vocabulary, developing comprehension strategies, and fostering critical reading skills. Structured peer collaboration and guided reading discussions can further enhance students' engagement and self-assurance in academic reading tasks (Helfrich and Clark 2016).

These findings underscore the critical role of language educators in shaping reading self-efficacy through instructional design. Teacher education programs must move beyond basic literacy instruction and offer explicit training in metacognitive strategies, academic vocabulary, and scaffolded engagement with challenging texts. Embedding these components into coursework not only builds technical reading proficiency but also empowers pre-service teachers to trust their ability to navigate complex material. In

doing so, they are better prepared to model confident reading behaviors, instill a growth mindset in their future students, and support learners across varying literacy levels.

Reading strategies. The study revealed a strong understanding of reading strategies among respondents, evident in their utilization of various techniques like looking up unknown words and summarizing main ideas. This suggests that pre-service teachers are aware of effective reading approaches and are willing to employ them. Their positive responses to tutorials further emphasize the value of seeking assistance, reflecting a proactive approach to learning (Akyol and Ulusoy 2010; Clark 2016; Aşıkcan et al. 2018).

However, the effectiveness of these strategies may vary depending on individual differences such as learning styles, linguistic background, and academic preparedness. As such, further research is needed to examine how these factors influence the application and success of reading strategies among pre-service teachers. A deeper understanding of these variations can help educators refine instructional methods to meet diverse learner needs more effectively.

These findings underscore the need for teacher education programs to formalize strategy instruction, not only by introducing techniques but also by contextualizing when, how, and why they should be used. Language educators should embed metacognitive strategy training into reading pedagogy, enabling pre-service teachers to reflect on their reading processes and adjust their approaches to suit different text types and learning contexts. Developing flexible, strategic readers will better prepare pre-service teachers to scaffold reading for their own students, particularly in classrooms with diverse language and literacy backgrounds.

Reading habits. The findings indicated diverse reading habits among pre-service elementary education students, with positive inclinations towards pleasure reading and post-reading writing. This suggests that many students find reading enjoyable and are willing to engage with it for personal fulfilment. However, variability in engagement across different reading activities stems from a complex interplay of factors, including personal preferences, academic demands, and individual routines. This highlights a mix of intrinsic and extrinsic motivations driving their reading habits (Uzum and Alincak 2021; Neno et al. 2022; Wijayanti et al. 2022).

Yet, some respondents expressed uncertainty around reading frequency and library usage, possibly due to limited exposure to diverse genres or a lack of consistent reading routines. This indicates a need to provide varied, accessible, and relevant reading materials to support engagement across academic and personal contexts (Akçay 2017; Jaelani and Holisah 2021). Promoting a wider range of reading experiences can help pre-service teachers expand their literary horizons and deepen their connection with text.

These findings emphasized the role of teacher education programs in cultivating sustainable and reflective reading habits among future educators. Language educators should implement structured reading routines, genre exploration projects, and reading journals to foster habitual reading practices. Moreover, encouraging pre-service teachers to reflect on their own reading behaviors allows them to become more conscious readers—capable not only of improving their own literacy, but of intentionally modeling strong reading practices for their future students.

Reading challenges. The results revealed common reading challenges among pre-service teachers, including difficulty concentrating, summarizing, confusion with letter-sound relationships, and connecting ideas within a passage. These challenges pose significant obstacles to comprehension and learning, particularly in classrooms where teacher preparation is limited or learning environments are overcrowded. Such issues emphasize the need for targeted instructional support and the promotion of reflective reading practices to improve literacy outcomes (Kodi and Phindane 2022).

Additionally, uncertainties related to punctuation, responding to questions, and linking texts to personal experiences may result from varying cognitive and language proficiency levels. These challenges are particularly critical for educators teaching English Language Learners (ELLs), where effective instruction requires sensitivity to varied linguistic backgrounds and alignment with diverse cognitive processes (De Jong et al. 2013; Solikhah 2018).

These findings highlighted the urgent need for teacher education programs to embed diagnostic and remedial reading strategies into their training models. Language educators should equip pre-service teachers with tools to identify and respond to common reading difficulties—not only in their students but in themselves. This includes instruction in phonological awareness, summarization techniques, metacognitive strategies, and text-to-self connections. Furthermore, institutions must ensure that remedial supports such as reading clinics, peer tutoring, and strategy workshops are accessible to teacher candidates.

Reading Competency of the Respondents

Reading rate. The reading assessment revealed that a significant number of pre-service teachers have below-average reading rates, suggesting challenges in fluency and speed that may hinder their ability to process and comprehend text efficiently. These struggles are often associated with difficulties in concentration, summarization, and vocabulary acquisition (Washburn et al. 2011). Inadequate

exposure to diverse reading materials and limited opportunities for fluency-building practice may further contribute to these challenges, negatively affecting comprehension and overall academic performance (Macalister 2014). Individual differences in reading habits and cognitive engagement also influence fluency levels (Aybala 2017).

Importantly, these limitations have practical implications for future teaching practice, as reading fluency is critical in delivering instruction, modeling reading strategies, and supporting learners—especially those with reading difficulties (Meeks et al. 2016). Fluency is also essential for effectively teaching ELLs where pacing, prosody, and clarity play key roles in comprehension (Dixon et al. 2014; Calet et al. 2017).

These findings underscore the necessity for teacher education programs to integrate systematic fluency development into their literacy training. Language educators should incorporate guided repeated reading, performance-based reading (e.g., reader's theater), and speed-reading strategies as part of instructional coursework. Additionally, pre-service teachers should be provided with formative assessments and individualized feedback to monitor their progress and address fluency deficits early. Enhancing reading rate not only supports academic success but also ensures that pre-service teachers are well-prepared to serve as fluent, confident reading models for their future students.

Oral reading level. The oral reading level of pre-service teachers that was assessed through miscue analysis, reveals high proficiency, indicating fluency and confidence in vocalizing written text. This skill is fundamental to effective teaching, particularly in modeling proper pronunciation, phrasing, and reading flow for students (Dennis et al. 2016). However, despite strong oral performance, the disparity between fluent oral reading and slower silent reading rates highlights the need for balance between fluency and comprehension. Oral reading may mask underlying comprehension challenges, making it important to analyze both expressive ability and cognitive understanding (Albertini and Mayer 2011; Latham Keh 2016).

Those classified as independent readers, scoring between 97–100, exhibit strong fluency, which can support better comprehension outcomes. Yet, students scoring at the instructional level may still require targeted support to develop decoding, vocabulary, and automaticity, reinforcing the need for differentiated fluency instruction (Badawy 2024). Additionally, the assessment method's focus on miscues overlooks critical components such as intonation, pacing, and dialectal variation, all of which impact the effectiveness of oral reading.

These findings highlighted the importance of broadening oral reading assessments in teacher education to include prosody, expression, and clarity—skills that are central to engaging classroom reading. Language educators should implement pronunciation training, expressive reading activities (e.g., poetry recitation, reader's theater), and peer feedback sessions to help pre-service teachers refine their delivery. Furthermore, addressing dialect-related articulation challenges through culturally responsive instruction can empower future teachers to read confidently and authentically model fluent, expressive reading that resonates with diverse learners.

Reading comprehension check. The comprehension assessment uncovered a range of scores among pre-service teachers, with most falling short of expected proficiency levels. Notably, 71.05% scored below 79, indicating inadequate comprehension. This aligns with Canoy and Loquias' (2022) study, revealing similar challenges among students in achieving reading comprehension standards. The emphasis on reading quantity over quality in some educational settings may hinder comprehension (Dickens and Meisinger 2016). While oral reading skills may excel, Trainin et al. (2015) note that comprehension levels can vary between oral and silent reading. This discrepancy suggests a need for balanced reading practices to foster comprehensive understanding.

Limited vocabulary and background knowledge can impede comprehension for pre-service teachers, impacting their ability to derive meaning from texts. Benevides and Peterson (2010) link higher comprehension scores to reading habits, early exposure to enjoyable reading, and frequent library visits. Enhancing reading comprehension demands a collaborative approach among researchers, educators, and policymakers, emphasizing the development of background knowledge, vocabulary, inferencing abilities, and comprehension monitoring skills throughout learners' educational journey (Elleman and Oslund 2019). Improving guidance on comprehensive reading strategies and promoting diverse text exposure can enhance pre-service teachers' comprehension skills.

The mean score of 63.42 underscores the group's overall struggle to meet expected reading comprehension levels, signalling a need to enhance the pre-service teachers' skills for effective teaching. The findings indicate the necessity for targeted support and interventions to boost reading comprehension abilities through innovative resources, training, and strategies. Reading comprehension involves complex cognitive skills, including word recognition and inference-making (Corso et al. 2024). Interventions targeting executive functions have demonstrated significant improvements in reading performance, particularly for students from low socioeconomic backgrounds (Corso et al. 2024). Programs that incorporate comprehension strategies, such as prediction and summarization, have

been effective. For instance, a study showed that 24 sessions of targeted reading comprehension intervention improved both fluency and comprehension, especially in narrative texts (Yang and Kim 2023).

Furthermore, research has shown that repeated reading aloud and immediate questioning post-reading significantly enhance comprehension levels among seventh-grade students, with notable progress in their reading abilities (Caabay et al. 2024). These findings underscore the urgency for teacher education programs to implement structured, research-based interventions that strengthen reading comprehension. Language educators must move beyond surface-level fluency instruction by integrating explicit strategy training—including metacognitive reflection, vocabulary development, and content-based reading across genres. Embedding practices such as reciprocal teaching, repeated reading with feedback, and scaffolded questioning into coursework can equip pre-service teachers with the cognitive and pedagogical tools needed for deep, reflective reading.

The study highlights a notable paradox: while many pre-service elementary education teachers demonstrate strong oral fluency, most struggle with reading rate and comprehension. This raises concerns about whether current programs adequately support the higher-order demands of reading, such as analysis, inference, and critical reflection. These gaps may stem from early reading experiences focused more on speed and accuracy than meaning-making. Without adequate exposure to academic texts or instruction in comprehension strategies, fluency may mask significant weaknesses in understanding.

This disconnect has critical implications for teacher preparation. Pre-service teachers who struggle with comprehension may lack the confidence and ability to effectively support student learning, potentially undermining literacy development in line with Sustainable Development Goal 4: Quality Education. Addressing these challenges requires a balanced, reflective approach to reading instruction—one that fosters fluency, deep comprehension, and lifelong learning habits.

To this end, teacher education programs should implement evidence-based strategies that build vocabulary, strengthen text analysis, and enhance metacognitive skills. Personalized instruction and diversified assessments—including evaluations of silent reading and prosody—are essential for meeting the needs of diverse learners. Reflective practices such as reading journals and peer discussions can further promote self-awareness and instructional readiness.

Although limited by a small sample size and simplified assessment tools, this study emphasizes the need for robust, inclusive literacy training. Future educators should be equipped with both technical and critical reading skills to ensure that they are well prepared to foster meaningful, lifelong learning in their classrooms.

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GENERATIVE AI STATEMENT

The researcher used generative artificial intelligence (AI) tools, including Grammarly and QuillBot, solely for language refinement and editing to improve clarity and readability. All intellectual content, data analysis, interpretations, and conclusions remain the original work of the authors, who take full responsibility for the integrity of the manuscript.

ETHICAL CONSIDERATIONS

In this study, the researcher further explained to the respondents the purpose of the study and confidentiality procedures, and requested their signatures of consent. Respondents' names remained anonymous and interview transcripts were assigned a random number as well as secure in the personal laptop with a password. The researcher assured the respondents to not engage in deception about the nature of the study nor share personal experiences with respondents during the survey.

DECLARATION OF COMPETING INTEREST

The author declares no competing interests.

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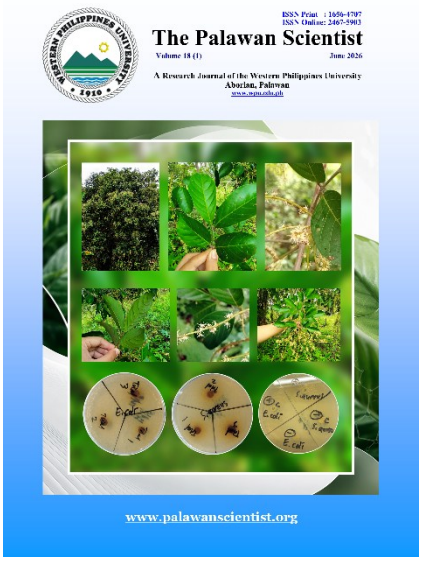

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Anti-inflammatory, antioxidant, and phytochemical profiles of pineapple (*Ananas comosus* L. var. MD2) juice and wine produced through *Saccharomyces cerevisiae* (Desm.) Meyen batch fermentation

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ABSTRACT

This study examined the anti-inflammatory and antioxidant activities, total phenolic content (TPC), and high-performance thin-layer chromatography (HPTLC) profile of fresh pineapple (*Ananas comosus* L. var. MD2) juice, wine, and aged wine produced through *Saccharomyces cerevisiae* (Desm.) Meyen batch fermentation to evaluate their nutraceutical potential. Pineapple wine was produced by fermenting pasteurized juice at 20˚, 25˚, and 30˚ Brix under anaerobic conditions for three weeks, followed by a one-month maturation period for aged wine. Anti-inflammatory activity, total antioxidant capacity (TAC), and TPC were determined using the fluorescence cyclooxygenase (COX) inhibition assay, phosphomolybdenum method, and Folin–Ciocalteu assay, respectively. The HPTLC analysis was performed using a Chemie-Erzeugnisse und Adsorptionstechnik Muttenz AG (CAMAG) system, and consumer acceptability was assessed using a 9-point hedonic scale. Fermentation reduced juice acidity by 4–17% and yielded alcohol concentrations ranging from 5.17–11.8%. Both juice and wine inhibited COX-1 and COX-2 by over 50%, indicating significant anti-inflammatory activity. The TAC decreased by 32% from juice (2241.03 ± 55.22 mg AAE/L) to wine (1527.82 ± 92.52 mg AAE/L), and by 37% to aged wine (1416.03 ± 12.70 mg AAE/L). Similarly, TPC declined by 2% from juice (430.10 ± 5.08 mg GAE/L) to wine (422.57 ± 5.95 mg GAE/L) and by 19% to aged wine (348.30 ± 5.92 mg GAE/L). The HPTLC analysis revealed distinct chromatographic profiles in wine and aged wine, indicating the formation of metabolites. Among the aged wines, the 30˚ Brix sample received the highest sensory score (7.75). Therefore, fermentation preserves pineapple bioactivity and may enhance its nutraceutical value through the generation of unique metabolites.

Keywords: 9-point hedonic scale, bioactive compounds, cyclooxygenase inhibition, nutraceutical potential, phytochemicals

INTRODUCTION

Pineapple (*Ananas comosus* L.) is a highly valued tropical fruit and the most economically important species of the Bromeliaceae family. It is widely consumed in various forms, including fresh, cooked, juiced, or preserved (Adebayo-Tayo and Akpeji 2016). Known for its pleasant aroma and flavor,

pineapple is also a rich source of phytochemicals, dietary fiber, and essential nutrients, such as vitamins C, B1, and B6, and minerals including magnesium, calcium, and copper (Palachum et al. 2021). Pineapple also stands out for its remarkable health benefits, particularly its potent anti-inflammatory and antioxidant properties (Joy 2015). Bromelain, a proteolytic enzyme complex found predominantly in pineapple stems and juice, plays a pivotal role in modulating inflammation and has been extensively studied for its therapeutic effects (Varilla et al. 2021). Regular consumption of pineapple has also been shown to reduce hypercholesterolemia-induced cardiac lipid peroxidation and suppress pro-inflammatory responses *in vivo* (Seenak et al. 2021).

The Philippines is the world's second-largest pineapple producer after Costa Rica, with an estimated total export of 692,365 MT in 2024 (Business World 2025). Northern Mindanao led pineapple production in the Philippines in 2023, contributing 391.16 thousand MT to the country's production (PSA 2023). However, like many other fruits, pineapples are highly perishable due to their high moisture and nutrient content, which lead to significant post-harvest losses ranging from 20% to 50% (Kasso and Bekele 2018). The average post-harvest loss for pineapples in the Philippines is among the highest among agricultural products, at 30-40% (Mopera 2016). These losses can occur at various stages, including on-farm handling, transportation, storage, retail, processing, and at the consumer level. Therefore, preservation methods are considered essential to reduce post-harvest losses in the pineapple industry. Fermentation has been an effective method for food and beverage preservation for millennia (Ross et al. 2002). Fermenting fruit juices, such as pineapple, not only preserves the fruit but also increases its value by transforming it into new products like wine, which has a longer shelf life (Boondaeng et al. 2021).

Although pineapple is ideal for fermentation (Chanprasartsuk et al. 2010), and there is growing interest in pineapple wine production for local and export markets, limited research has examined its anti-inflammatory activity and high-performance thin-layer chromatography (HPTLC) profile. Previous studies mainly focused on its antioxidant properties and phenolic composition in fresh fruit or juice (Adeboyejo et al. 2018; Candrawinata et al. 2012), but little is known about whether fermentation and aging alter these bioactivities. Additionally, wine fermentation might concentrate or produce new metabolites that enhance its nutraceutical potential, creating an opportunity to set pineapple wine apart from other fruit wines. Such discoveries could increase the market appeal of pineapple wine, and help reduce post-harvest losses while enhancing the competitiveness of the Philippine pineapple industry.

This study aimed to examine the physicochemical properties (pH and total soluble solids), anti-inflammatory activity, total antioxidant capacity, total phenolic content, and HPTLC profile of fresh pineapple (*A. comosus* L. var. MD2) juice, wine, and aged wine produced through *S. cerevisiae* (Desm.) Meyen batch fermentation. It also sought to assess the consumer acceptability of the aged pineapple wine. Overall, the study provided a comprehensive evaluation of the biochemical properties, phytochemical profile, and potential health benefits of pineapple wine produced via *S. cerevisiae* batch fermentation.

METHODS

Pineapple Juice Extraction and Preparation

The fully ripe pineapple fruits (*A. comosus* var. MD2) were obtained from Wao, Lanao del Sur. Fruits were selected based on uniform size, yellow external peel color, and high sweetness aroma as indicators of ripeness. This ensured the homogeneity of the samples and minimized variability due to the maturity stage. The fruits were then washed with running tap water, peeled, and sliced into pieces. The juice was extracted using a high-quality kitchen juicer (Koii KMK-168) and filtered through filter paper to remove solid particles. It was then transferred to glass bottles with water valves to serve as fermentation containers. The total soluble solids (TSS) of the extracted juice were measured using a refractometer. To standardize the sugar content, granulated white sugar was added to adjust the TSS to fixed levels of 20˚, 25˚, and 30˚ Brix. This standardization allowed for controlled comparison of fermentation performance and product quality across treatments. Finally, the juice was pasteurized at 83˚C for 3 min and allowed to cool to approximately 50˚C before the addition of the yeast culture (Qi et al. 2017).

Wine Fermentation Set-up

A total of 2.5 g of active dry yeast (*S. cerevisiae*) was added to 500 mL of the pasteurized juice to initiate the fermentation. The mixture was stirred thoroughly. A control setup in which the juice underwent the same preparation steps, including sugar content adjustments, but without the addition of yeast, was also prepared. Fermentation was carried out for three weeks under anaerobic conditions using a fermentation valve to allow gas release while preventing air entry. After 3 weeks, the sediments were removed, and the supernatant was transferred to another bottle. Fermentation was stopped using sodium metabisulfite (1.32 mL/L wine). The resulting wine was then aged for one month.

Physicochemical Analysis (pH and total soluble solids)

The physicochemical parameters, such as pH and TSS of fresh juice, wine, and aged wine, were determined using a digital pH meter (HANNA HI6221) and a hand refractometer (THE01502), respectively. The alcohol content of the aged wine was determined using an ebulliometer (LDS Ref. 160250D). The alcohol contents of the 20˚ and 25˚ Brix control samples were not measured due to the limited sample volume after processing. Monitoring pH, TSS, and alcohol content provided key indicators of fermentation progress, acidity changes, and ethanol yield, which directly affected product quality and stability.

Determination of Anti-Inflammatory Activity of Fresh Pineapple Juice and Wine

The assay followed the methodology described by Bonner and Fry (2012). It was chosen to evaluate the anti-inflammatory potential of pineapple juice and wines by measuring their ability to inhibit cyclooxygenase enzymes, especially cyclooxygenase (COX)-2, which is a key enzyme involved in inflammation. First, 5,184 μL of 100 mM Tris buffer (pH 8) was transferred to a clean vial, followed by the addition of 96 μL of COX-2 and COX-1 enzymes (250 *u*/mL each) and 480 μL of 20 μM hemin to prepare the enzyme-cofactor solution. Next, 120 μL of this enzyme-cofactor solution was pipetted into each well of a 96-well microplate prefilled with 50 μL of 100 mM Tris buffer. To initiate the reaction, 10 μL of the test samples (diluted in ethanol to final concentrations of 50 mL/L and 100 mL/L) were added to the wells. Indomethacin (4 mM in 100% DMSO) served as a positive control. The plates were incubated at 25˚C for 15 min, after which 10 μL of 200 μM Amplex Red and 10 μL of 2000 μM arachidonic acid were added to each well. The solutions were mixed thoroughly and purged with nitrogen gas to remove oxygen. Fluorescence readings were taken every 12 s for 3 min using a CLARIOstar® microplate reader (BMG LABTECH) set at an excitation wavelength of 535 nm and an emission wavelength of 590 nm. The COX inhibitory activity was quantified based on the relative change in the slope of fluorescence intensity over time.

The positive control and the percentage inhibition of the samples were calculated using the average slope of each replicate using the following formula:

$$\% \text{ Inhibitory Activity} = \frac{Slope\ uninhibited - Slope\ inhibited}{Slope\ unhinhibited} \text{ x } 100\%$$

where $Slope_{uninhibited}$ represents the slope of the fluorescence vs. time plot for the negative control group, while $Slope_{inhibited}$ refers to the slope of the fluorescence vs. time plot for the samples or positive control (Ang et al. 2022).

Determination of Total Antioxidant Capacity (TAC) of Fresh Pineapple Juice, Wine, and Aged Wine

The TAC of the pineapple juice, wine, and aged wine samples was evaluated using the phosphomolybdenum method, following the procedure described by Prieto et al. (1999). This method was used as a broad measure of overall antioxidant capacity, indicating the samples' ability to donate electrons and potentially reduce oxidative stress. Briefly, 100 μL of test samples (diluted in ethanol to a final concentration of 50 mL/L) were mixed with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated at 95ºC for 90 min. Then, 200 μL of the resulting solutions were transferred to the designated microplate wells in quadruplicate. The absorbance of the solutions was measured at 695 nm using a Spectramax 250 Microplate Reader against a blank after cooling to room temperature. The antioxidant capacity was expressed as milligrams of ascorbic acid equivalents per liter (mg AAE/L), based on a calibration curve generated using ascorbic acid.

Quantification of Total Phenolic Content (TPC) of Fresh Pineapple Juice, Wine, and Aged Wine

The TPC of the pineapple juice, wine, and aged wine samples was quantified using the Folin–Ciocalteu colorimetric method described by Ainsworth and Gillespie (2007). Phenolics are major contributors to antioxidant and anti-inflammatory effects in fruits and wines; therefore, quantifying TPC provides a direct measure of their nutraceutical potential. Briefly, 200 μL test samples (125 mL/L) were mixed with 200 μL of 10% of Folin-Ciocalteu reagent in a microcentrifuge tube and allowed to stand for 5 min. Subsequently, 800 μL of 10% sodium carbonate was added, and the mixture was incubated at room temperature for 30 min. The reaction mixture was centrifuged at 11,000 rpm for 3 min. Then, 200 μL of the resulting solution was transferred to the designated microplate wells in quadruplicate. The absorbance was measured at 750 nm using a Spectramax 250 Microplate Reader. The TPC was expressed as milligrams of gallic acid equivalents per liter (mg GAE/L), based on a calibration curve generated using gallic acid.

HPTLC Analysis of Fresh Pineapple Juice, Wine, and Aged Wine

An HPTLC system (CAMAG or Chemie-Erzeugnisse und Adsorptionstechnik Muttenz AG, Switzerland), equipped with an automatic TLC Sampler ATS 4, Automatic Developing Chamber ADC 2, Scanner 4, TLC Visualizer, Immersion Device 3, Plate Heater, and visionCATS 2.5 software, was used for analysis. Four (4) μL of test samples were applied onto an HPTLC aluminum-backed plate (silica

gel 60 F254; dimensions 20 × 10 cm; sourced from Merck) under a stream of nitrogen. Following application, the plate was developed in a pre-saturated twin-trough glass chamber (20 × 10 cm) maintained at a relative humidity of 33%. The mobile phase consisted of ethyl acetate, formic acid, and water (80:10:10). The chromatogram was visualized under both white light and UV light at wavelengths of 254 nm and 366 nm. Subsequently, the plate was immersed in a natural product (NP) reagent (2-aminoethyl diphenylborinate) (immersion speed: 5 cm/s; dwell time: 1 s), air-dried for 5 min under a fume hood, and visualized again under UV light at 366 nm (Ang et al. 2022; Jug et al. 2018). The HPTLC profiling enabled the detection and tentative identification of phenolic compounds, providing insight into the compositional changes and formation of novel metabolites during fermentation and aging.

Sensory Evaluation of Aged Pineapple Wine

The sensory evaluation for the aged wine was conducted in triplicate by a panel of 15 randomly selected and trained Bachelor of Science in Food Technology graduates from Central Mindanao University. The evaluation assessed the descriptors and acceptability of attributes, including color, aroma, taste, and overall acceptability of the wine (Boondaeng et al. 2021), utilizing a 9-point hedonic scale as follows: 1 - dislike extremely, 2 - dislike very much, 3 - dislike moderately, 4 - dislike slightly, 5 - neither like nor dislike, 6 - like slightly, 7 - like moderately, 8 - like very much, and 9 - extremely like.

Statistical Analysis

The effects of fermentation on TSS over time were analyzed using a two-way analysis of variance (ANOVA) to evaluate the main effects of time and Brix levels and their interaction. A mixed-effects model (Type III Wald F tests) with the Kenward-Roger method was used to improve the accuracy of F statistics and p-values (Kuznetsova et al. 2017). Prior to ANOVA, the assumptions of normality were evaluated using quantile–quantile (Q–Q) plots for graphical inspection and the Shapiro–Wilk test for statistical assessment.

A two-way ANOVA was also used for pairwise comparisons to assess differences between treatment groups. Additionally, a Kruskal-Wallis test was conducted ($P < 0.05$) to evaluate overall group differences (Nwiyi et al. 2023). For multiple comparisons, Tukey's honest significant difference (HSD) test and the Bonferroni correction were applied to adjust for multiple testing and ensure statistical robustness (Cosme et al. 2024; Ruppert et al. 2021). All statistical analyses were performed using R software, with statistical significance set at $P < 0.05$ and additional significance thresholds ($P < 0.001$, $P < 0.05$).

These statistical approaches were applied to ensure a robust evaluation of the main effects, interactions, and group differences while accounting for assumption checks and controlling Type I error.

RESULTS

Physicochemical Properties (pH and Total Soluble Solids)

The fresh pineapple juice exhibited a pH of 3.94 ± 00 and a TSS of 15˚ Brix. After 21 days of fermentation, the pH of the resulting pineapple wine slightly decreased, indicating increased acidity. The final pH of the control and treated samples at 20˚ Brix were 3.70 ± 00 and 3.73 ± 0.03, respectively. For the 25˚ Brix samples, the pH decreased to 3.75 ± 00 (control) and 3.77 ± 0.01 (treated). At 30˚ Brix, the control had a final pH of 3.28 ± 00, while the treated sample had a pH of 3.73 ± 0.03. The Total Soluble Solids (TSS) decreased after fermentation (Figure 1). The juices with 20˚ Brix and 25˚ Brix decreased to 7 ± 0.00˚ Brix and 9 ± 0.00˚ Brix, respectively. Meanwhile, the juices with 30˚ Brix showed a final TSS values of 21.6 ± 0.00˚ Brix for the control and 14˚ Brix for the wine (Table 1). These findings indicate that fermentation reduced the TSS (i.e., sugar concentration) of the juice, leading to the formation of alcohol.

The statistical analysis using a mixed-effects model (Type III Wald F tests with Kenward-Roger degrees of freedom) revealed significant effects of fermentation time and Brix levels on the TSS of the juice (Table 2). The main factors – Week to Month (time), Brix concentration, and juice type - all had highly significant effects ($P < 0.001$), confirming that these variables significantly influenced TSS reduction. Furthermore, the interactions between week to month × Brix, week to month × juice, and Brix × juice were also highly significant ($P < 0.001$), indicating that the effect of fermentation on TSS depended on both initial sugar concentration (Brix) and juice type. The three-way interaction week to month × Brix × juice was also highly significant ($P < 0.001$), suggesting that the combined effects of fermentation duration, initial sugar concentration, and juice type played a crucial role in TSS reduction.

The alcohol content of the wines ranged from 5.17 ± 0.12% to 11.8 ± 0.00%. The wine produced from 30˚ Brix juice exhibited the highest alcohol concentration at 11.8 ± 0.00%, while the lowest was observed in the wine produced from 20˚ Brix juice, with 7.17 ± 0.06%. The wine from the control group (30˚ Brix juice) had an alcohol content of 5.17 ± 0.12% (Figure 2). The alcohol content for the wines produced from 20˚ and 25˚ Brix juice control groups was not determined due to limited sample volume after processing.

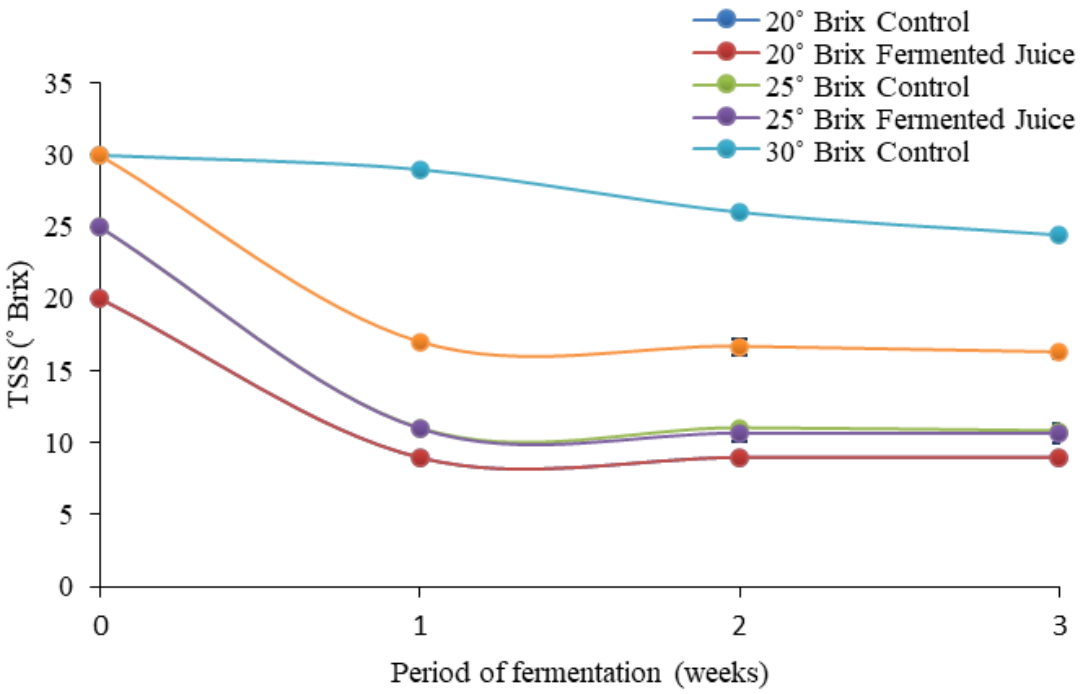

Figure 1. Effect of fermentation period, levels of initial total soluble solids on the rate of fermentation.

Table 1. Initial and final pH, total soluble solids (TSS) and alcohol content of juice and the resulting pineapple wine after aging (mean ± SD, n = 3).

Sample	pH		TSS (° Brix)	
	Initial (n = 3.94 ± 00)	Final	Initial	Final (Aged)
20° Brix Control	3.94 ± 00	3.70 ± 00	20 ± 0.00	7 ± 0.00
20° Brix	3.94 ± 00	3.73 ± 0.03	20 ± 0.00	7 ± 0.00
25° Brix Control	3.94 ± 00	3.75 ± 00	25 ± 0.00	9 ± 0.00
25° Brix	3.94 ± 00	3.77 ± 0.01	25 ± 0.00	9 ± 0.00
30° Brix Control	3.94 ± 00	3.28 ± 00	30 ± 0.00	21.60 ± 0.00
30° Brix	3.94 ± 00	3.73 ± 0.03	30 ± 0.00	14 ± 0.20

Table 2. Analysis of deviance table for mixed effects model (Type III Wald F Tests with Kenward-Roger df) on total soluble solids. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Factor	F Value	Df	Df. Residual	*P*-value
Week to Month	884.47	3	36	< 2.22e-16***
Brix	278.71	2	12	8.76E-11***
Juice	184.35	1	12	1.21E-08***
Week to Month: Brix	215.19	6	36	< 2.22e-16***
Week to Month: Juice	265.92	3	36	< 2.22e-16***
Brix: Juice	985.1	2	12	4.92E-14***
Week to Month: Brix: Juice	168.58	6	36	< 2.22e-16***

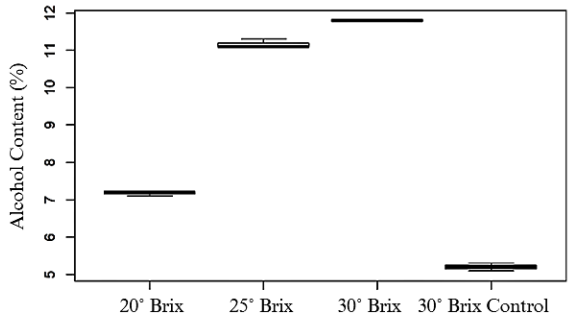

Figure 2. Alcohol content of the resulting aged pineapple wines. Values represent the mean of triplicate fermentations.

A Kruskal-Wallis test, a non-parametric alternative to ANOVA, was conducted to evaluate differences in alcohol content among the Brix levels (20˚, 25˚, 30˚, and 30˚ control). The test indicated a statistically significant difference among the groups (χ^2 = 10.607, df = 3, *P* = 0.01405), suggesting that alcohol content varied with the initial sugar concentration. Pairwise comparisons revealed that only the 30˚ Brix wine and the 30˚ Brix control differed significantly (*P* = 0.0060), demonstrating that fermentation, rather than sugar content alone, was a key factor in determining the final alcohol content at this sugar level compared with the non-fermented control (Table 3).

Table 3. Kruskal-Wallis rank sum test and post hoc Bonferroni comparison of alcohol content of aged pineapple wines. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Comparison	Value	*P*-value
Kruskal-Wallis	χ^2= 10.607	0.01405
Group Comparison	**Z-scores**	***P*-value**
25˚ Brix vs. 20 Brix	-1.0299s	0.9092
30˚ Brix vs. 20 Brix	-2.0598	0.1182
30˚ Brix vs. 25 Brix	-1.0299	0.9092
30˚ Brix vs. 20 Brix	1.0299	0.9092
30˚ Brix vs. 25 Brix	2.0598	0.1182
30˚ Brix vs. 30 Brix	3.0897	0.0060*

Anti-inflammatory Activity of Fresh Pineapple Juice and Wine

Fresh pineapple juice and wine, both produced from 30˚ Brix juice fermented with *S. cerevisiae*, were tested for their inhibitory effects on COX-1 and COX-2 enzymes at concentrations of 50 mL/L and 100 mL/L. The results showed that fresh pineapple juice inhibited COX-2 by 76.73% ± 2.49 at 50 mL/L and 84.53 ± 0.28% at 100 mL/L, while the wine exhibited inhibition rates of 79.77 ± 1.42% and 80.44 ± 1.83%, respectively (Table 4). Notably, at the higher concentration of 100 mL/L, fresh pineapple juice demonstrated greater COX-2 inhibitory activity than the standard drug indomethacin, which showed a COX-2 inhibition rate of 83.90 ± 3.22%. For COX-1 inhibition, fresh juice showed inhibition rates of 61.72 ± 9.45% and 76.33 ± 1.26% at 50 mL/L and 100 mL/L, respectively, while the wine exhibited lower inhibition rates of 49.72 ± 0.25% and 74.31 ± 1.14% (Figure 3).

A two-way ANOVA was performed to examine the effects of COX enzyme type (COX-1 and COX-2) and concentration on inhibition rates. The analysis revealed statistically significant main effects for both COX type (F = 104.44, *P* = 2.19e-09) and concentration (F = 28.36, *P* = 5.43e-08), indicating that each factor significantly affected enzyme inhibition. More importantly, a significant interaction effect was observed between COX type and concentration (F = 14.28, *P* = 1.16e-05) (Table 5). This interaction indicates that the inhibitory effects of fresh juice and wine on COX enzymes varied across concentrations, meaning that the effect of one factor depended on the level of the other.

Pairwise comparisons using Tukey's honestly significant difference (HSD) test revealed significant differences in COX inhibition among treatments. Fresh pineapple juice at 100 mL/L showed significantly higher COX-1 inhibition compared with that at 50 mL/L (*P* = 0.0003), while wine at 100 mL/L demonstrated significantly greater COX-1 inhibition than wine at 50 mL/L (*P* < 0.0001). Fresh juice at 50 mL/L showed significantly lower COX-1 inhibition than indomethacin (*P* < 0.0001), indicating that although pineapple juice exhibited anti-inflammatory potential, its COX-1 inhibitory activity at lower concentrations was weaker than that of the standard drug. However, the higher COX-1 inhibition observed in wine at higher concentrations suggested that fermentation may increase or preserve certain bioactive compounds responsible for COX-1 inhibition, thereby enhancing its effectiveness at higher doses. For COX-2 inhibition, no significant differences were found among most treatments (*P* > 0.05), except for fresh juice at 100 mL/L, which showed slightly higher inhibition than fresh juice at 50 mL/L (*P* = 0.0727) (Table 6). The absence of statistical significance in COX-2 inhibition suggests that both fresh juice and wine maintained similar potency across different concentrations, with fermentation likely helping to preserve their anti-inflammatory activity.

Table 4. COX-2 and COX-1% inhibition of fresh juice (50 mL/L and 100 mL/L) and 30˚ Brix pineapple wine (50 mL/L and 100 mL/L).

Sample	Concentration	COX-2 (%)	COX-1 (%)	COX-2: COX-1
Indomethacin	4 mM	83.90 ± 3.22	79.70 ± 1.37	1.05
Fresh Juice	50 mL/L	76.73 ± 2.49	61.72 ± 9.45	1.24
Wine	50 mL/L	79.77 ± 1.42	49.72 ± 0.25	1.60
Fresh Juice	100 mL/L	84.53 ± 0.28	76.33 ± 1.26	1.11
Wine	100 mL/L	80.44 ± 1.83	74.31 ± 1.14	1.08

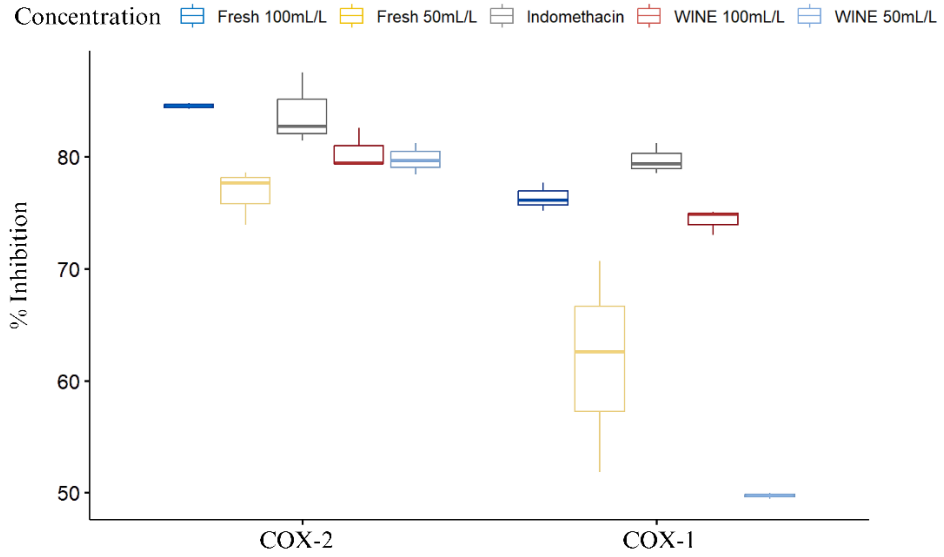

Figure 3. Boxplot showing the inhibitory activity of the fresh juice and wine from 30˚ Brix juice against COX-1 and COX-2.

Table 5. Two-way ANOVA results showing the significant effects of COX type, concentration, and their interaction on inhibition rates. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Source of Variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
COX	1	1212.6	1212.6	104.44	2.19e-09 ***
Concentration	4	1317.3	329.3	28.36	5.43e-08 ***
COX: Concentration	4	663.1	165.8	14.28	1.16e-05 ***
Residuals	20	232.2	11.6		

Table 6. Pairwise comparisons of COX-1 and COX-2 inhibition. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Contrast	COX-1 (*P*-value)	COX-2 (*P*-value)
Fresh 100 mL/L - Fresh 50 mL/L	0.0003^{***}	0.0727^{ns}
Fresh 100 mL/L - Indomethacin	0.7453^{ns}	0.9993^{ns}
Fresh 100 mL/L - Wine 100 mL/L	0.9476^{ns}	0.5904^{ns}
Fresh 100 mL/L - Wine 50 mL/L	0.0004^{***}	0.4485^{ns}
Fresh 50 mL/L - Indomethacin	$<0.0001^{***}$	0.1343^{ns}
Fresh 50 mL/L - Wine 100 mL/L	0.0017^{**}	0.6774^{ns}
Fresh 50 mL/L - Wine 50 mL/L	0.0028^{**}	0.8088^{ns}
Indomethacin - Wine 100 mL/L	0.3304^{ns}	0.5836^{ns}
Indomethacin - Wine 50 mL/L	$<0.0001^{***}$	0.7480^{ns}
Wine 100 mL/L - Wine 50 mL/L	$<0.0001^{***}$	0.9002^{ns}

Both fresh juice and wine showed selectivity for COX-2 over COX-1, with COX-2/COX-1 inhibition ratios greater than 1.0 (1.08 - 1.60). These results indicate strong COX-2 inhibitory effects of pineapple juice and wine, with fresh juice showing more potent activity at higher concentrations. The fermentation process appeared to maintain the anti-inflammatory properties of pineapple, although small variations in COX enzyme inhibition were observed.

Total Antioxidant Capacity (TAC) and Total Phenolic Content (TPC) of Fresh Pineapple Juice, Wine, and Aged Wine

The TAC of fresh pineapple juice was notably higher (2241.03 ± 55.22 mg AAE/L) compared with wine (1527.82 ± 92.52 mg AAE/L) and aged wine (1416.03 ± 12.70 mg AAE/L) (Table 7; Figure 4). A Kruskal-Wallis test revealed a significant difference among the groups ($\chi^2 = 6.25$, $P = 0.04$). Post hoc analysis using the Bonferroni correction showed that the TAC of fresh juice was significantly higher than that of aged wine ($P = 0.0203$). Meanwhile, no significant differences were observed between fresh juice and wine ($P = 0.1740$) or between wine and aged wine ($P = 0.5536$) (Table 8; Figure 5).

Similarly, the TPC of fresh juice was the highest (430.10 ± 5.08 mg GAE/L), compared with wine (422.57 ± 5.95 mg GAE/L) and aged wine (348.30 ± 5.92 mg GAE/L) (Table 7). A Kruskal-Wallis test confirmed a significant difference among the groups ($\chi^2 = 6.4889$, $P = 0.03899$). Post hoc analysis with Bonferroni correction showed that fresh juice had significantly higher TPC than aged wine ($P = 0.0169$), while no significant differences were observed between fresh juice and wine ($P = 0.4451$) or between wine and aged wine ($P = 0.2041$) (Table 9).

HPTLC Profile of Fresh Pineapple Juice, Wine, and Aged Wine

The analysis was performed on pineapple juice, wine (30˚ Brix), and aged wine (30˚ Brix) to evaluate their phytochemical profiles. The HPTLC analysis of these samples revealed chromatographic profiles with 7, 10, and 10 blue fluorescent bands, respectively, under UV light at 366 nm. These bands were characterized by distinct Rf values, ranging from 0.02 to 0.83 for fresh juice, 0.03 to 0.84 for wine, and 0.02 to 0.84 for aged wine (Table 10). The blue fluorescence observed in these bands was commonly associated with phenolic acids. Additionally, the presence of these fluorescent bands in all samples suggests that phenolic compounds remained stable during fermentation and aging. However, differences in Rf values and band intensities indicated changes in the relative amounts of individual phytochemicals (Figure 6).

Sensory Evaluation of Aged Pineapple Wine

The average sensory evaluation scores of aged pineapple wines, assessed using a 9-point hedonic scale, are presented in Table 11. Among the samples, the wine produced from 30˚ Brix juice achieved the highest ratings across all attributes, including color (7.64 ± 0.04), aroma (7.31 ± 0.27), taste (7.73 ± 0.07), and overall acceptability (7.75 ± 0.08).

Table 7. Total antioxidant capacity (50mL/L) and total phenolic content (125mL/L) of fresh pineapple juice, wine and aged wine.

Samples	TAC (mg AAE/L)	TPC (mg GAE/L)
Fresh Juice	2241.03 ± 55.22	430.10 ± 5.08
Wine	1527.82 ± 92.52	422.57 ± 5.95
Aged Wine	1416.03 ± 12.70	348.30 ± 5. 92

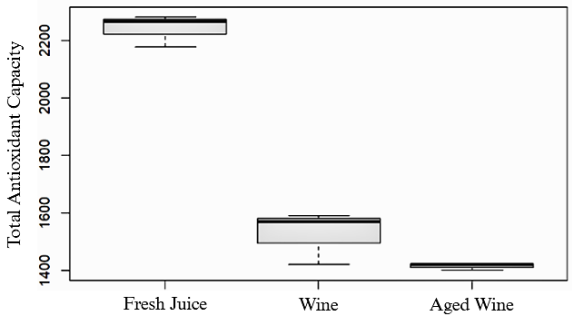

Figure 4. Boxplot showing the total antioxidant capacity (50mL/L) of fresh pineapple juice, wine and aged wine.

Table 8. Kruskal-Wallis test and post hoc pairwise comparisons of total antioxidant capacity among pineapple juice, wine, and aged wine. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Comparison	Value	*P*-value
Kruskal-Wallis Test	$\chi^2 = 6.2521$	0.04
Pairwise Comparisons	**Z-score**	***P*-value (adjusted)**
Aged Wine vs. Juice	-2.469987	0.0203*
Aged Wine vs. Wine	-0.898177	0.5536
Juice vs. Wine	1.57181	0.1739

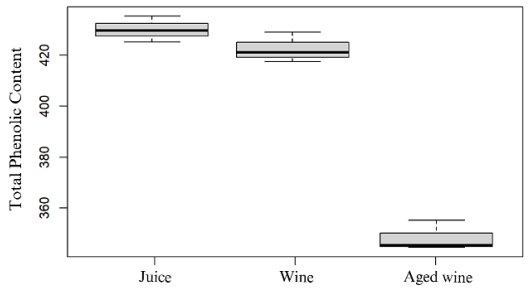

Figure 5. Boxplot showing the total phenolic content (125mL/L) of fresh pineapple juice, wine and aged wine.

Table 9. Kruskal-Wallis test and post hoc pairwise comparisons of total antioxidant capacity among pineapple juice, wine, and aged wine. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Comparison	**Value**	***P*-value**
Kruskal-Wallis Test	$\chi^2 = 6.4889$	0.04
Pairwise Comparisons (Bonferroni)	**Z-score**	***P*-value**
Juice vs. Aged Wine	-2.53421s	0.0169*
Wine vs. Aged Wine	-1.490711	0.2041
Juice vs. Wine	1.043498	0.4451

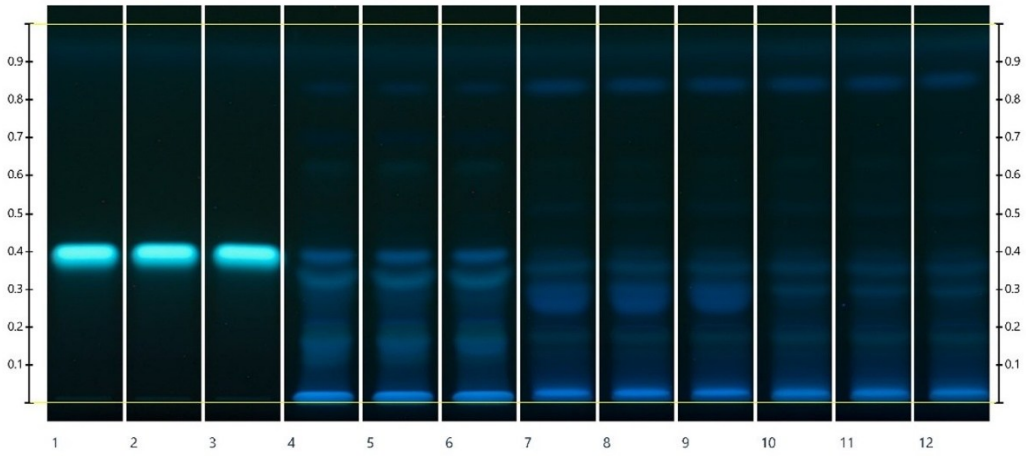

Figure 6. High-performance thin layer chromatography profiles of Chlorogenic acid (track 1-3), fresh pineapple juice (track 4-6), wine (track 7-9), and aged wine (track 10-12) visualized under ultraviolet light at 366 nm following elution with an ethyl acetate: formic acid: water (80:10:10) and derivatization using natural product reagent.

Table 10. High-performance thin layer chromatography profile of the fresh juice, wine and aged wine visualized under ultraviolet light at 366 nm ethyl acetate: formic acid: water (80:10:10) under 366 nm as mobile phase and natural product as a derivatizing agent.

Range of Rf Values	**Chlorogenic Acid**		**Fresh Juice**		**Wine**		**Aged Wine**	
	Rf	**Color**	**Rf**	**Color**	**Rf**	**Color**	**Rf**	**Color**
0.00-0.20			0.02	Blue	0.03	Blue	0.02	Blue
			0.16	Blue	0.17	Blue	0.17	Blue
0.21-0.40					0.26	Blue	0.27	Blue
					0.29	Blue	0.29	Blue
			0.32	Blue	0.36	Blue	0.35	Blue
	0.4	Blue	0.39	Blue	0.39	Blue	0.39	Blue
0.41-0.60					0.52	Blue	0.52	Blue
					0.57	Blue	0.57	Blue
0.61-0.80			0.62	Blue	0.63	Blue	0.64	Blue
			0.70	Blue				
0.80-11.00			0.83	Blue	0.84	Blue	0.84	Blue

The 30˚ Brix control wine received slightly lower but still favorable scores, with an overall acceptability of 7.47 ± 0.07, corresponding to the "liked moderately " to "liked very much" range. In contrast, wines from the 20˚ Brix control (6.93 ± 0.13), 20˚ Brix juice (6.91 ± 0.27), 25˚ Brix juice (6.88 ± 0.08), and 25˚ Brix control (6.80 ± 0.24) were rated lower, generally falling between "like slightly" and "like moderately." These results suggest that a higher initial sugar concentration (30˚ Brix) contributed positively to sensory attributes and consumer acceptability of aged pineapple wine. Furthermore, pineapple wine produced from 30˚ Brix juice exhibited the most favorable sensory qualities, particularly in taste and overall acceptability, making it more appealing to consumers than wines produced from lower Brix levels.

A two-way ANOVA revealed statistically significant effects of Brix level (F = 18.96, $P < 0.001$), acceptability factors (F = 8251.65, $P < 0.001$), and their interaction (F = 60.41, $P < 0.001$) on sensory evaluation scores. Simple main effects analysis indicated significant differences in aroma (F = 46.86, $P < 0.001$), color (F = 61.32, $P < 0.001$), and taste (F = 49.16, $P < 0.001$). Post hoc pairwise comparisons showed that color and taste differed significantly ($P < 0.001$), whereas aroma and taste did not differ significantly ($P = 0.1368$). In terms of descriptive analysis, aroma-color ($P < 0.001$), aroma-taste ($P < 0.001$), and color-taste ($P < 0.001$) all displayed significant differences (Table 12). These results suggest that the juice's sweetness level (measured by Brix) significantly influenced consumer perception of pineapple wine, with higher sugar content leading to improved color, taste, and overall sensory appeal.

Panelists' color descriptions ranged from 7.29 ± 0.10 to 7.64 ± 0.04, reflecting moderate acceptance. The hues were identified as shades between Buttered Rum (#A16D0A) and Muddy Brown (#945C06). Bitterness was noted in wines from 20˚ Brix and 25˚ Brix juices and their corresponding controls, while wines from 30˚ Brix juices exhibited a balanced bittersweet to moderately sweet flavor profile.

This analysis confirmed that higher Brix levels (30˚ Brix) led to significantly higher acceptability scores compared to lower Brix levels (20˚ and 25˚ Brix), aligning with the preference for sweeter and more balanced flavor profiles in pineapple wine (Figure 7).

Table 11. Sensory evaluation of pineapple wine with various sugar level. Desc – Description, Acc – Acceptability.

Sample	Color		Aroma		Taste		Overall Acceptability
	Desc.	Acc.	Desc.	Acc.	Desc.	Acc.	
20˚ Brix Control	2.09 ± 0.28	7.44 ± 0.14	2.84 ± 0.32	7.28 ± 0.20	1.76 ± 0.21	6.64 ± 0.14	6.93 ± 0.13
20˚ Brix	2.02 ± 0.17	7.47 ± 0.18	2.76 ± 0.23	7.04 ± 0.21	1.87 ± 0.06	6.69 ± 0.43	6.91 ± 0.27
25˚ Brix Control	1.98 ± 0.25	7.49 ± 0.10	2.6 ± 0.18	6.98 ± 0.28	1.58 ± 0.10	6.56 ± 0.15	6.80 ± 0.24
25˚ Brix	1.40 ± 0.20	7.29 ± 0.10	2.71 ± 0.25	7.11 ± 0.10	1.93 ± 0.07	6.73 ± 0.13	6.88 ± 0.08
30˚ Brix Control	1.09 ± 0.08	7.36 ± 0.25	3.2 ± 0.37	7.27 ± 0.12	4.24 ± 0.20	7.58 ± 0.10	7.47 ± 0.07
30˚ Brix	1.82 ± 0.23	7.64 ± 0.04	2.69 ± 0.14	7.31 ± 0.27	3.07 ± 0.17	7.73 ± 0.07	7.75 ± 0.08

Table 12. Two-Way ANOVA and pairwise comparisons on sensory evaluation. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Analysis	Factor(s)	F/t Value	*P*-Value
Two-Way ANOVA	Factors	18.96	7.27E-09***
	Acceptance	8251.65	< 2e-16***
	Factors × Acceptance	60.41	< 2e-16***
Simple Main Effects	Aroma	46.855	< 0.0001***
	Color	61.321	< 0.0001***
	Taste	49.16	< 0.0001***
Pairwise Comparisons (Acceptability)	Aroma - Color	-3.02	0.0072**
	Aroma - Taste	1.908	0.1368^{ns}
	Color - Taste	4.928	< 0.0001***
Pairwise Comparisons (Description)	Aroma - Color	11.446	< 0.0001***
	Aroma - Taste	4.213	0.0001***
	Color - Taste	-7.233	< 0.0001***

Figure 7. Fermented pineapple juice (20˚ Brix: 1-R1, 2-R2, 3-R3, 4-control; 25˚ Brix: 5-R1, 6-R2, 7-R3, 8-control; 30˚ Brix: 9-R1, 10-R2, 11-R3, 12-control).

DISCUSSION

Physicochemical Properties (pH and Total Soluble Solids)

The observed physicochemical changes in pineapple juice and wine are consistent with previous studies, supporting the use of pineapple juice as a substrate for winemaking. The decrease in pH across all samples, from an initial 3.94 ± 00 in fresh juice to a range of 3.28 ± 00 to 3.77 ± 0.01 in wines, corresponds to a decrease of approximately 0.17 to 0.66 pH units (equivalent to 4–17%), and aligns with the typical increase in acidity during fermentation (Boondaeng et al. 2021). This acidification is important for improving the wine's stability and flavor profile (Van Man 2021). The small variations in wine pH may be attributed to differences in yeast metabolic activity and the initial sugar content of the juice.

The total soluble solids (TSS) decreased significantly during fermentation, particularly in the first week, reflecting the rapid consumption of sugars by *S. cerevisiae*. This aligns with previous studies, which indicate that the initial stages of fermentation are the most active, with sugar levels dropping rapidly as glucose and fructose are converted into ethanol and carbon dioxide (Van Man 2021). The final stabilization of TSS values, usually around the second week, indicates the near completion of sugar consumption and marks the end of fermentation.

Interestingly, the TSS in the control group (no yeast) also declined, probably due to the activity of native yeasts and bacteria in the juice, such as *Pichia guilliermondii* Wickerham and *Hanseniaspora uvarum* (Niehaus) Shehata, Mrak & Phaff ex M.T. Sm. (Di Cagno et al. 2010; Chanprasartsuk et al. 2010). This underscores the potential role of native microbial flora in driving spontaneous fermentation processes, even without the addition of *S. cerevisiae*. Additionally, the presence of Firmicutes on pineapple peel, particularly the genera *Weissella, Lactobacillus,* and *Lactococcus* (Tallei et al. 2022), may have facilitated spontaneous fermentation in the control group.

The alcohol content of the wines ranged from 5.17 ± 0.12% to 11.80 ± 0.00%, with higher sugar content in the juice resulting in higher ethanol levels. This positive correlation between TSS and alcohol content is well-documented in many fermentation studies (Van Man 2021). Notably, the 30˚ Brix juice sample, which contained the highest sugar concentration, yielded the highest alcohol content (11.80 ± 0.00%), demonstrating the direct influence of sugar availability on ethanol production. The fermentation process, driven by *S. cerevisiae*, efficiently converted the sugars into ethanol, confirming the yeast's crucial role in alcohol yield.

The lower alcohol content in the 30˚ Brix juice no-yeast control group (5.17 ± 0.12%) compared to its yeast-inoculated counterpart (11.80 ± 0.00%) further highlights the importance of using a controlled yeast strain to achieve higher ethanol production. While natural fermentation can occur through native microbial activity, it is significantly less efficient at producing high alcohol concentrations without the addition of a selected yeast strain.

Anti-inflammatory Activity of Fresh Pineapple Juice and Wine

Both fresh pineapple juice and the resulting wine showed COX-2 selective inhibition of COX-2, indicating potential anti-inflammatory effects. The higher COX-2/COX-1 inhibition ratio suggests that

these samples specifically target COX-2 over COX-1, which is a desirable trait in anti-inflammatory agents, as COX-1 inhibition can cause adverse gastrointestinal effects (Radi and Khan 2006). In contrast, COX-2 is an inducible isoform mainly found in inflammatory cells and tissues in response to stimuli such as cytokines, mitogens, endotoxins, hormones, tumor promoters, carcinogens, wounding, and ultraviolet (UV) radiation (Jain et al. 2008; Rundhaug and Fischer 2011). The assay's reliability is supported by its consistency with the positive control, which usually shows 60-80% inhibition at 4 mM (Opog and Amor 2019).

Despite fermentation, which typically alters the chemical composition of the juice, the anti-inflammatory activity of pineapple juice was retained, indicating that key bioactive compounds responsible for COX-2 inhibition remained stable. The slight decrease in COX-2 and COX-1 inhibitory activities at higher concentrations (100 mL/L) may indicate a threshold effect, beyond which diminishing returns or a balancing effect on both COX enzymes may occur. The selective increase in COX-2 activity at lower concentrations (50 mL/L), along with the decrease in COX-1 inhibition, demonstrates a concentration-dependent effect on enzyme inhibition.

The anti-inflammatory effects observed in both fresh pineapple juice and wine may be attributed to bioactive phytochemicals that modulate key enzymatic pathways involved in inflammation. Hidaka et al. (2008) demonstrated that bromelain from pineapple can suppress the expression of CD25, a transmembrane protein usually upregulated in activated T cells, and can also inhibit cyclooxygenase-2 (COX-2) expression. These mechanisms support the potential anti-inflammatory and antitumor properties of bromelain (Bhui et al. 2009; Secor et al. 2009). In addition to these bioactive compounds, pineapple is a rich source of essential nutrients and vitamins, including ascorbic acid, folate (DFE), niacin, vitamin B6, riboflavin, thiamin, and vitamins A, E, and K. It also contains important minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc (USDA-NRCS 2014). These nutritional components further contribute to the wide range of health benefits associated with pineapple consumption.

Total Antioxidant Capacity and Total Phenolic Content of Fresh Pineapple Juice, Wine and Aged Wine

Free radicals can cause oxidative damage that may build up over time and potentially lead to degenerative diseases (Zubia and Dizon 2019). Antioxidants are substances capable of neutralizing free radicals. The observed decreases in TAC and TPC values may be attributed to various factors related to juice processing, including clarification, filtration, and pasteurization. These processes can remove phenolic compounds associated with dietary fiber and pectin in pineapple (Candrawinata et al. 2012). Furthermore, thermal processing has been shown to degrade anthocyanins in grapes (Kechinski et al. 2010).

Khalid et al. (2016) reported that pineapple juice inhibits the activity of cytochrome P450 2C9, an enzyme that plays a crucial role in the oxidation and metabolism of many therapeutic drugs. Additionally, pineapple contains the alkaloid 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-α-carboline, which exhibits antioxidant properties (Herraiz and Galisteo 2003). It is widely accepted that much of the antioxidant activity in pineapple juice is attributable to its phenolic compounds (Khalid et al. 2016). Li et al. (2014) identified the primary polyphenolics in pineapples as gallic acid, catechin, epicatechin, and ferulic acid. In addition, flavonoids such as catechin, epicatechin, and myricetin in pineapples are considered effective antioxidants because of the number and position of hydroxyl groups (Domínguez et al. 2018).

Previous phytochemical analyses of pineapple extracts revealed that the phenolic content, expressed as caffeic acid equivalents, was highest in the methanol extract, followed by the ethyl acetate extract and the water extract. The antioxidant capacity of these extracts, expressed in terms of ascorbic acid equivalents (μmol g^{-1} of extract), ranked in descending order: methanol extract, ethyl acetate extract, and water extract (Hossain and Rahman 2011). Adeboyejo et al. (2018) highlighted that pineapple cultivars grown in Nigeria are particularly rich in antioxidants, characterized by high levels of flavonoids, phenolic content, and overall antioxidant activity. In a related study, Boondaeng et al. (2021) reported an increase in phenolic content from the first to the last day of fermentation, accompanied by a decrease in antioxidant capacity. Overall, these findings revealed the potential of fresh pineapple juice and, to a lesser extent, pineapple wine, as valuable sources of natural antioxidants.

High-Performance Thin Layer Chromatography (HPTLC) Profile of Fresh Pineapple Juice, Wine and Aged Wine

Pineapple juice is a valuable commercial product; however, its phenolic profile largely remains uncharacterized (Khalid et al. 2016). To address this gap, HPTLC analysis was conducted on samples of pineapple juice, wine, and aged wine.

The presence of blue fluorescence observed in these bands was commonly attributed to phenolic acids (Bernardi et al. 2019; Ang et al. 2022). Further analysis showed that the Rf values for the test samples (0.39) closely matched those of the standard chlorogenic acid (0.40). Khan et al. (2020) and Samreen et al. (2020) demonstrated that pineapples contain chlorogenic acid. Therefore, it is likely that the

band at an Rf value of 0.39 corresponds to chlorogenic acid. However, confirmation of this identification using gas chromatography-mass spectrometry (GC-MS) is necessary to verify that the compound is indeed chlorogenic acid.

New bands appeared after fermentation, with an Rf value of 0.52 observed in both wine and aged wine. Procopio et al. (2013) showed that yeast fermentation breaks down glycosidic precursors, releasing bioactive compounds such as monoterpenoid alcohols, terpene oxides, and diols. This enzymatic activity is important in changing the chemical composition of fermented products. Similarly, during pineapple wine fermentation, yeast may have broken down glycosidic precursors in the juice, generating new metabolites that contributed to the observed bands. This biochemical change could explain the appearance of new bands in the chromatographic profile, indicating structural modifications or the formation of new metabolites. These findings suggest that fermentation not only improves the volatile content and sensory qualities of pineapple wine but also alters its phytochemical profile, potentially enhancing its functional properties.

Sensory Evaluation of Aged Pineapple Wine

Sensory evaluation of pineapple wines prepared from different pineapple juice-to-water ratios revealed that these ratios significantly affected the wines' overall acceptability. Ratios of 2:1, 1:1, and 1:2 produced acceptability scores of 6.35, 6.08, and 5.58, respectively (Boondaeng et al. 2021), indicating that the concentration of pineapple juice plays a key role in shaping sensory perceptions and consumer satisfaction. In a related study, wines were produced using a fixed ratio of pineapple must to sugar (1:4) across four different formulations: Recipe A used only natural yeast; Recipe B included granulated sugar and natural yeast; Recipe C incorporated both baker's yeast and granulated sugar along with natural yeast; and Recipe D (control) contained only granulated sugar and baker's yeast. The sensory characteristics of the control wine (Recipe D) closely resembled those of natural palm wine, demonstrating that variations in yeast compositions and sugar levels can significantly influence the wine's flavor profile and preservation qualities. According to Idise (2012), pineapple wines produced using Recipes A through C are suitable for immediate consumption or preservation through refrigeration, highlighting the flexibility of pineapple winemaking methods to suit various taste preferences and storage conditions. This evidence highlights the importance of ingredient ratios and fermentation techniques in pineapple wine production, influencing its sensory qualities, consumer appeal, and storage potential.

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GENERATIVE AI STATEMENT

This manuscript was prepared with the assistance of ChatGPT (OpenAI; GPT-5.2) to improve clarity and language. All scientific content, data interpretation, and conclusions are the author's original work.

ETHICAL CONSIDERATIONS

The study followed both institutional and national ethical guidelines for research involving human participants. Approval from the Institutional Ethics Review Committee (IERC) was obtained before conducting the research. All participants, who were food technologists, were informed about the study's goals, procedures, and potential risks. Informed consent was collected from all participants to ensure voluntary participation and data confidentiality. The study did not include minors or individuals below 18 years of age.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests among the authors.

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Evaluation of the antioxidant and phytochemical analysis of Tabon-tabon (*Atuna racemosa* Raf) methanol fruit extract

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ABSTRACT

Atuna racemosa Raf, locally known as "tabon-tabon" in the Philippines, is a fruit-bearing tree used as a souring agent for native Filipino dishes in Mindanao. Previous reports have shown its antimicrobial efficacy. However, limited studies have demonstrated its antioxidant potential. The study determined the phytochemical components of *A. racemosa* methanol fruit extract and evaluated its antioxidant profile using various *in vitro* systems. The antioxidant profile of *A. racemosa* methanol fruit extract was determined by radical scavenging (DPPH, superoxide radical, hydroxyl radical), reducing capacities (FRAP and MTT), and chelation activities. Methanol fruit extract of *A. racemosa* demonstrated a potent DPP radical inhibition comparable to ascorbic acid. It has also exhibited concentration- dependent hydroxyl radical scavenging activity and reducing power. However, low iron chelation and superoxide anion inhibition were observed, even at the highest concentration. Qualitative phytochemical analyses revealed the presence of carbohydrates, cardiac glycosides, steroids, terpenes/terpenoids, quinones, anthraquinones, flavonoids, phenolics, and tannin compounds, which may explain *A. racemosa* strong antioxidant behavior. These findings indicate that *A. racemosa* holds strong antioxidant potential, making it a vital natural antioxidant source that could be efficiently used in treating oxidative stress conditions.

Keywords: DPPH radical scavenging, hydroxyl radical, metal chelation, reducing capacities, superoxide radical

INTRODUCTION

Normal metabolism spontaneously forms unstable species and molecular oxygen intermediates with unpaired electrons, recognized as reactive oxygen species (ROS) (Juan et al. 2021). Prolonged exposure to high concentrations of these reactive intermediates can result in the oxidation of biological structures (Afzal et al. 2023). Damage to oxidative components due to cell component malfunction can cause disorders like atherosclerosis, cancer, and diabetes (Arfin et al. 2021; Salekeen et al. 2022; Caturano et al. 2023).

To reduce the harmful consequences of oxidation, the human body makes use of protective mechanisms comprised of antioxidants (Afzal et al. 2023). Antioxidants are substances that manage excess free radicals by halting the commencement and progression of oxidizing chain reactions. Humans

have employed intricate approaches to manage redox homeostasis (Sharifi-Rad et al. 2020). The human body is equipped with endogenous antioxidants, either enzymatic or non-enzymatic systems, which could reverse their harmful effects (Sharifi-Rad et al. 2020; Juan et al. 2021). In contrast, external sources such as food and supplements offer exogenous antioxidants (Afzal et al. 2023). Among the food sources, plants are the richest source of natural exogenous antioxidants (Jafri et al. 2022; Afzal et al. 2023).

Plants are rich source of various secondary metabolites that have remarkable antioxidant properties. These compounds include flavonoids, alkaloids, phenolics, tannins, glycosides, and steroids (Jafri et al. 2022). As these phytochemicals work independently or synergistically, their antioxidant activity stabilizes high ROS by their ability to donate electrons or hydrogen, producing stable intermediates (Chaves et al. 2020). These phytochemicals are highly beneficial as natural antioxidants, especially in developing countries where plants are cheap and abundant (Leite et al. 2018).

Atuna racemosa Raf., commonly called "tabon-tabon" in the Philippines, belongs to the Chrysobalanaceae family, indigenous and native to selected Mindanao regions (Nadayag et al. 2019). Its fruit, characterized by its stiff flesh and strong astringent flavor, is used to balance the acidity and fishy taste of raw seafood in the Filipino dish, "kinilaw" (Rathi et al. 2019). Outside of the culinary application, the tree has been traditionally used in massage oil preparations to relieve pain, acting as a natural anti-inflammatory agent (Prance 2004). Moreover, *A. racemosa* has been noted to hold promising antimicrobial properties (Buenz et al. 2007; Nadayag et al. 2019; Tila et al. 2022). However, despite its extensive history of use and reported bioactivity, the fruit's antioxidant capacities remain underexplored. Investigating the bioactivity of *A. racemosa* fruit not only aids in the promotion of sustainable, culturally-embedded health solutions but also aligns with the United Nations Sustainable Development Goals (UN SDG), particularly the promotion of good health and well-being (SDG 3) and responsible consumption of natural resources (SDG 12). Thus, this study evaluated the antioxidant activity of *A. racemosa* methanol fruit extract in various antioxidant assays and determined the phytochemicals present *in vitro*. The findings of this research might contribute to wider efforts aimed at encouraging local communities by highlighting the benefit of traditional knowledge and promoting the utilization of endemic plant resources for health applications. Moreover, this might emphasize the value of preserving biodiversity, which aligns with the global health goals of integrative and sustainable solutions to healthcare challenges.

METHODS

Chemicals and Reagents

Reagents for antioxidant testing such as potassium ferricyanide [$K_3Fe(CN)_6$], 1,1-Diphenyl-2-picrylhydrazyl (DPPH), sodium nitroprusside (SNP), naphthylethelenediaminedihydrochloride (NED), sulfanilamide, nitro blue tetrazolium (NBT), nicotinamide adenine dinucleotide reduced form (NADH), phenazinemethosulfate (PMS), as well as the standards for each antioxidant tests, were acquired from Sigma. Methanol (ACS Grade, Scharlau) was procured from Belman Laboratories. Other analytical grade (AR) reagents and solvents utilized were procured from local suppliers.

Fruit Collection and Preparation of Fruit Extract

The fruits of *Atuna racemosa* were gathered from the local market of the Municipality of Maramag in Bukidnon, Philippines (7°45'37.16" N, 125°0'10.74" E). The identity of fruit specimen was verified and authenticated by the Institute of Biology Herbarium - University of the Philippines, Diliman. The whole fruit (Figure 1) was cut in half, and the flesh was taken out. The fruit flesh was subjected to air-drying and subsequently pulverized using blender until a coarse powder was obtained.

Approximately 200 g of dried pulverized *A. racemosa* were immersed in absolute methanol (99.9%) (500 mL) at room temperature for 12 hours. Methanol was used as the solvent in the study for good extraction efficiency (as it extracts wide range of hydrophilic and lipophilic compounds), high penetrability, and low boiling point, allowing ease of removal from active components for downstream testing (Lee et al. 2024). The methanol extract was then filtered and dried at 40˚C through vacuum-assisted evaporation system (Buchi Rotavapor R-200). The residue was re-soaked twice using the same proportions. All extracts were stored in airtight containers at 4°C until further usage.

Figure 1. Images of *Atuna racemosa* Raf fruit. A) Whole mature fruits with a rough, woody pericarp with ellipsoid to subglobose shape, approximately 5.5 cm long in diameter; (B) Longitudinal section of the fruit showing the distinctive brain-like pattern of the endosperm.

Antioxidant Tests

DPPH scavenging assay. Ten microliters (10 μL) of prepared extracts/standards at different concentrations (250-1000μg/mL) were loaded into microwell plate. Subsequently, 140 μL of 0.0683 mM DPPH was added and was allowed to remain at room temperature (RT) for 30 minutes. Absorbances were determined at 517 nm. The median inhibitory concentration (IC_{50}) of DPPH was computed from regression analysis (Itam et al. 2021). The positive control used was ascorbic acid (Bueno et al. 2023).

Ferric ions (Fe^{3+}) reducing antioxidant power assay. An aliquot of seventy microliters (70 μL) of varying extracts/standard concentrations (250-1000 μg/mL) was combined with 176.5 μL of 0.2 M phosphate buffer (pH = 7.4) and 176.5 μL of 1% potassium hexacyanoferrate (III). The mixture was incubated for 20 min in a 50 °C water bath. After incubation, the reaction mixtures were cooled, acidified with 176.5 μL of trichloroacetic acid (10%), and centrifuged for 10 min at 650 x g. Two hundred seventy-three microliters (273 μL) supernatant was aliquoted, combined with equal volumes of distilled water. Fifty-five microliters (55 μL) of 0.1% ferric chloride were added, and absorbances were read at 700 nm. The median effective concentration (EC_{50}) value (μg extract/mL) was determined for reducing power through interpolation from the linear regression analysis (Zhang et al. 2011). The positive control used was butylated hydroxytoluene (Bueno et al. 2023).

MTT assay. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent (1 mg/mL) was dissolved in water. An aliquot of 190 μL of MTT was reacted with 10 μL extracts/standards (250-1000 μg/mL) in DMSO was mixed in a glass tube for 1 minute. The reaction mixture was kept inside the incubator for 6 hours at 37°C. Absorbances were read at 570 nm. The positive control used was curcumin (Yu et al. 2020).

Metal chelation. Twenty microliters (20 μL) of different extract/standard concentrations (250-1000 μg/mL) were reacted with 0.2 mM ferrous chloride (100 μL) in a 96-microplate. Afterward, 40 μL of ferrozine (5 mM) was placed in each well and allowed to sit at RT for 10 min. Absorbances were read at 562 nm. The positive control used was ethylenediaminetetraacetic acid (EDTA) (Yu et al. 2020).

Superoxide radical scavenging assay. Ten microliters (10 μL) of extracts/standards (250-1000 μg/mL) were loaded into a 96-well plate. To the plate, 100 μL of nicotinamide adenine dinucleotide reduced (0.468 mM), 100 μL of nitroblue tetrazolium (0.156 mM), and 50 μL phenazine methosulfate (0.06 mM) were sequentially added. The mixture was kept at RT for 5 min. Absorbance was measured at 560 nm. Quercetin was used as the positive control (Bueno and Yu 2021).

Hydroxyl radical scavenging assay. Five hundred microliters (500 μL) of extracts/standards (250-1000 μg/mL) dissolved in buffer were placed into the clean test tube. The prepared mixture, containing 100 μL 2-deoxyribose (28 mM in 20 mM KH_2PO_4 buffer, pH 7.4), 200 μL EDTA-ferric chloride mixture (1.04 mM EDTA and 200 μM $FeCl_3$, 1:1), 100 μL 1 mM hydrogen peroxide, and 100 μL 1 mM

ascorbic acid were added. The reaction vessel was kept for 1 hour at 37°C. One milliliter (1 mL) of thiobarbituric acid (1%) and 1 mL of trichloroacetic acid (2.8%) were sequentially added. The tubes were incubated for 20 minutes at 100°C. The resultant mixture was cooled and absorbances were determined at 532 nm against a blank containing deoxyribose and buffer. The positive control used was quercetin (Yu et al. 2020).

Phytochemical Screening

The phytochemical screening of fruit crude extracts, such as phenolics, flavonoids, tannins, saponin, alkaloids, reducing sugars, coumarins, steroids, terpenoids, anthraquinones, and quinones, was conducted using the standard procedures described in a previous study (Bueno and Yu 2021).

Statistical Analysis

Linear regression analysis was performed to calculate the IC_{50} values for scavenging assays, and the EC_{50} values for both reduction and chelation assays (Zhang et al. 2011; Itam et al. 2021). Statistical significance was assessed through one-way analysis of variance (ANOVA). Each assay was carried out in triplicates (Rumpf et al. 2023). Data were presented as the mean value with corresponding standard deviation (mean ± SD). A p-value of $P < 0.05$ was considered statistically significant difference.

RESULTS

Antioxidant Activities

DPPH scavenging activity. Methanol extracts of *A. racemosa* (tabon-tabon) fruit (86.13 ± 1.13%) exhibited excellent scavenging activity against DPP radical, like that of ascorbic acid (85.59 ± 3.65%) at 1000 μg/mL (Figure 2). Moreover, *A. racemosa* extract was notably effective, extending its efficacy at 85.32 ± 0.617% comparable to ascorbic acid ($P > 0.05$) even at the lowest dose of 250 μg/mL. The calculated median inhibitory concentrations (IC_{50}) for crude methanol extract and ascorbic acid were found to be 82.36 and 48.60 μg/mL, respectively.

Ferric ions (Fe^{3+}) reducing antioxidant power activity. Excellent reducing ability was observed in the *A. racemosa* methanol extract compared to that of butylated hydroxytoluene (BHT), achieving iron reductions of 94.56 ± 0.497% and 96.47% ± 0.788% at 1000 μg/mL, respectively. Notably, the extract displayed no statistical difference ($P > 0.05$) in activity compared to BHT at the highest concentration. The extract maintained its ~70% iron reduction activity even at 250 μg/mL (Figure 3). The calculated median effective concentration (EC_{50}) for crude methanol extract was 235.66 μg/mL while BHT demonstrated EC_{50} even lower than <100 μg/mL.

MTT reduction activity. Superior MTT reduction was displayed by *A. racemosa* methanol extract. At 250 μg/mL, *A. racemosa* methanol extract produced more formazan than curcumin, achieving MTT reductions of 62.62 ± 15.90% and 41.68 ± 9.064%, respectively. Notably, the extract displayed no statistical difference ($P > 0.05$) in activity compared to curcumin at the highest concentration (Figure 4). The extract maintained its good MTT reduction with an average of ~70% across all concentrations. The EC_{50} for crude methanol extract < 250 μg/mL while curcumin demonstrated EC_{50}= 426.94 μg/mL.

Metal chelation. Significantly low iron chelating activity (< 20%) was demonstrated by A. racemosa compared to EDTA ($P < 0.05$) (Figure 5). It showed relatively consistent chelation with inhibitions falling from 5.82 ± 0.091% – 10.86 ± 0.975%. On the other hand, EDTA showed significantly high iron chelation ($P < 0.05$), keeping its activity by > 90% at 250 μg/mL. The EC_{50} for crude methanol extract is >1000 μg/mL whereas EDTA had EC_{50} even lower than <100 μg/mL.

Superoxide radical scavenging activity. Low scavenging activity (< 20%) was observed in *A. racemosa* methanol extract at 1000 μg/mL (Figure 6). Quercetin demonstrated a dose-response radical scavenging activity of 33.71 ± 8.31% at 250 μg/mL. The scavenging potential of *A. racemosa* was only observed when concentrations were increased to 750 μg/mL (7.93 ± 3.86%) and 1000 μg/mL (17.53 ± 0.780%). The IC_{50} for crude methanol extract was >1000 μg/mL while quercetin displayed an IC_{50}=540.74 μg/mL.

Hydroxyl radical scavenging activity. Dose-dependent inhibition was demonstrated by *A. racemosa* methanol extract against hydroxyl radical (Figure 7). Quercetin standard sustained high scavenging rate of hydroxyl radical at ~70% even at 250 μg/mL. Though it exhibited significantly lower activity compared to quercetin ($P < 0.05$), it was still able to reach 50.20 ± 6.03% inhibition at 1000 μg/mL. The IC_{50} for crude methanol extract was 937.52 μg/mL while quercetin displayed an IC_{50} <100 μg/mL.

Phytochemical Content

Atuna racemosa holds a wide range of primary and secondary metabolites, which include carbohydrates, cardiac glycosides, steroids, terpenes/terpenoids, quinones, anthraquinones, flavonoids, phenolics, and tannin compounds as summarized in Table 1.

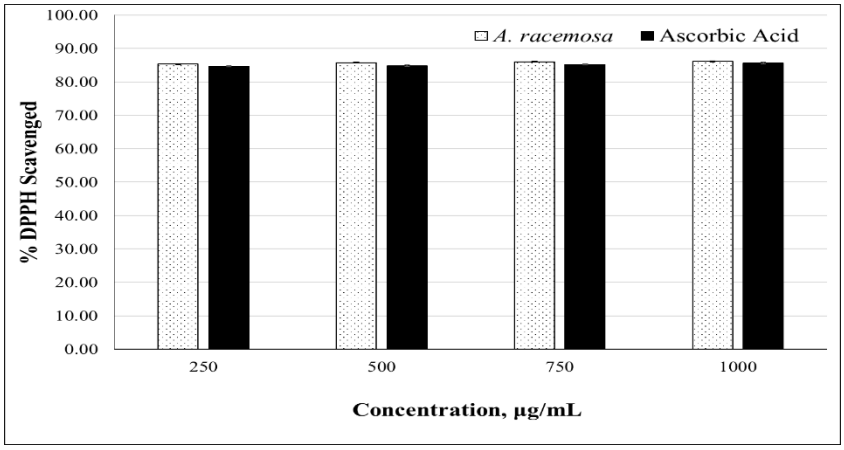

Figure 2. DPPH scavenging assay of *Atuna racemosa* Raf methanol fruit extract. Both *A. racemosa* and ascorbic acid show high percentages of DPPH scavenging across all concentrations tested (around 85-90%), indicating strong antioxidant activity. Each value represents mean ± standard deviation. Means were compared against the crude extract using one-way with $*P < 0.05$.

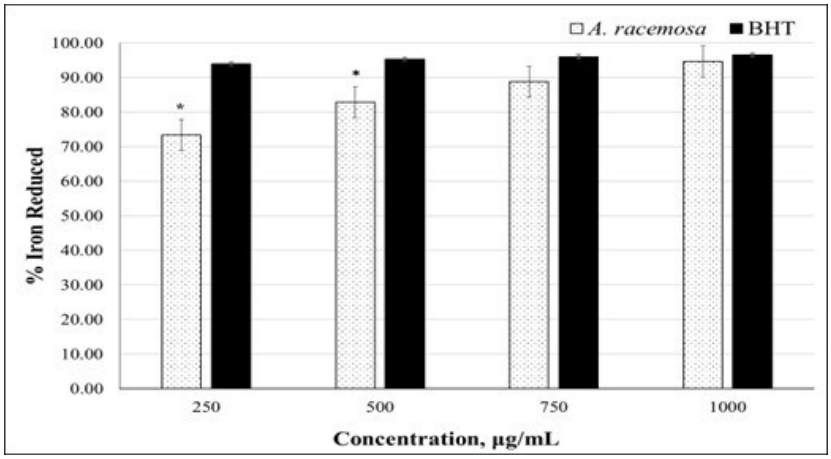

Figure 3. Reducing Power of *Atuna racemosa* Raf methanol fruit extract. *A. racemosa* methanol extract showed strong reducing power iron reduction comparable to butylated hydroxytoluene (BHT) at 1000 μg/mL and maintained approximately 70% reducing activity at 250 μg/mL. Each value represents mean ± standard deviation. Means were compared against the crude extract using one-way with $*P < 0.05$.

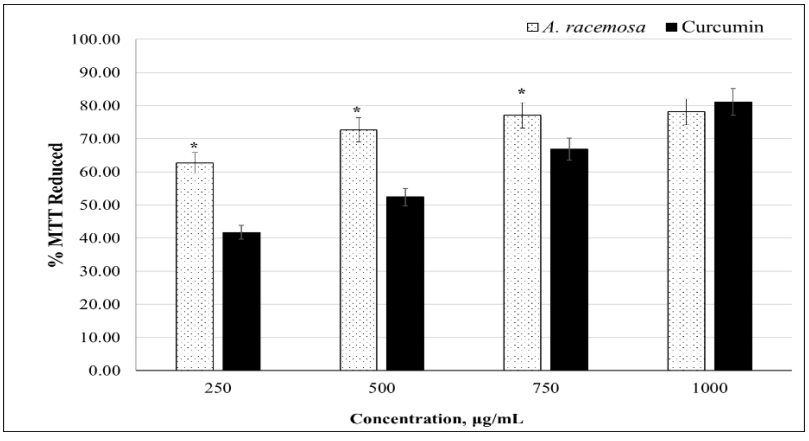

Figure 4. MTT Reducing Activity of *Atuna racemosa* Raf methanol fruit extract. *A. racemosa* methanol extract showed higher MTT-reducing activity than curcumin at 250 μg/mL and maintained good reduction across concentrations. Each value represents mean ± standard deviation. Means were compared against the crude extract using one-way with $*P < 0.05$.

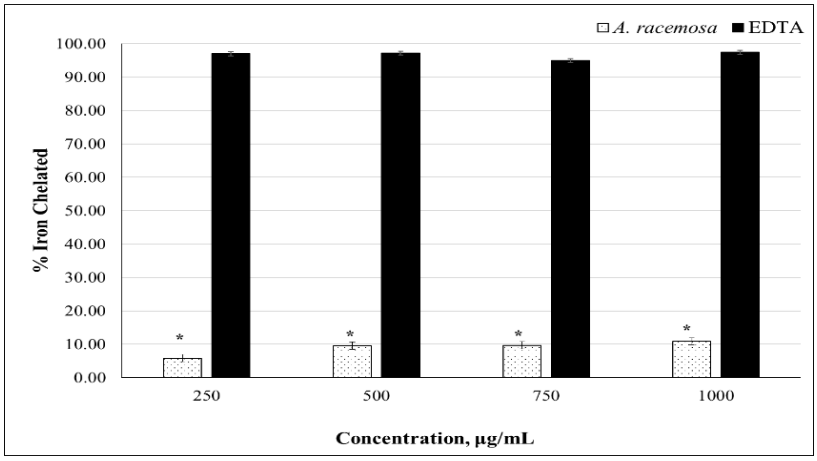

Figure 5. Metal chelating Activity of *Atuna racemosa* Raf methanol fruit extract. Significantly low iron chelating activity of less than 20% was demonstrated by *A. racemosa* compared to EDTA ($P < 0.05$). Each value represents mean ± standard deviation. Means were compared against the crude extract using one-way with $*P < 0.05$.

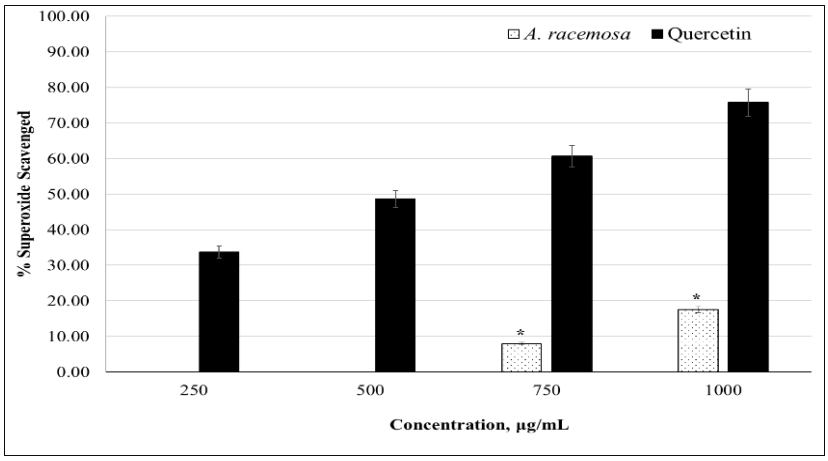

Figure 6. Superoxide radical scavenging activity of *Atuna racemosa* Raf methanol fruit extract. *A. racemosa* methanol extract displayed weak radical scavenging (<20% at 1000 μg/mL) than that of quercetin which exhibited stronger activity. Each value represents mean ± standard deviation. Means were compared against the crude extract using one-way with $*P < 0.05$.

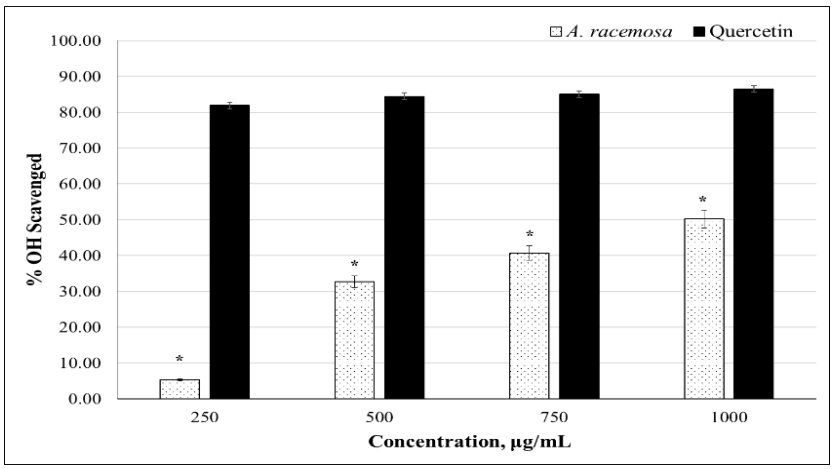

Figure 7. Hydroxyl radical scavenging assay of *Atuna racemosa* Raf methanol fruit extract. A. *racemosa* extract demonstrated increasing hydroxyl radical scavenging activity but has lower activity than quercetin across all concentrations tested. Each value represents mean ± standard deviation. Means were compared against the crude extract using one-way with $*P < 0.05$.

Table 1. Summary of phytochemical constituents present in *A. racemosa* methanol fruit extract. Legend: (−) not detected; (+) detected in low amount with low color intensity; (++) detected in large amounts with high color intensity.

Phytochemicals	Presence (+) or Absence (-)
Reducing Sugars and Carbohydrates	++
Proteins and amino acids	-
Alkaloids	-
Glycosides and cardiac glycosides	++
Steroids	+
Terpenes and terpenoids	+
Quinones	+
Anthraquinones	+
Flavonoids	++
Phenolics	++
Tannins	+
Saponins	-

DISCUSSION

Antioxidant Activity

Plants play a central role in nutrition and human well-being (El-Ramady et al. 2022). They are important sources of nutrients that support growth and development, and they have also been implicated in promoting plant-based remedies for disease prevention (Phan et al. 2018). Studies have highlighted that the mitigation of elevated oxidative stress brought about by excessive ROS may impede the progression of long-term medical conditions such as cancer, diabetes, obesity, and other chronic inflammatory problems (Khutami et al. 2022; Luo et al. 2022; Blagov et al. 2024).

Various assays *in vitro* were carried out to evaluate the antioxidant potentials of *A. racemosa* methanol fruit extract, as these test models vary in their mechanisms of action. Generally, antioxidant systems are classified either as primary antioxidants (ROS terminators and scavengers) or as secondary antioxidants (transition metal ion chelators) (Apak et al. 2016). Under primary antioxidant systems, radical termination involves the sacrificial consumption of antioxidants that may be inhibited by either single electron (e–) transfer (SET) or hydrogen atom transfer (HAT) mechanism (Siddeeg et al. 2021). Thus, antioxidant systems should not be limited to a single test (Bueno and Yu 2021).

General antioxidant tests, DPPH, FRAP, and MTT, were measured for *A. racemosa* methanol extract. DPPH, a stable, synthetic free radical, is a popular test to determine the extracts' antioxidant capacity (Sridhar and Charles 2019). This method is a highly relevant test as it provides a simple, cost-effective, and robust way to screen natural products. This involves the reduction of deep purple DPPH radical to a yellow-colored solution upon interaction with antioxidant compounds (Gulcin and Alwasel 2023). The current findings showed that *A. racemosa* methanol extracts displayed high scavenging activity against DPPH radicals, which conform with other studies on its polar extracts (Gicole et al. 2019; Nadayag et al. 2019). On the other hand, reducing ability was assessed using FRAP and MTT assays. The FRAP assay quantifies antioxidant capacities based on the conversion of Fe^{3+} to Fe^{2+} by electron donation, resulting in a blue complex formation (Bueno et al. 2023). The MTT assay, commonly used for cell viability experiments, has also been developed as a non-cell-based method to effectively screen antioxidant activity. In this system, antioxidant compounds reduce the yellow MTT compound to a purple formazan, providing association to inhibitory effects against lipid peroxidation (Liu and Nair 2010). In both FRAP and non-cell-based MTT, higher absorbance for FRAP and MTT relates to a stronger reducing power. Superior reductions of ferric ion (Fe^{3+}) and MTT were observed in *A. racemosa*, indicating good single electron donation with potential oxidants and converting them into stable metabolites (Ou et al. 2002; Bueno et al. 2023). The fruit of *A. racemosa* fruit demonstrated significant DPPH scavenging activity and reducing power, which can be attributed to its ability to donate electrons and hydrogen atoms. By conversion of free radicals to a non-reactive form, this maintains cellular redox homeostasis and keeps biomolecules structural integrity from detrimental effects of oxidative damage (Chandimali et al. 2025).

Secondary antioxidants retard oxidation through the process of chelation (Gulcin and Alwasel 2022). Transition metals, like iron and copper, can also initiate oxidative damage at elevated concentrations

through Fenton the reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH\bullet$) (Apak et al. 2016). In biological system, this process increases the speed of lipid peroxidation, producing elevated levels of lipid hydroperoxides (LOOH). In this study, metal chelation was assessed by monitoring the reduction of the red violet Fe^{2+}/ferrozine complex formation (Gulcin and Alwasel 2022). Although *A. racemosa* demonstrated limited iron chelation, it may still complement the action of primary antioxidants in neutralizing ROS.

In normal cellular processes, ROS regulates diverse biochemical signaling networks, namely inflammatory responses, cell replication, and apoptosis (De Almeida et al. 2022). The ROS comprises a range of species, from unpaired electron types, like superoxide anions and hydroxyl radical radicals, to molecules with strong oxidizing properties, such as hydrogen peroxide (H_2O_2). Superoxide ($O_2\bullet^-$) is a one-electron radical byproduct of normal aerobic metabolism derived from molecular oxygen (Fujii et al. 2022). While it is short-lived, it stimulates the formation of oxygen-related intermediates and other radical species such as hydroxyl radicals (Abdal Dayem et al. 2017). Hydroxyl radical (HO•) is a strong oxidizing agent (known for its high 1-electron reduction potential) produced from the Fenton reaction (Tvrdá and Benko 2020). While ROS are crucial for normal cellular processes, their overproduction can disturb redox equilibrium, which may lead to harmful interaction with biomolecules (Juan et al. 2021). Elevated concentrations may reduce disulfide bonds, inducing abnormal protein folding (Bueno et al. 2023). Hence, the elimination of these species is deemed crucial.

In this study, superoxide and hydroxyl radicals were generated *in vitro* and their scavenging were evaluated through colorimetric tests. Superoxide radicals were created through reduction of phenazine methosulfate (PMS) by NADH, subsequently reducing nitroblue tetrazolium (NBT) to form a colored blue formazan (Bueno and Yu 2021). Hydroxyl radicals were produced via Fenton-like reaction from the interaction of Fe^{3+} ions and hydrogen peroxide in the presence of ascorbic acid. The resulting HO• reacts with 2-deoxy-D-ribose to form malondialdehyde, followed by reaction with thiobarbituric acid (TBA) to produce a pink chromogen. Antioxidant capacities were gauged by the reduction in absorbance, proportional to radical scavenging activity (Richards and Chaurasia 2022). Scavenging of both superoxide and hydroxyl radicals was demonstrated by *A. racemosa* methanol extract. Moreover, it was able to reduce hydroxyl radicals in a concentration-dependent manner. This suggests that *A. racemosa* possesses bioactive metabolites that can quench specific radicals, which may eliminate the overall ROS-associated damage in biological systems (Hussain et al. 2016).

Atuna racemosa is a native plant widely distributed species in the Malay Peninsula and the Pacific Islands (Prance 2004). Literature has mentioned that *A. racemosa* is also known in other synonymous names such as *Parinari laurina* A. Gray*, Parinari curranii Merr., Parinari glaberrima* Hassk.*,* and *Cyclandrophora glaberrima* Hassk. to name a some (Steenis and Steenis-Kruseman 1950; Kew Science 2016). Though *A. racemosa* has been explored for its pharmacological benefits, most studies focused on its antimicrobial activities (Buenz et al. 2007; Pacaña and Galarpe 2017; Nadayag et al. 2019; Tila et al. 2022). Limited studies have studied its detailed antioxidant mechanism. Ang and Deocampo (2019) reported that good DPPH inhibition as well as iron reduction were exhibited by acidified, aqueous endosperm extracts of *A. racemosa* exposed at various storage temperature and time points. Baltazar et al. (2024) mentioned that the fruit (177.99 mg AAE/g) exhibited the highest total antioxidant capacity, an amount even doubled among the other plant parts (leaves, buds and twigs) measured via phosphomolybate method. The polar leaf extracts of *A. racemosa,* both queous and hydroalcoholic, showed superior antiradical activity extending ~90% potency even at 500ppm (Gicole et al. 2019). Methanol fruit pulp extract of *Atuna* related species, *Parinari curatellifolia,* exhibited potent DPPH inhibition and strong FRAP value (Nkosi et al. 2022; Mwamatome et al. 2023). Thus, this highlights the importance of the current study as it employed a range of antioxidant assays, ensuring a comprehensive evaluation of the *A. racemosa* antioxidant properties.

Phytochemical Content

Plant-based foods hold not only necessary macro- and micronutrients but also high amounts of bioactive secondary metabolites, known as phytochemicals (Elshafie et al. 2023). Many of these phytochemicals work as superior antioxidant systems and are vital in neutralizing reactive oxygen species (ROS) (Chen et al. 2022). In the study, methanol extract of *A. racemosa* contained various secondary metabolites where its antioxidant activity is accounted. These phytochemicals present corroborate with the previous study of Pacaña and Galarpe (2017) where the methanol extracts contained flavonoids, tannins, terpenoids, and anthraquinones. Baltazar et al. (2024) revealed that polyphenol is abundant in *A. racemosa* plant parts, with their amounts high in fruit followed by buds, leaves, and twigs. Gicole et al. (2019) determined that flavonoids and tannins are rich polar leaf extracts of *A. racemosa.* Its bark extract contained high levels of polyphenolic compounds, specifically flavonoids (Nadayag et al. 2019). Prance (2004)

mentioned that *Atuna* oil, tradionally used as a massage oil for inflammation, has flavan-3-ol derivatives of flavonoids (4'-MeO-(-)-gallocatechin and (+)-gallocatechin). The *Parinari* genus, a closely related genus to Atuna, contains several phytochemicals, which include flavonoids, phenolic compounds, tannins, anthraquinones, triterpenoids, steroidal and kaurene-type diterpenoid compounds (Brew-Daniels and Harrison 2025). Particularly, phenolic acids (ellagic acids, chlorogenic acids, 3-O-p-coumaroylquinic acid, and caffeic acid) and flavonoids (quercetrin, rutin, and kaempferol) are reported to be abundant (Nkosi et al. 2022; Kaseke et al. 2025). Polyphenolic compounds, as well as flavonoids, greatly contribute to overall antioxidant activity (Rudrapal et al. 2022). The presence of the double bonds within the aromatic ring and the corresponding hydroxyl groups attached to it facilitates polyphenol inactivation of the resident radical (Huang et al. 2023). Radical scavenging activities of flavonoids are greatly attributed to the presence of hydroxy and oxo groups attached to the B and C ring system respectively (Hassanpour and Doroudi 2023). The -OH groups found at the 3rd and 5th position on quercetin glycosides and procyanidins aids in the scavenging mechanism (Kaseke et a. 2025). Moreover, Kaseke et al. (2025) stated that this scavenging roles of polyphenolic compounds allows them to also chelate metals, preventing metal-mediated enzymatic reactions that may contribute to DNA damage and lipid peroxidation. Tannins and terpenoids, as identified in the study and coherent with the phytochemical analysis of Tila et al. (2022), may also be contributing factors to the observed antioxidant potential. Research mentioned that tannins' ability to neutralize radical species to their polyhydroxyl functional groups while the exact mechanism is still not known. On the other hand, the conjugated double bonds of terpenoids allow radical termination (Gutiérrez-del-Río et al. 2021).

Collectively, these results revealed the *A. racemosa* fruit extract contained promising antioxidant compounds that mitigate reactive oxygen species effectively. The bioactive compounds neutralize ROS and terminate radical chain reactions by acting as hydrogen donors, radical quenchers, metal chelators, or combinations of these roles (Nadayag et al. 2019; Siddeeg et al. 2021; Tila et al. 2022). These extracts' capacity to relieve oxidative stress may play a central role in addressing ROS-mediated health conditions such as cancer, diabetes, and other chronic inflammatory problems (Khutami et al. 2022; Luo et al. 2022; Blagov et al. 2024). These results offer a foundational knowledge of the fruit's antioxidant ability, underlining its possible application as a natural source of health-enhancing molecules. Moving forward, it is recommended that specific bioactive components in this fruit extract be isolated and characterized through chromatographic and spectroscopic techniques. Future studies may also focus on exploring the potential synergistic interactions among phytochemicals, as combinatorial effects may improve overall bioactivity. Another good avenue for research entails the application of *A. racemosa* fruit extract in *in vivo* studies to corroborate its effects in a biological system.

In conclusion, the methanol extract of *A. racemosa* fruit exhibited high antioxidant activities through scavenging relevant radicals, reducing chemical species, and chelating metal ions. These bioactivities were attributed to various antioxidant-rich phytochemical components within *A. racemosa* fruit. This study features the *A. racemosa* fruit as a natural source of antioxidants and may be considered as a promising candidate for innovation as a new food ingredient with functional benefits that may aid the prevention of oxidative stress-related disorders.

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This document was prepared without the use of generative artificial intelligence for content creation. Language-based AI tools such as Grammarly and QuillBot were only used for grammar checking. The ideas, analyses, interpretations, and conclusions presented in this document are the original work of the authors.

DECLARATION OF COMPETING INTEREST

The authors declare that there is no competing interest to any authors.

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Inhibition of ESKAPE bacterial pathogens by endolichenic fungi *Nemania bipapillata* and *Xylaria badia* associated with the fruticose lichen *Ramalina* spp.

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ABSTRACT

Endolichenic fungi (ELF), filamentous fungi that live asymptomatically within the lichen thalli, hold promise for combating ESKAPE bacteria — *Enterococcus faecalis* Schleifer and Kilpper-Bälz 1984, *Staphylococcus aureus* Rosenbach 1884, *Klebsiella pneumoniae* Trevisan 1887, *Acinetobacter baumannii* Bouvet and Grimont 1986, *Pseudomonas aeruginosa* Migula, and *Enterobacter aerogenes* Hormaeche and Edwards 1960 (current name: *Klebsiella aerogenes* Tindall et al. 2017). Twelve ELF from *Ramalina* were cultured in rice medium and potato dextrose broth (PDB), extracted with ethyl acetate, and assessed for antibacterial activity using a disc diffusion assay against ESKAPE bacterial pathogens. Specifically, 40 μL of 10 mg/mL crude extracts were impregnated onto each disc and placed on 15 mL Mueller-Hinton agar plates previously swabbed with 1 mL of bacterial suspension equivalent to a 0.5 McFarland standard. Crude culture extracts of *Xylaria badia* Pat. grown in PDB showed promising inhibitory activities against *E. faecalis* and *S. aureus*, with zones of inhibition (ZOI) of 18.17 mm and 21.70 mm, respectively. In addition, crude culture extracts of *Nemania bipapillata* (Berk. & M.A.Curtis) Pouzar grown in rice medium showed weak inhibition against *A. baumannii* (10.23 mm ZOI). These findings support the potential of endolichenic fungi, particularly *X. badia* and *N. bipapillata,* as sources of secondary metabolites active against ESKAPE bacterial pathogens.

Keywords: bioprospecting, disc diffusion assay, fruticose lichen, Philippines

The fruticose lichen *Ramalina* is widespread globally (Kirk et al. 2010), with extensive studies on its bioactivity, e.g., antimicrobial (Moreira et al. 2015), herbicidal (Gazo et al. 2019), cytotoxic (Koopaie et al. 2023), and insecticidal (da Silva et al. 2021). Moreover, endolichenic fungi associated with the thalli of *Ramalina* showed antioxidant activities (Galinato et al. 2021). In the study of Santiago et al. (2021a, 2022), endolichenic fungus (ELF) produced metabolites that inhibited Gram-negative bacteria, as opposed to the lichen acids produced by their lichen hosts, which target mainly Gram-positive bacteria.

The fungus *Nemania* has been isolated as an ELF in the fruticose lichen *Usnea* (Santiago et al. 2021a, 2022), as well as in the foliose *Parmotrema rampoddense* (Nyl.) Hale. (Tan et al. 2020), although it can also occur as an endophytic fungus in *Aquilaria sinensis* (Lour.) Spreng. (Kumarihamy et al. 2019; Tibpromma et al. 2021). On the other hand, *Xylaria* is known as an endophyte, e.g., of *Gingko biloba* L. (Suryelita et al. 2021), *Haematoxylum brasiletto* H. Karst (Sánchez-Ortiz et al. 2016), and *Geophila repens* (L.) I.M. Johnst. (Rajendran et al. 2023), and as an endolichenic fungus, e.g., of *Cladonia* (Cañón et al. 2019) and *Usnea* (Santiago et al. 2021a, 2022). Owing to the prolific production of secondary metabolites by ELF and the documented bioactivities of its lichen host, we assessed the antibacterial activities of selected *Xylaria* and *Nemania* species associated with the lichen *Ramalina* to contribute to the growing list of endolichenic fungi targeted for bioprospecting.

Endolichenic fungi were isolated from two *Ramalina* species, i.e., *Ramalina* sp. and *Ramalina* cf. *farinacea*, following the protocol of Paranagama et al. (2007). Briefly, lichen thalli were surface sterilized by successive dipping in 96% ethanol (10 sec), 0.5% NaOCl (2 min), and 70% ethanol (2 min), and then plated after rinsing on Dichloran Rose Bengal Chloramphenicol agar plates. Colonies of six *Nemania* and six *Xylaria* isolates were selected and characterized morphologically on Malt Extract Agar (MEA) and Corn Meal Agar (CMA). Colonial growth was measured daily, and the mean colony extension rates (MCER) were computed following the method of dela Cruz et al. (2006). To confirm species identity, the isolated ELF were sent to Macrogen, South Korea, for sequencing using the primer pairs internal transcribed spacer 4 (ITS4) and ITS5 for the nuclear ribosomal (ITS) region. For phylogenetic analysis, phylogenetic trees were generated and constructed using the Randomized Axelerated Maximum Likelihood program with 1000 bootstrap values through the TrEase Web Service.

The twelve *Ramalina*-ELF were cultivated using solid-state and submerged liquid-state fermentation to produce secondary metabolites. For solid-state fermentation, 60 g of long-grain white rice in 90 mL distilled water were prepared in culture bottles and inoculated with ten agar blocks of seven-day-old ELF cultures. The culture bottles, in duplicate, were incubated for 30 days at room temperature. For submerged liquid-state fermentation, ten mycelial agar blocks of each ELF isolate were aseptically inoculated into 120 mL potato dextrose broth (PDB) (in duplicate) and cultured for 14 days under a rotary shaker (100 rpm) at room temperature. Secondary metabolites were extracted with 120 mL analytical-grade ethyl acetate and concentrated in vacuo using a rotary evaporator (80 rpm) at 45°C. Finally, the crude culture extracts were air-dried and then reconstituted with methanol at a concentration of 100 mg/mL.

The antibacterial activity was tested using the following bacteria: *Enterococcus faecalis* Schleifer and Kilpper-Bälz 1984 UST-CMS 10029, *Staphylococcus aureus* Rosenbach 1884 UST-CMS 1090, *Klebsiella pneumoniae* UST-CMS 1194, *Acinetobacter baumannii* Bouvet and Grimont 1986 UST-CMS 10005, *Pseudomonas aeruginosa* Migula UST-CMS 10013, and *Enterobacter aerogenes* Hormaeche and Edwards 1960 UST-CMS 1021 (current name: *Klebsiella aerogenes* Tindall et al. 2017), which were generously provided by the University of Santo Tomas Collection of Microbial Strains. Each bacterium was prepared in Mueller-Hinton Broth as an inoculum equivalent to a 0.5 McFarland standard. Then, 1 mL of the standardized bacterial suspension was swabbed onto 15 mL Mueller-Hinton Agar (MHA) plates in triplicate, onto which paper disks (6 mm) containing 40 μL of the crude culture extracts were placed. Briefly, 40 μL of the crude culture extracts were impregnated into blank discs in two aliquots of 20 μL followed by a 30-minute interval between applications to allow the solvent to evaporate and ensure uniform absorption. Disks containing erythromycin (15 μg, positive control), methanol (negative control), and extracts of uninoculated rice medium or PDB (negative control) were used as controls. All culture plates were incubated at 37°C for 24 hours, and the zones of inhibition (ZOI) for each disk were measured.

The six *Ramalina*-ELF isolates described as *Xylaria* generally exhibited white-gray, opaque colonies with cottony and velvety surface textures on MEA, with a usually white-cream underside (Figure 1). The colony diameter ranged from 18 to 30 mm on day four. When grown on CMA, these *Ramalina*-ELF had a general colonial morphology that was cottony and powdery, with a white-gray surface color and a white-gray to white-yellowish underside. The colony diameter ranged from 14 to 22 mm. On the other hand, the six *Ramalina*-ELF described as *Nemania* were cottony and velvety on MEA, with colony diameters of 19 to 24 mm after four days of incubation (Figure 1). When grown on CMA, the colony diameter ranged from 11 to 28 mm. When the mycelial colony extension rate was computed, most *Ramalina*-ELF isolates exhibited faster growth on CMA than on MEA (Figure 2). This finding is unexpected, given the limited nutrient content of CMA compared with MEA.

Of the twelve *Ramalina*-ELF, only ten were successfully sequenced and submitted to National Center for Biotechnology Information for accession numbers. Phylogenetic analysis identified these as: *Xylaria badia* Pat. [D1A.2, PV413362], *Xylaria grammica* (Mont.) Mont. [D2A.5.2, PV425956], *Nemania* sp.1 [D2C.3.2, PV425957], *Xylaria laevis* Lloyd [D3D.4.1, PV425959], *Nemania primolutea* Y.M. Ju, H.M. Hsieh & J.D. Rogers [D4A.4, PV425958;

D6B.2, PV425978], *Nemania bipapillata* (Berk. & M.A. Curtis) Pouzar [D4D.1, PV425960; D5D.5.2, PV425962], *Nemania* sp.2 [D5C.2.2, PV425961], and *Xylaria feejeensis* (Berk.) Fr. [D6D.1.1, PV425977]. The *Nemania* isolates formed two clades (Figure 3). Two ELF clustered with *N. bipapillata* with high bootstrap support, while another *Nemania* formed a distant group. Two ELF also clustered with *N. primolutea*. In previous reports, the separation of *Nemania* from *Annulohypoxylon*, *Daldinia*, and *Hypoxylon,* and its close relationship with *Xylaria,* was established only by combined data of ITS and *rpb2* genes (Tang et al. 2007), but may require detailed morphological and multi-gene phylogenetic analyses using ITS, α-actin, *rpb2,* and β-tubulin genes (Pi et al. 2021); hence, the use of additional reference genes may shed light on the correct placement of our *Nemania* isolates.

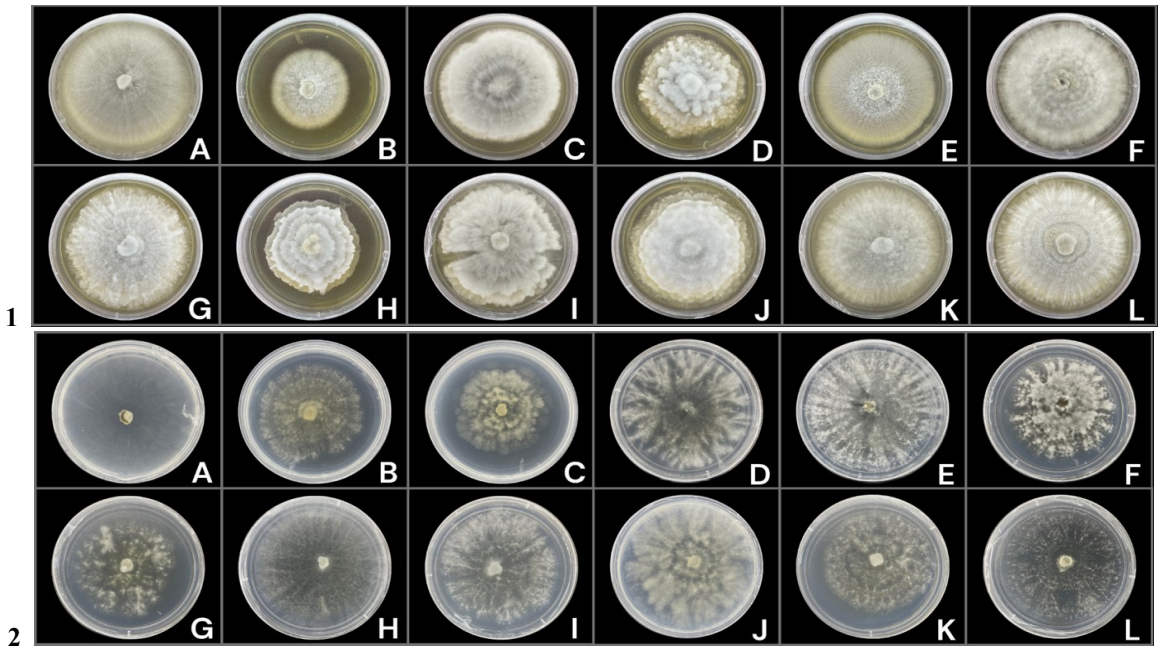

Figure 1. Colonial growth of the endolichenic fungi grown on (1) malt extract agar and (2) corn meal agar. A. *Xylaria badia* (D1A.2) B. *Xylaria* sp. 1 (D1B.3) C. *Xylaria grammica* (D2A.5.2) D. *Xylaria* sp. 2 (D2C.3.2) E. *Nemania* sp. 1 (D3C.5.1) F. *Xylaria laevis* (D3D.4.1) G. *Nemania primolutea* 1 (D4A.4) H. *Nemania bipapillata* 1 (D4D.1) I. *Nemania* sp. 2 (D5C.2.2) J. *Nemania bipapillata* 2 (D5D.5.2) K. *Nemania primolutea* 2 (D6B.2) L. *Xylaria feejeensis* (D6D.1.1)

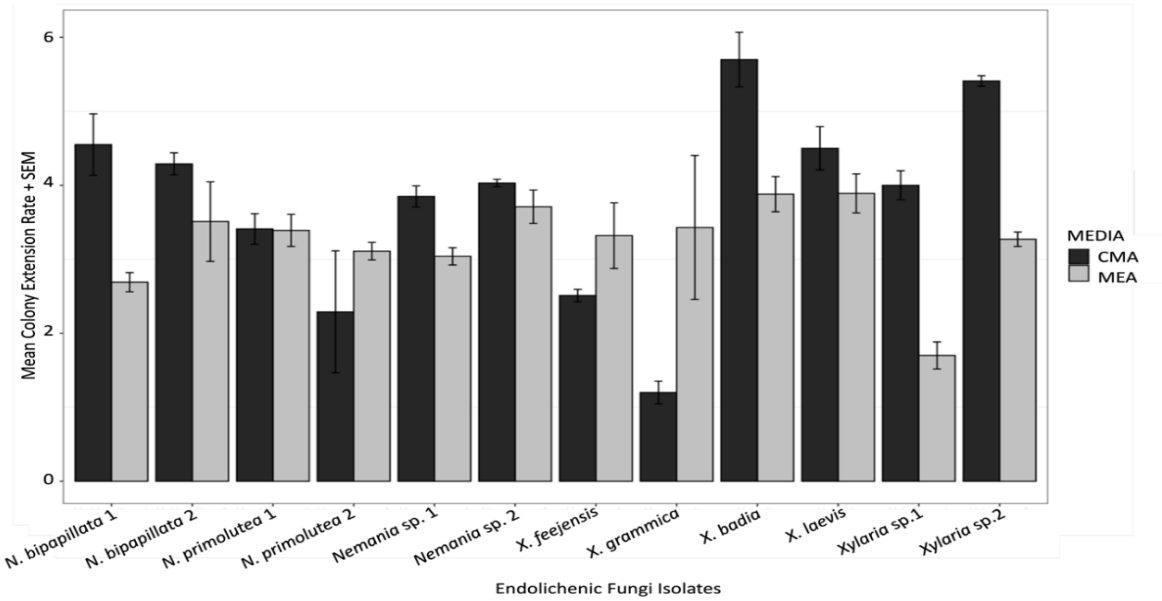

Figure 2. Growth rates of ELF isolates in corn meal agar (CMA) and malt extract agar (MEA) expressed as mean colony extension rates.

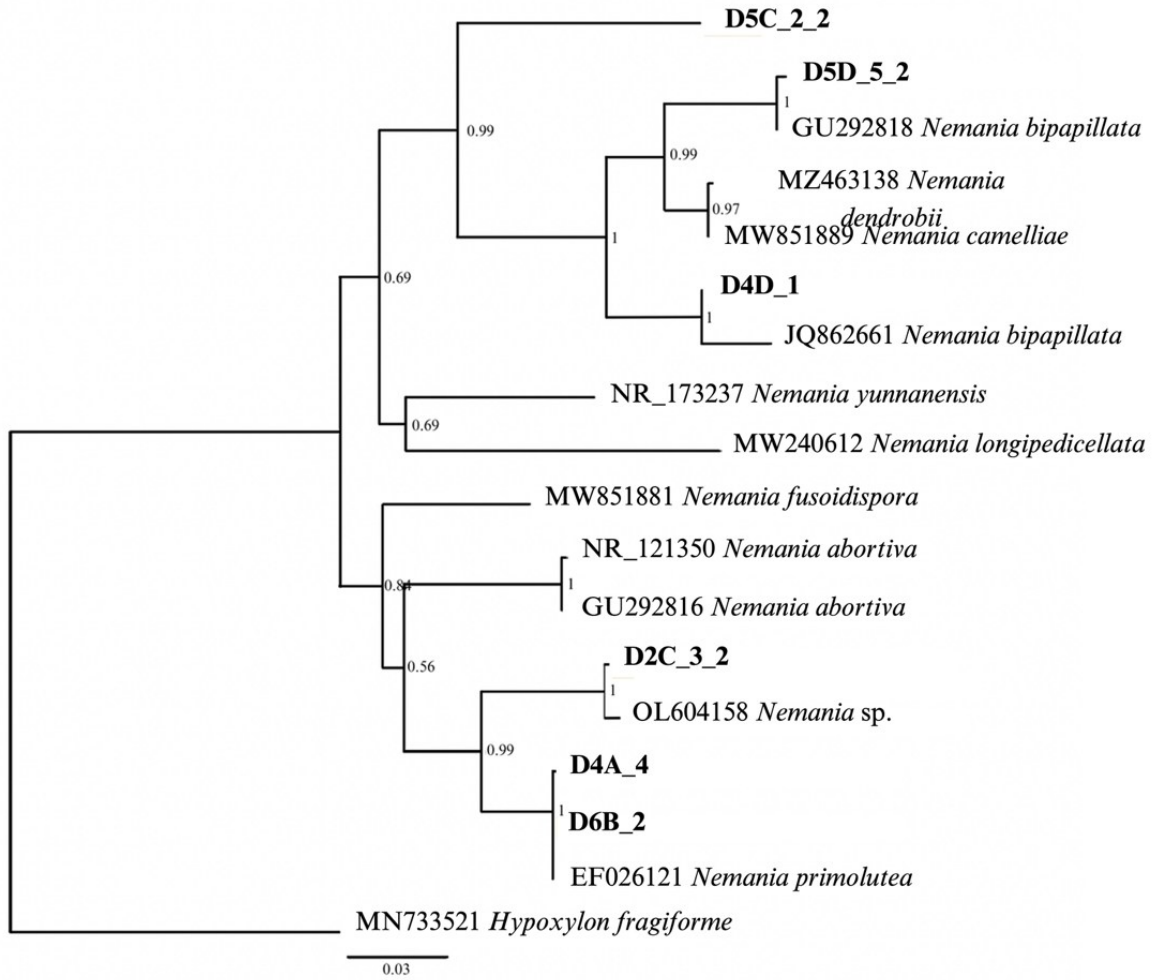

Figure 3. Phylogenetic tree of our isolated *Ramalina* endolichenic fungi within the genus *Nemania*. Accession codes are as follows: D4A.4 (PV425958), D6B.2 (PV425978), D2C.3.2 (PV425957), D4D.1 (PV425960), D5C.2.2 (PV425961), D5D.5.2 (PV425962).

Only four *Xylaria* isolates were successfully sequenced and were placed as closely related to *X. feejeensis* with moderately supported low posterior probability, to *X. grammica*, also with low support, and to *X. laevis* and *X. badia*, both with relatively good support (Figure 4). Similarly, ITS coupled with *rpb2* genes were used to resolve the identities of *Xylaria* (Lee et al. 2000; Pan et al. 2022).

Contrary to our expectation, only the crude culture extract of *X. badia* (D1A.2) grown in PDB showed inhibitory activities against *S. aureus* (ZOI = 21.7 ± 0.61 mm) and *E. faecalis* (ZOI = 18.17 ± 1.73 mm) (Figure 5). The crude culture extracts of *Nemania* sp. (D5C.2.2) grown in rice medium also showed minimal inhibitory activity against *A. baumanii* (ZOI = 10.23 ± 0.85 mm). These extracts were compared with the negative control, the solvent methanol, which showed no zone of inhibition as outlined in Guevara (2005). Nevertheless, our study confirmed the antibacterial activities of endolichenic fungi from *Ramalina,* specifically the crude culture extracts of *N. bipapillata* and *X. badia,* against ESKAPE bacterial pathogens, as similarly observed with those isolated from other lichen hosts, e.g., *Parmotrema* and *Usnea* (Tan et al. 2020; Santiago et al. 2021a, 2022). While inhibition was observed, the study does not report any detailed quantitative measures, e.g., MIC values or bacterial counts before and after treatment. Of particular interest are species of *Xylaria* that produce bioactive metabolites that inhibit pathogenic bacteria and are distinct from those of their lichen hosts (Santiago et al. 2021b). Endophytic *Xylaria* also showed varied bioactivities (Orachaipunlap et al. 2016). The ELF has also been shown to have other pharmacological potentials, e.g. antioxidant activities (Galinato et al. 2021). However, the varying degrees of bioactivity reported in our ELF culture extracts could be due to the media used for fermentation. For example, our *Ramalina*-ELF identified as *N. primolutea* did not exhibit any

antibacterial activity, whereas the same species from the lichen *P. rampoddense* showed antibacterial activity against *E. faecalis* and *S. aureus* (Tan et al. 2020). This indicates that the observed inhibitory activity was strain-dependent. In that study, malt extract broth was also used as a fermentation medium, as opposed to PDB and rice medium. Frisvad (2012) and Vandermolen et al. (2013) noted that growth media and incubation conditions greatly influence secondary metabolite production. Thus, it is recommended to characterize the bioactive metabolites in the crude extract to identify compounds responsible for the antibacterial activity. It is also recommended to study the possible mechanism of action for each of the bioactive crude culture extracts. Although there is no consensus on which medium is optimal for bioactive metabolite production, one should note that the use of varying media and conditions may lead to variations in secondary metabolite production and should therefore be considered in any bioprospecting research.

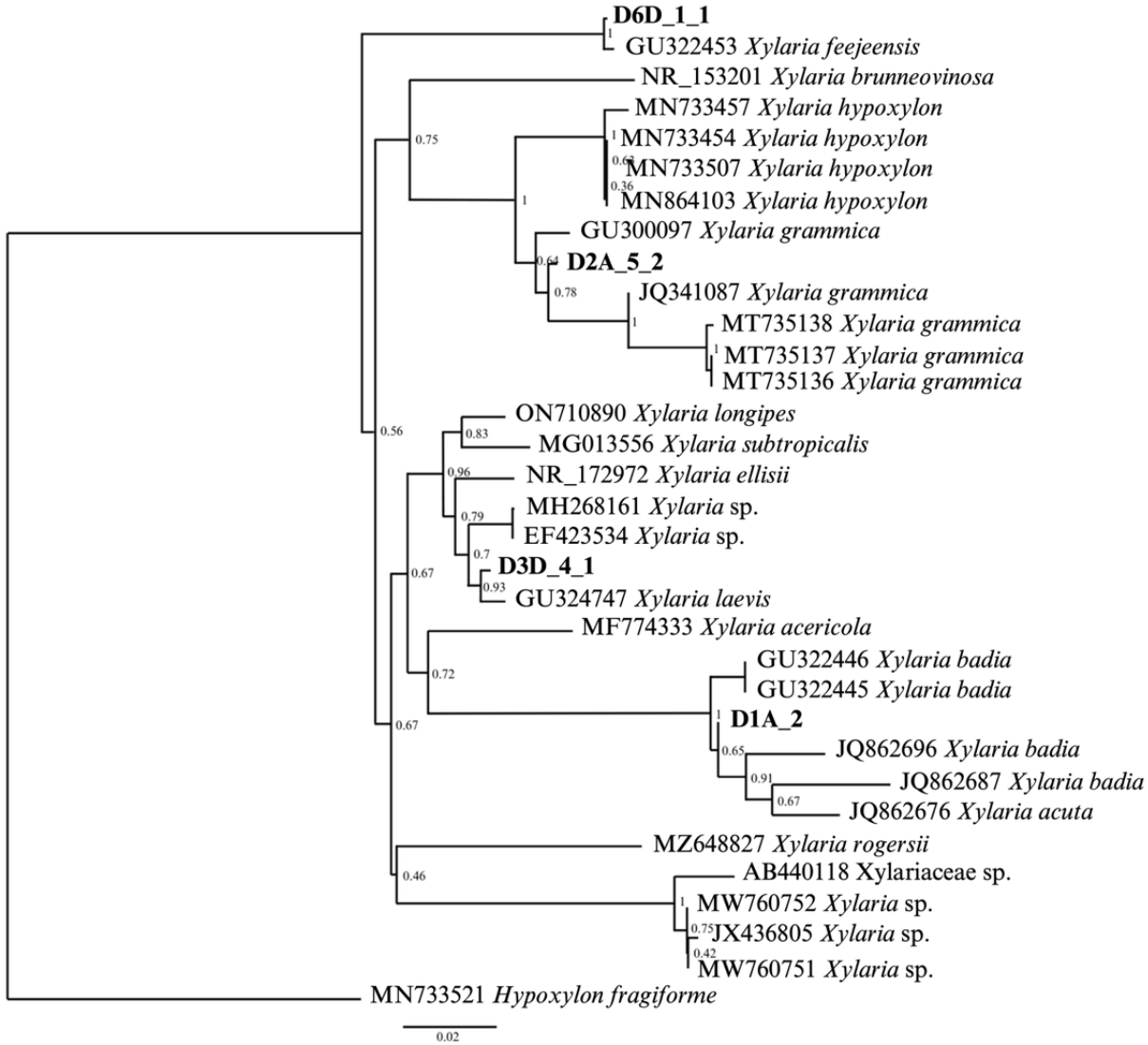

Figure 4. Phylogenetic tree of our isolated *Ramalina* endolichenic fungi within the genus *Xylaria*. Accession codes are as follows: D1A.2 (PV413362), D2A.5.2 (PV425956), D3D.4.1 (PV425959), D6D.1.1 (PV425977).

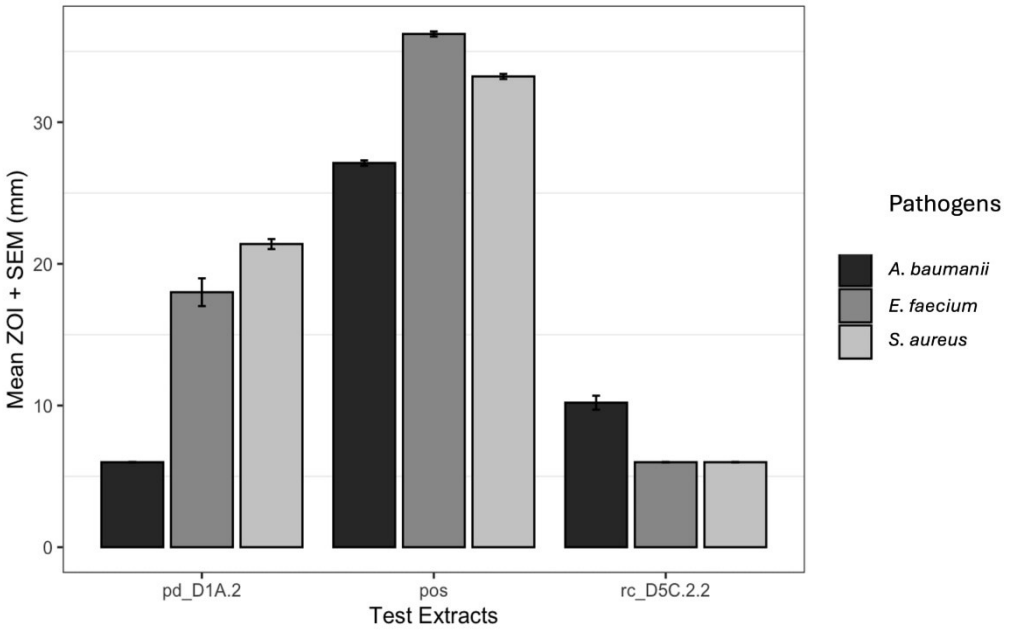

Figure 5. Mean zone of inhibition (ZOI), expressed in mm ± standard error of the mean (SEM), of the crude culture extracts of *Nemania bipapillata* (D5C.2.2) grown in rice medium (rc) and *Xylaria badia* (D1A.2) grown in PDA (pd), with erythromycin as the positive control (pos).

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GENERATIVE AI STATEMENT

We declare that this manuscript was prepared without the assistance of artificial intelligence. Hence, the content of this paper is original.

ETHICAL CONSIDERATIONS

No ethical clearance is required for this study.

DECLARATION OF COMPETING INTEREST

We declare no conflict of interest to disclose.

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Bacterial contamination of *Salmonella* spp. and *Escherichia coli* (Migula, 1895) in fresh chicken meat and chicken-based street food sold in the City of Mati

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ABSTRACT

The increasing popularity of chicken-based street food among low-to-middle-income consumers in the City of Mati, Davao Oriental, Philippines, raises serious health concerns due to the potential risk of foodborne illnesses. Despite rising poultry consumption, microbial assessment of street-vended chicken products is not routinely conducted, creating a significant knowledge gap in local food safety monitoring. This study aimed to determine the presence of *Salmonella* spp. in fresh chicken meat and isaw and *Escherichia coli* (Migula, 1895) in fried chicken, "isaw," and "kwek-kwek" sold by randomly selected street vendors. Samples were collected using standard aseptic techniques. Salmonella Shigella agar and Eosin methylene blue (EMB) agar were used to identify the target bacteria. The most probable number (MPN) method, based on Department of Agriculture-National Meat Inspection Service (DA-NMIS) Circular No. 9-2008-5, was used to detect the presence of *E. coli* in fresh chicken meat. Total plate count (TPC) was used to detect the presence of *Salmonella* spp. in fresh chicken meat and isaw and to detect *E. coli* in fried chicken, "isaw," and "kwek-kwek," following standards set by DA-NMIS and the Department of Health-Food and Drugs Administration (DOH-FDA) Circular No. 2022-12-2. The results showed *Salmonella* spp. in all fresh chicken and "isaw" samples, exceeding the DA-NMIS absence requirement in 25 g. *Escherichia coli* in fresh chicken meat was within the 500 MPN g^{-1} limit. However, TPC values in fried chicken, "isaw," and "kwek-kwek" exceeded the 100 CFU g^{-1} DOH-FDA limit. These findings revealed significant bacterial contamination in fresh chicken meat and popular chicken-based street foods, underscoring the urgent need for stronger implementation of regulation, regular microbial monitoring, and food safety education to support local public health efforts and guide future policy enhancement.

Keywords: bacterial load, food hygiene, food safety, health risk, microbial limit, street food

INTRODUCTION

Poultry meat is an important industry that flourished out of the necessity for affordable and low-fat protein sources. Currently, Philippine poultry production has increased and currently dominates the food industry, which reflects the growing demand for poultry products (DA-BAR 2022). Poultry meat is also a popular main ingredient for several street foods, such as barbecue, "isaw," (an offal-based food product made from processed and fried poultry intestines) and fried chicken, while chicken eggs are the base material

of the popular street food, "kwek-kwek" (deeped fried hardboiled egg coated with flour). These street foods are popular with low-to-middle-income consumers and students because of their cheap and ready-to-eat nature. However, bacterial contamination has been well established in these kinds of foods, thereby raising public health concerns (Rouger et al. 2017). For example, *Escherichia coli* (Migula, 1895) contamination was documented in "kwek-kwek" sold by vendors along the University of Eastern Philippines (Dagalea et al. 2021), while *E. coli* and *Salmonella* (Lignières, 1900) were detected in Laguna street-vended grilled and fried meat (Manguiat and Fang 2013).

Bacterial contamination in food is a public health threat because it can cause illness and death. The World Health Organization (2015) estimates 600 million foodborne disease cases, with 420,000 deaths a year resulting from unsafe food globally. The 2005-2018 consolidated data in the Philippines documented 209 foodborne disease outbreaks, and meat-based dishes account for 14.35% of these outbreaks (Azanza et al. 2019). Among the foodborne pathogens are coliform bacteria, which have notorious variants that can lead to health complications in humans. One such variant is the Shiga toxin-producing (STEC) strain, which produces toxins that can damage the intestinal lining, causing cramps and bloody diarrhea, and may progress into a life-threatening kidney problem called Haemolytic uraemic syndrome (HUS) (Costa 2013; Ishi and Sadowsky 2008; WHO 2018). *Salmonella* is also a top foodborne bacteria causing diarrheal outbreaks. The WHO reported that 17,000 people in the Philippines were recorded to have acute bloody diarrhea in 2018 (WHO 2018).

Given the risks of ingesting bacteria-contaminated food, the public must be informed and assured of the safety of the food they buy and consume. Republic Act 10611 (Food Safety Act of 2013) prescribes a mechanism to ensure this. An essential step in establishing this state is through microbiological assays and monitoring procedures of food items, such as the most probable number (MPN) test for detecting *E. coli* and the total plate count (TPC) method for assessing *Salmonella* spp. contamination. To date, local health authorities reported that their monitoring has not reached a rigorous level (B.H. Catbagan, personal communication, 04 May 2022). These bacteriological studies on chicken meat and selected street foods were done to assist the local health authorities and the local government in setting and implementing programs and actions. The study examined the presence of bacterial contamination, particularly *E. coli* and *Salmonella*, in fresh chicken meat and selected chicken-based street food sold in the City of Mati, Davao Oriental, Philippines.

METHODS

Sampling Areas and Sample Collection Sites

The locale of the study was the community of the City of Mati., Davao Oriental, Philippines. Samples of fresh chicken meat were bought from Madang Public Market (Site 1). Samples of chicken-based street food were bought from Burgos Street along Baywalk Park (Site 2) and from the stalls and eateries near the main campus of Davao Oriental State University (DOrSU) (Site 3) (Figure 1). A total of 45 pieces of samples were collected from these sites per sampling period (Table 1). These areas were selected due to their popularity among low-to-middle consumers and students, attributed to their accessibility and the ready-to-eat nature of the food offerings. Baywalk Park, situated in the center of the city, is a common gathering place for people to socialize and relax. Students frequently visit the eateries near DOrSU because of their proximity to the campus and the affordability of the food. Studies have shown that taste, convenience, and price significantly influence street food consumption among students and low-middle-middle income groups (Chang et al. 2020; Beniwal and Mogra 2023; Tacardon et al. 2023).

Sample Data-gathering procedure

Table 1 presents the replication of meat samples used for the assay in the laboratory. Sample collection strictly observed the aseptic process. Each bought/collected fresh/cooked sample from each stall packed in labeled sterile plastic zip-lock bags, sealed, and placed in an ice box. These were immediately transported to the laboratory for microbiological analysis. The number of stalls and replications varied due to the sampling limits specified in the Food and Drugs Administration Circular No. 2022-12-2 (DOH-FDA 2022) and Department of Agriculture-National Meat Inspection Service Circular No. 9-2008-5 (DA-NMIS 2008), which require five sample units. Additionally, the number of stalls included in the study was influenced by the limited availability of open stalls at the time of sampling.

Laboratory Analysis of Samples

Measure of presence. The laboratory analyses of the samples began within 60 min of collection. The MPN method was used to analyze the occurrence of *E. coli* in the fresh chicken (drumsticks) meat. The MPN method is a statistical, multistep assay consisting of presumptive, confirmed, and completed phases (Capuccino and Sherman 2013).

In the presumptive test, 20 g of meat from each drumstick were homogenized using a sterile mechanical blender. The homogenized sample was then diluted to 180 mL of sterile water and thoroughly mixed by manually shaking the Erlenmeyer flask. A total of 200 mL mixture was transferred to the test

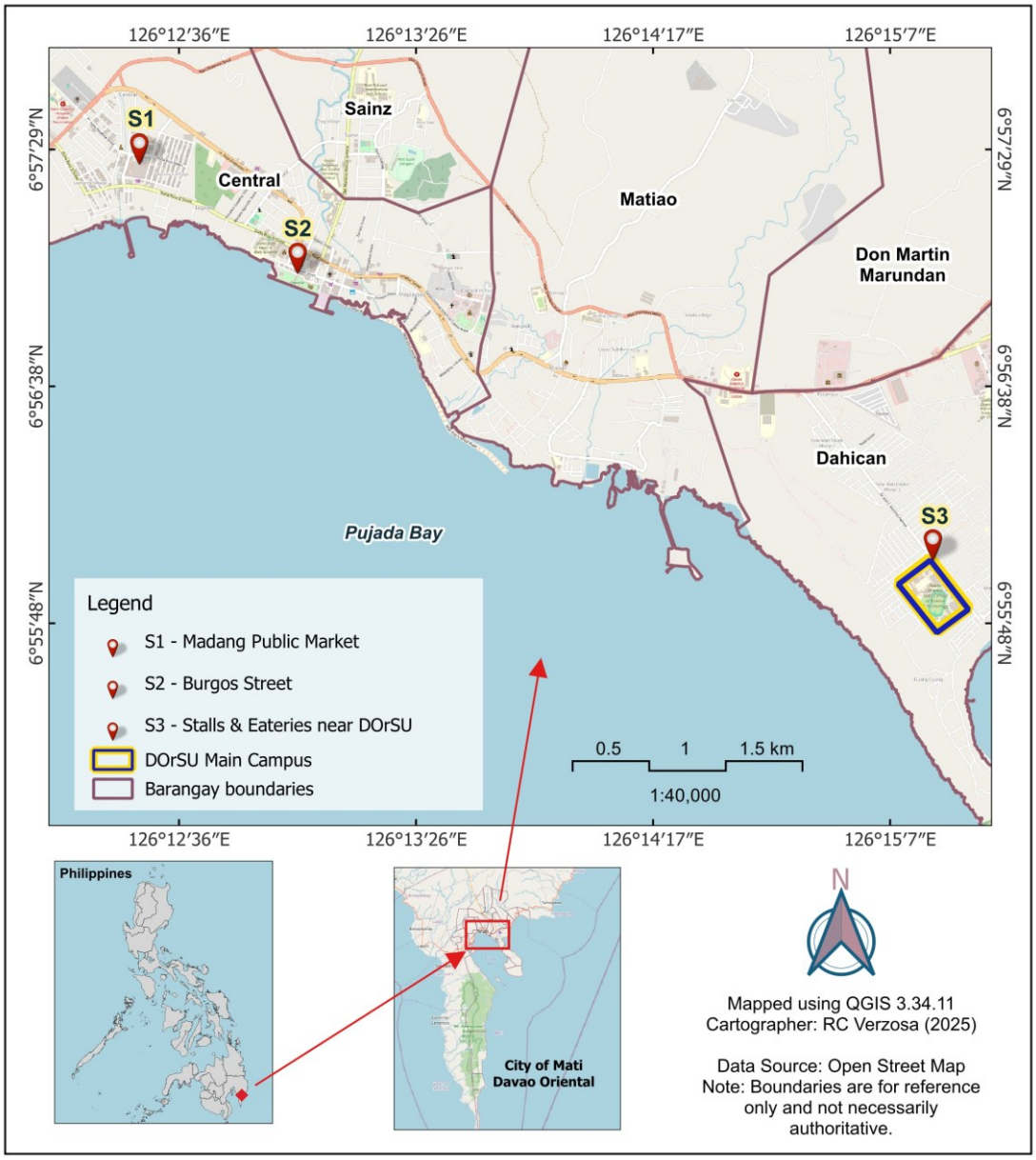

Figure 1. The research area showing the three sampling sites within the City of Mati, Davao Oriental, Philippines. **S1**-Madang Public Market (enclosed public marketplace) located in the central area of the city; **S2**-Burgos Street (open-air vending along Baywalk Park); and **S3**- includes stalls and eateries near Davao Oriental State University (DOrSU) Main Campus in Barangay Dahican. Red location pins indicate sites, while DOrSU is outlined in yellow. Barangay boundaries are shown in purple, and Pujada Bay is depicted in blue. Insets show the location of the study area within Mindanao and the Philippines.

Table 1. Sample sources (Madang Public Market and DOrSU) and replication.

Food Samples	No. of Store/Stall Sources	Replication per Store/Stall (Pieces)
Fresh chicken meat (drumsticks) (MPM)	2	5
Chicken intestines (“isaw”): cooked (MPM)	2	10
Chicken intestines (“isaw”): uncooked (Baywalk)	2	10
Chicken intestines (“isaw”): fried (DOrSU)	3	10
Fried chicken	3	5
“Kwek-kwek”	3	5

tubes containing the double-strength lactose broth (DSLB) and the single-strength lactose broth (SSLB). For each sample, five test tubes for DSLB and 10 test tubes for SSLB were used, amounting to 50 DSLB tubes and 100 SSLB tubes overall. Each culture tube is placed with Durham tubes to trap gas. The tubes with lactose medium were inoculated using a micropipette with 0.1 mL DSLB, 0.01 mL SSLB, and 0.001 mL SSLB aliquots of the homogenized meat sample. Incubation was then carried out at 37°C for 48 h. The presence of gas/bubble formation in Durham tubes indicated a presumptive positive result of coliform bacteria. The number of positive tubes at each dilution allows for the MPN estimation (Blodgett 2020; Capuccino and Sherman 2013).

The confirmed test was conducted to validated the presence of coliform. In this phase, a loopful of inoculum from the positive lactose broth tubes from the presumptive test was streaked into the EMB agar plate (HIMEDIA®). Control plates were also set up to check for cross-contamination. After streaking, the inoculated and the control plates were incubated in an inverted position at 37°C for 24 h. They were then examined for colonies with dark centers and green metallic sheen, which is a positive indication of *E. coli* (Aryal 2019).

The completed test was conducted to examine the coliform colonies in the HIMEDIA® EMB agar plates used in the confirmed test. An isolated colony from the confirmatory test was inoculated into lactose broth tubes and streaked into the nutrient agar slant to perform the gram-staining process. They were then incubated at 37°C for 24 h. The culture tubes that showed acid and gas formation in the lactose broth positively confirmed *E. coli* (Capuccino and Sherman 2013).

The TPC test was used to determine the *Salmonella* population for uncooked and cooked chicken intestines "isaw" and chicken meat to conform to the specified unit in the DA-NMIS guideline (DA-NMIS 2008). It also determined *E. coli* load in other chicken-based street foods. The analysis used 25 g from each sample (chicken meat, uncooked isaw, and chicken-based street food), homogenized, placed in a HIMEDIA® nutrient broth, and incubated for 24 h. This is to recover many bacteria (Elumba et al. 2018). Following incubation, a 10-fold serial dilution was made to decrease a dense cell culture to a more acceptable concentration and facilitate colony counting (Sapkota 2021). In 10-fold serial dilution, the concentration was reduced by a multiple of ten, and it was completed with a ratio of 1:10, of which 1 represents the amount of sample added, and 10 represents the total volume of the final sample. A 1.0 mL aliquot from the stock solution was transferred into a 9.0 mL diluent and mixed by gently shaking the test tubes. The procedure involved five series of 10-fold dilutions to minimize the number of colonies and make them countable (Werner 2023). After serial dilution, 0.1 mL aliquots were plated into dupli-petri HIMEDIA® EMB agar plates and HIMEDIA® Salmonella Shigella (SS) agar plates. The inocula were spread on the surface using a sterile metal spreader. The bacterial cultures were then incubated in a BIOBASE incubator at 37℃ for 24 h and examined for distinctive *Salmonella* and *E. coli* colony features.

The *E. coli* and *Salmonella* colonies were counted per culture plate to determine the degree of contamination. The results are presented as colony-forming units (CFU) per gram of the sample for *E. coli* and per 25 grams for *Salmonella*, following the standards. The number of colonies per plate was calculated based on the dilution factor used to prepare the samples (Capuccino and Sherman 2013). The FDA's Bacteriological Analytical Manual (BAM) (2021) was followed to interpret where, for a plate that surpasses 250 CFU, the counts were recorded as too numerous to count (TNTC) and, if less than 25, the counts were recorded as too few to count (TFTC).

Data Processing and Analysis

The colony count was compared to the recent Philippine FDA Circular No. 2022-12-2 guidelines. This circular was used as a reference to assess the bacteriological quality of food cooked immediately before sale or consumption. The FDA's acceptable level of chicken-based food for *E. coli* was 20 to 100 CFU g^{-1}, and the DA-NMIS acceptable limit was 100–500 MPN g^{-1}. *Salmonella* must not be detected or absent in chicken meat and in all chicken-based street food. Hence, food cooked immediately before sale must not exceed the microbial limit to avoid potential health hazards.

RESULTS

Evidence of Presence of *E. coli* and *Salmonella* in Samples

Table 2 and Table 3 show the result of the examination of culture plates based on the diagnostic colony features of *E. coli* and *Salmonella*. As shown, it can be seen that all samples examined manifested these diagnostic features, which are a green metallic sheen for *E. coli* colonies and transparent colonies with black centers for *Salmonella* as compared to the bacterial control. *Salmonella* is, thus, detected in fresh chicken meat samples and cooked and uncooked *isaw* sold in one stall along the city's main public market (S1) and in another stall located along the Baywalk area (S2). On the other hand, *E. coli* is detected in all chicken-based street food examined and bought from sellers near the DOrSU main campus (S3).

The fresh chicken meat and "isaw" bought from S1 and in Burgos Street along S2 have

Salmonella spp. exceeding the requirement of absence in the 25 g sample as per DA-NMIS Circular No. 9-2008-5 guidelines (Table 4). Although there were colonies that were TFTC in cooked "isaw" in the first and third sampling period, confirmed presence of *Salmonella* spp. renders the sample non-compliant with DA-NMIS standards, which require complete absence in 25 g samples. In this study, both TNTC and TFTC colony growths were confirmed to contain *Salmonella* spp. and as such all positive samples regardless of bacterial load were classified as exceeding the acceptable limit. The *E. coli* from fresh chicken meat was within the acceptable limit of 500 MPN g^{-1} based on the DA-NMIS set standards. However, the chicken-based street foods, including fried chicken, "isaw", and "kwek-kwek" bought near DOrSU (S3) are generally way above the acceptable limit set by DOH-FDA Circular No. 2022-12-2 (Table 5). The permissible limit for *E. coli* in takeaway food (such as fried chicken, "isaw", and "kwek-kwek"*)* based on DOH-FDA Circular No. 2022-12-2 set standards is 20 CFU g^{-1} and should not exceed 100 CFU g^{-1}.

Table 2. Result of sample examination to detect the presence of *Salmonella* spp.

Sample Source		***Salmonella*** **Diagnostic Colony Features** **(colorless or transparent colonies, usually with black centers in SS Agar plates)**
Fresh Chicken Meat (Madang Public Market)	Stall 1	Positive
	Stall 2	Positive
Uncooked "Isaw"	Stall 1 (Madang Public Market)	Positive
Cooked "Isaw"	Stall 2 (Baywalk Park)	Positive

Table 3. Result of sample examination to detect the presence of *E. coli* in Madang Public Market (MPM) and Davao Oriental State University (DOrSU).

Sample Source		***Escherichia coli*** **Diagnostic Colony Features (Green metallic sheen colonies in EMB Agar plates)**
Fresh Chicken Meat (Madang Public Market)	Stall 1	Positive
	Stall 2	Positive
Fried Chicken meat (DOrSU vicinity)	Eatery 1	Positive
	Eatery 2	Positive
	Eatery 3	Positive
"Isaw" (DOrSU vicinity)	Stall 1	Positive
	Stall 2	Positive
	Stall 3	Positive
"Kwek-kwek" (DOrSU vicinity)	Stall 1	Positive
	Stall 2	Positive
	Stall 3	Positive

Table 4. *Salmonella* spp. mean load in fresh chicken meat and chicken products across three sampling periods (DA-NMIS standard: absent in 25 g sample). Note: Regardless of colony count, the confirmed presence of *Salmonella* spp. in any amount violates DA-NMIS guidelines, which require complete absence in 25 g sample. Thus, even TFTC samples are classified as exceeding the acceptable limit. (TNTC=too numerous to count; TFTC = too few to count).

Sample Source		**1st Sampling**	**2nd Sampling**	**3rd Sampling**	**Standard Limit (Based on DA-NMIS: must be absent in g^{-25})**
Fresh Chicken Meat (Madang Public Market)	Stall 1	TNTC	TNTC	TNTC	Exceeds acceptable limit
	Stall 2	TNTC	TNTC	TNTC	Exceeds acceptable limit
Uncooked "Isaw"	Stall 1 (Madang Public Market)	TNTC	TNTC	TNTC	Exceeds acceptable limit
	Stall 2 (Baywalk Park)	TNTC	TNTC	TNTC	Exceeds acceptable limit
Cooked "Isaw"	Stall 1 (Madang Public Market)	TNTC	TNTC	TFTC	Exceeds acceptable limit
	Stall 2 (Baywalk Park)	TFTC	TNTC	TFTC	Exceeds acceptable limit

Table 5. *E. coli* mean load in the examined fresh chicken meat and chicken-based products across three sampling periods, with reference to Department of Agriculture-National Meat Inspection Service and Food and Drugs Administration microbial limits. (TNTC = too numerous to count; TFTC = too few to count).

Sample Source		Unit	Mean Bacterial Load Per Sampling Period			Standard Limit (Based DA-NMIS: ≤500 MPN g^{-1}) and FDA: ≤100 CFU g^{-1})
			1st Sampling	2nd Sampling	3rd Sampling	
Fresh Chicken Meat (Madang Public Market)	Stall 1	MPN g^{-1}	103.20	105.10	398.00	within acceptable limit
	Stall 2		10.10	86.80	354.00	within acceptable limit
Fried Chicken meat (DOrSU vicinity)	Eatery 1	CFU g^{-25} Sample	TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)
	Eatery 2		TNTC	TNTC	TNTC	Exceeds acceptable limit
	Eatery 3		TNTC	TFTC	TNTC	Exceeds acceptable limit (1st & 3rd); within limit (2nd)
"Isaw" (DOrSU vicinity)	Stall 1	CFU g^{-25} sample	TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)
	Stall 2		TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)
	Stall 3		TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)
"Kwek-kwek" (DOrSU vicinity)	Stall 1	CFU g^{-25} sample	TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)
	Stall 2		TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)
	Stall 3		TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)

DISCUSSION

Presence of *Salmonella* spp. and *E. coli* in Samples

Based on personal communications with students and colleagues, incidents of gastrointestinal discomfort were reported following the consumption of takeaway foods purchased from local food establishments. This study presents preliminary evidence that these takeaway foods are contaminated with *Salmonella* spp. and *E. coli*. However, data gathered did not establish a causal link between the isolated bacteria. Some *E. coli* strains are commensals and natural residents in the gut of warm-blooded animals (WHO 2018). However, some harmful strains cause diarrhea, so its possible link to the reported cases cannot be ruled out. Further studies, such as strain identification and pathogenicity testing, would be necessary to confirm whether the isolated bacteria were responsible for the gastrointestinal symptoms. The presence of *E. coli* and *Salmonella* in the foods examined (Table 2 and Table 3) probably resulted from fecal contamination that could be attributed to poor sanitation and improper food handling (Birgen et al. 2020). Although the takeaway foods are cooked, *E. coli* is generally killed at 70°C (Li and Ganzle 2016; WHO 2018), including *E. coli* O157:H7, the most health-threatening *E. coli* variant to humans (WHO 2018). The presence of *E. coli* and *Salmonella* spp was exceedingly high. Table 4 and Table 5 beyond the permissible limit from set guidelines on these food may be attributed to the ability of these bacteria to withstand various environmental stresses, such as heat and acidity. While *Salmonella* was killed at 74°C (Ansah 2023) or less than this temperature given a more prolonged time exposure, some *E. coli* strains demonstrated heat-resistance such as *E. coli* AW1.7 (Li and Ganzle 2016) or produce toxins that are persistent in food (FSIS-USDA 2021). Moreover, *E. coli* can adapt to heat (Wang et al. 2021) and acidic environments (van Elsas et al. 2011), allowing it to survive under harsh conditions and rapidly reproduce when favorable conditions return. Similarly, enteric *Salmonella* has been found to endure heat treatments and desiccation (Wang et al. 2021), with specific variants exhibiting resistance to heat, acid, and other stresses (Thames and Sukumaran 2020). Given these factors, preparers and vendors must be aware of these survival mechanisms and implement proper food handling, preparation, and storage practices to prevent

contamination and the reintroduction of pathogens during food processing and selling.

Improper food handling increases bacterial contamination anywhere during chicken handling, slaughtering, and meat processing (Bhaisare et al. 2014). Another percentage of bacterial load is added during food preparation and selling (Moloi et al. 2021; Wardhana et al. 2021). The presence and high levels of *E. coli* and *Salmonella* in the samples could be due to unsanitary handling practices, contamination sources, and vendor hygiene conditions. According to WHO (2018), *E. coli* is typically present in the digestive tract of warm-blooded animals; thus, its presence in fresh chicken meat and cooked chicken food indicates fecal contamination. This could result from poor handling practices, such as improper washing of raw chicken, using contaminated water for washing, or cross-contamination from raw to cooked food during preparation. Birgen et al. (2020) established a direct link between high bacterial load and the hygiene conditions of vendors. Factors such as lack of food covers to prevent flies and other vectors, waste accumulation in vending areas, improper hand hygiene, and unclean clothing among food handlers all contributed to bacterial contamination. This supports the idea that *E. coli* and *Salmonella* in the samples may result from inadequate sanitary practices in food establishments in the local community (Marquez and Bureros 2022). Given these findings, food sellers and establishments should be assessed for compliance with health and hygienic standards to prevent foodborne contamination and illnesses.

In early 2022, a policy brief was generated from the findings of these early studies by DOrSU Bachelor of Science Biology students with close supervision of their faculty advisers. The policy brief was presented to the Sangguniang Panlungsod of Mati in March 2022; however, concrete action is yet to be felt by the local authorities. With the findings of additional studies done in 2023 and the current year, as consolidated in this paper, it is hoped that this material provides enough scientific proof that local authorities need to enforce the necessary action to implement sanitary inspections, mandatory food safety training for handlers, strengthening local food safety ordinances, and establishing routine microbial monitoring to protect public health and ensure safer street food practices.

Salmonella is a leading cause of foodborne disease outbreaks in humans. On the other hand, *E. coli*, a natural resident in the digestive tract of humans and warm-blooded animals, indicates fecal contamination in food. Although the study did not identify specific variants of *E. coli* present, the high amounts detected in food samples are still a significant concern. The latter has pathogenic variances that are responsible for a high number of disease outbreaks in humans. Both bacteria are present in fresh chicken meat and chicken-based street food commonly sold in certain stores in Mati to a degree way beyond the acceptable limit set by FDA Circular No. 2022-12-2 and DA-NMIS Circular No. 9-2008-5, which should alert and trigger the local authorities to implement the necessary and immediate action to address the situation. Regular monitoring of food establishments should ensure strict compliance with health standards and sanitary practices must be followed. Further, building the capacity of local/barangay leaders in the assessment of the actions employed by the food establishments as well as people involved in food-based economic activities should be done through information and education. Collaboration with the university and other institutions to assess the strategies implemented by the Local Government Unit is also highly encouraged.

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GENERATIVE AI STATEMENT

The authors affirm that no generative AI was used in the design of the study, data acquisition, analysis, or interpretation of the results. Grammarly profreader was solely used for enhancing the clarity of the manuscript document. All the content, ideas, and conclusions are the work and responsibility of the authors.

ETHICAL CONSIDERATIONS

There were no experimental animals used in this study.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests for any of the authors.

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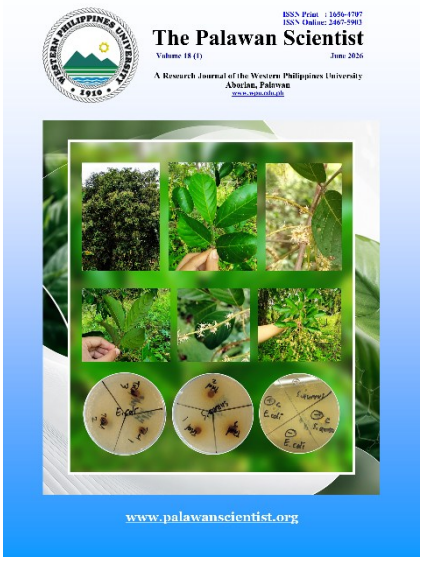

Floral assessment and conservation prioritization in Dugo Watershed, Mountain Province, Northern Philippines

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ABSTRACT

Forests play a key role in biodiversity conservation supporting local livelihoods. These ecosystems face threats from agricultural expansion, forest fires, and unsustainable land use practices. This study focused on assessing woody species diversity, their conservation status, biogeography, and local use values, and established a geospatial database in Dugo Watershed in Tadian, Mountain Province. The goal was to provide baseline data for conservation planning of the watershed. Nineteen plots recorded 81 woody species from 60 genera and 36 families. Dominant species included *Pinus kesiya* Royle ex Gordon and *Ficus benguetensis* Merr. The Shannon-Wiener Diversity Index measured 2.84, indicating moderate species diversity in the area. There are 14 species classified as threatened woody species consisting of: three endangered, four near threatened, two other threatened, and five vulnerable. High-priority species such as endangered *Guioa acuminata* Radlk. and vulnerable *Saurauia bontocensis* Merr. are endemic with specific habitat requirements, making them highly sensitive to disturbance. The presence of several endemic species further underscores the watershed's conservation value. Documented species use range from ecological functions to economic and cultural purposes, indicating strong potential for community-based stewardship. Geographic Information System (GIS) showed the distribution of species to support monitoring and management. The findings emphasize the need for community-based conservation efforts, habitat restoration, and regular monitoring to strengthen the watershed's biodiversity and ecological resilience.

Keywords: biodiversity, community-based conservation, GIS, local use values, woody species

INTRODUCTION

The Philippines is recognized as one of the 18 mega-biodiverse countries of the world (CBD 2020). According to Pelser et al (2011), Philippine vascular plants comprise approximately 290 families and 10,220 recorded species, of which about 51.3% are endemic. This rich biodiversity provides resources and services to local communities, including food, clean water, climate regulation, and cultural values (Shin et al. 2022; Sharma and Birman 2024). However, human activities such as agricultural expansion, forest conversion, and unsustainable resource use, combined with climate change, continue to threaten these

ecosystems (Wang et al. 2021; Prakash and Verma 2022). As a result, there is a need to focus conservation efforts on areas that are important for preserving biodiversity.

The Philippine biodiversity conservation priorities identified critical areas for conservation and the importance of conducting floral assessment to guide these initiatives (Ong 2002). The urgency of these efforts is underscored by the fact that only 3% remains as the country's primary vegetation which is the lowest percentage among the world's biodiversity hotspots (Campbell et al. 2016). Moreover, the ongoing discovery of endemic species underscores the increasing value of the remaining fragments of both primary and secondary native vegetation as natural capital for the Philippines (Peng et al. 2017).

Despite the presumed rich biodiversity of the Cordillera region publicly available and site-specific biodiversity data remain limited (Baoanan et al. 2020). This knowledge gap is particularly evident in small watersheds like Dugo Watershed where baseline information on species composition, distribution, and conservation status is lacking. Establishing such baseline data is essential for guiding conservation planning and sustainable management because forest ecosystems are vital for maintaining biodiversity and supporting the livelihoods of nearby communities (Oldekop et al. 2020). As these ecosystems lose functionality, the flow of these services is disrupted, posing risks to both biodiversity and human well-being. Therefore, protecting these areas requires a comprehensive approach that considers both ecological and socio-economic factors, including the integration of community-based strategies (Bauyot et al. 2024).

The Cordillera Administrative Region is home to diverse plant life, which includes species found in lowland and montane forests (Baoanan et al. 2020). However, these forests are under threat from various activities, such as forest fires and expansion to vegetable farms (Paltiyan-Bugtong et al. 2022). Tadian is a rural municipality in the southwestern part of Mountain Province in the Northern Philippines (Figure 1). It is currently facing similar biodiversity conservation challenges. Anthropogenic activities have impacted the biodiversity of these areas, sometimes before species can be properly documented, and some species may have already been lost.

Dugo Watershed is a communally managed forest within Tadian that plays a critical role in supplying water to nearby communities and among the few remaining forested areas in the municipality. Although the watershed is locally managed, it is not formally protected under the National Integrated Protected Areas System (NIPAS) and E-NIPAS system. Current management practices focus primarily on water resource protection and land-use regulation, while species-level biodiversity assessment and conservation prioritization remain limited. Furthermore, the area faces ongoing threats from agricultural expansion, forest fires, and unsustainable land use practices. As van Beijnen and Jose (2020) showed in a similar case, the absence of formal protective status in a watershed allows disturbances to pose serious threats to endemic species, underscoring the urgency of establishing protection measures for Dugo Watershed.

This study assessed the woody species diversity, evaluated their conservation status, biogeography, and local use values, and established a geospatial database for monitoring to support future conservation activities in Dugo Watershed. Baseline data on woody species diversity and spatial distribution in Dugo Watershed will provide essential information to monitor ecosystem changes over time (Castillo et al. 2021). Species status and geographical data were documented (BMB and GIZ Gmbh 2017) to identify taxa requiring immediate conservation action. In this study, woody species were defined functionally as plants with a persistent above-ground stem that remains over time and through changing environmental conditions (Gray 1887; FitzJohn et al. 2014). Only trees and shrubs were included, while lianas, palms, tree ferns and bamboo were excluded.

The outcomes of this study are intended to support the development of conservation strategies for the watershed. The findings will help shape community-based conservation initiatives and support decision-making by the DENR and Local Government Units (LGUs) in making informed decisions. The study will also serve as a resource for researchers and students working on floral biodiversity and conservation planning in the future.

METHODS

Study Site

The municipality of Tadian is located in the southwestern part of Mountain Province, Northern Philippines (Figure 1). It is bounded on the west by the municipality of Cervantes, Ilocos Sur, on the east by the municipality of Bauko, Mountain Province, on the south by the municipality of Mankayan, Benguet, and on the north by the municipality of Besao, Mountain Province. Being at the center of different forest types, like pine forests and broad-leaf forest, diversity could be higher in this area. The municipality has an approximate land area of 14,942 ha (Tadian LGU 2000).

The Dugo Watershed is in Tadian, Mountain Province, and covers about 440 hectares. It consists of secondary forest with Benguet pine (*Pinus kesiya* Royle ex Gordon, 1840) and broadleaf trees. Other land uses include agroforestry areas, rice fields, vegetable gardens, and residential zones. The

watershed lies at an elevation of 1,000 to 1,860 meters above sea level and is one of the few remaining forested areas in the municipality. The terrain includes steep to moderately steep slopes. Based on Corona's system of classification, the area falls under Climatic Type I, with two seasons: the wet season ("deam") and the dry season ("tiagew") (Tadian LGU 2000).

Sampling Sites

Nineteen (19) sampling plots were established in the existing forested watershed area. Potential plot locations were first systematically generated in QGIS Ver. 3.22.9 at 300 m intervals. This systematic generation resulted in 26 potential plot locations. From these points, 19 plots were randomly selected using QGIS randomization tools to ensure unbiased representation across the forested watershed area. The number of plots was determined using Cochran's formula (1977) for a population of 26 potential plot locations, with a confidence level of 90%, a precision of ±10%, and an estimated proportion of 0.5, resulting in 19 plots. Each plot measured 40 m × 40 m.

Diversity Assessment

All woody species (trees and shrubs) found within each plot were identified, documented, and geotagged. Species identification was conducted in the field, with scientific names and classifications verified using Co's Digital Flora of the Philippines (Pelser et al. 2011) and Plants of the World Online (POWO 2024). Species that could not be identified were further verified with the help of plant taxonomist. No herbarium specimens were collected, but field observations and reference verification ensured accurate species identification. Density, frequency, and dominance were used to compute the importance value of woody species (Guron et al. 2019).

Diversity Indices

Species diversity in the Dugo Watershed was assessed using several diversity indices (Guron et al. 2019). These included the Shannon-Wiener Diversity Index (H'), Simpson's Diversity Index (D), and Margalef's Richness Index (R). Computations were performed using Microsoft Excel 2016. The formulas for each diversity index were as follows:

Shannon-Wiener diversity index (H)

$H = \sum_{i=1}^{S} pi(\ln pi)$

where: H = Shannon-Wiener diversity index
pi = Number of individuals of species i/ total number of samples
S = Number of species or species richness
ln = the natural logarithm

Simpson's diversity index (D)

$D = 1 - \sum_{i=1}^{S} \frac{ni(ni-1)}{N(N-1)}$

where: ni = total individual of species i
N = total number of individual of all species
S = number of species or species richness

Margalef's Richness Index (R)

$R = \frac{(S-1)}{\ln(N)}$

where: R = richness
S = number of species
N = number of individuals (of all species)

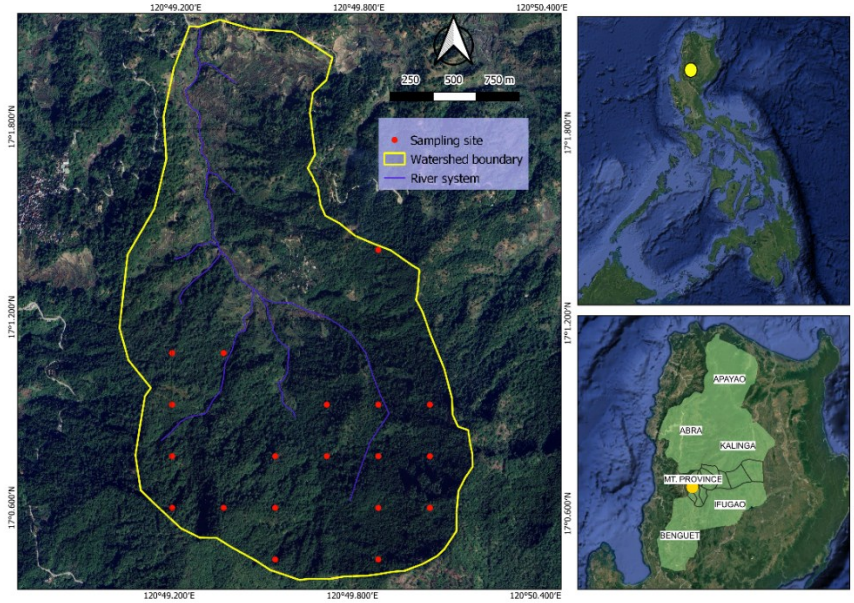


Figure 1. Map of Philippines, the Cordillera Administrative Region, showing the sampling sites.

Conservation Status, Biogeography and Use Value

The conservation status of all recorded woody species was assessed using the IUCN Red List of Threatened Species (IUCN 2025) and the DENR Administrative Order (DAO) 2017-11: Updated National List of Threatened Philippine Plants (DENR 2017). Species that were not assessed or were absent from the IUCN Red List and national conservation records were designated as Not Evaluated**.** Distribution status was assessed using Co's Digital Flora of the Philippines to determine whether species are endemic, indigenous, or introduced. Documenting endemic species is critical for identifying conservation priorities as their restricted range makes them more vulnerable to extinction (Manes et al. 2021; Orsenigo et al. 2018). Indigenous and introduced species were also recorded to complete understanding of the local flora. Information on species uses including food, medicine, construction, cultural significance, and ecological services were obtained through interviews with local communities. This information provides context for the perceived conservation value and potential threats to plant species within the watershed.

GIS Mapping and Database

Field data were processed using QGIS to map the locations of all identified woody species within each sampling plot. A geospatial database was developed in QGIS to store species attributes, including diameter at breast height (DBH), scientific name, family, distribution status, and documented uses. The database provides a baseline for visualizing spatial patterns and supporting biodiversity monitoring in the watershed.

RESULTS

Woody Species Composition and Diversity

A total of 81 woody species (Table 1) with 60 genera and 36 families were documented in Dugo Watershed (Figure 2). Among these, the genus *Ficus* had nine, which was the highest number of species. On the other hand, the genera *Ardisia* and *Pittosporum* each had three species. At the family level, Moraceae had nine species, followed by Rubiaceae, with seven, and Euphorbiaceae, with five.

In terms of importance value (IV), results showed that *P. kesiya* had the highest IV at 111.38, indicating its dominant presence and influence on the community structure (Table 2). Following this, *Ficus benguetensis* Merr., (1905), has an IV of 9.98, which also reflects its importance. Other notable species include *Pipturus asper* Wedd., (1854), with an IV of 8.19, and *Eurya coriaceae* Merr., (1910), at 7.85.

The diversity indices used for Dugo Watershed were a Shannon-Weiner Diversity Index of 2.84, a Margalef's Species Richness Index of 11.78, a Pielou's Evenness Index of 0.65, and a Simpson's Dominance Index of 0.81.

Conservation Status, Biogeography and Use Value

Several species found in the watershed were listed as threatened (Figure 3). The results revealed that three species were classified as endangered, four nearly threatened, two as other threatened, and five as vulnerable. The rest are categorized as least concern, which indicates that they were not currently at risk, and not evaluated, meaning they have not been assessed or evaluated by the reports used. Key species classified as Endangered include *Guioa acuminata* Radlk., (1914), *Pterocarpus indicus* Willd (1802), and *Wendlandia philippinensis* Cowan, (1932). These species were at high risk of extinction due to specific habitat requirements and threats such as habitat loss. Species classified as Near Threatened include *E. coriacea, Leea philippinensis* Merr., (1906), *Mussaenda benguetensis* Elmer, (1906) and *Vaccinium benguetense* S. Vidal, (1886). These species were at risk, requiring monitoring and habitat protection to prevent further declines. Species classified as Other Threatened include *Aphanamixis polystachya* (Wall.) R. Parker, (1931) and *Pittosporum ramosii* Merr., (1920) which face specific threats such as habitat loss. Species classified as Vulnerable

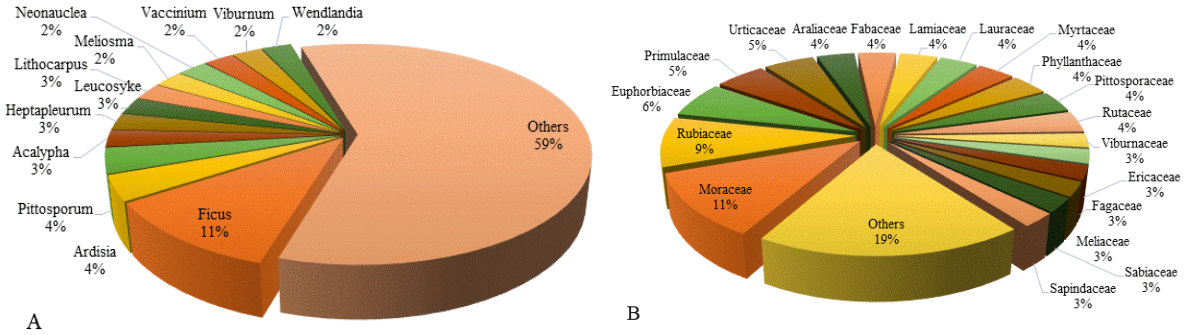


Figure 2. Proportion of documented genera (A) and families (B) in the study site. Categories with less than 1.2% of the total were grouped as "Others".

Table 1. List of woody species identified.

	Scientific name	Family Name	Conservation status
1	*Acalypha angatensis* Blanco	Euphorbiaceae	Not Evaluated
2	*Acalypha cardiophylla* Merr.	Euphorbiaceae	Least Concern
3	*Alnus japonica* (Thunb.) Steud.	Betulaceae	Least Concern
4	*Alphitonia excelsa* (Fenzl) Reissek ex T. Mitch.	Rhamnaceae	Least Concern
5	*Aphanamixis polystachya* (Wall.) R.Parker	Meliaceae	Other Threatened
6	*Aralia bipinnata* Blanco	Araliaceae	Least Concern
7	*Archidendron clypearia* (Jack) I.C.Nielsen	Fabaceae	Least Concern
8	*Ardisia pyramidalis (Cav.) Pers.*	Primulaceae	Not Evaluated
9	*Ardisia* Sw.	Primulaceae	Not Evaluated
10	*Ardisia warburgiana* Mez	Primulaceae	Not Evaluated
11	*Bischofia javanica* Blume	Phyllanthaceae	Least Concern
12	*Boehmeria* Jacq.	Urticaceae	Not Evaluated
13	*Bridelia* Willd.	Phyllanthaceae	Not Evaluated
14	*Buddleja asiatica* Lour.	Scrophulariaceae	Least Concern
15	*Callicarpa pedunculata* R.Br.	Lamiaceae	Least Concern
16	*Cinnamomum* Schaeff.	Lauraceae	Not Evaluated
17	*Clausena anisum-olens* (Blanco) Merr.	Rutaceae	Least Concern
18	*Croton* L.	Euphorbiaceae	Not Evaluated
19	*Crypteronia paniculata* Blume	Crypteroniaceae	Least Concern
20	*Decaspermum parviflorum* (Lam.) A.J.Scott	Myrtaceae	Least Concern
21	*Eugenia* L.	Myrtaceae	Not Evaluated
22	*Euonymus cochinchinensis* Pierre	Celastraceae	Least Concern
23	*Eurya coriacea* Merr.	Pentaphylacaceae	Near Threatened
24	*Ficus ampelos* Burm.f.	Moraceae	Least Concern
25	*Ficus benguetensis* Merr.	Moraceae	Least Concern
26	*Ficus concinna* (Miq.) Miq.	Moraceae	Least Concern
27	*Ficus cuneiformis C.C.Berg*	Moraceae	Not Evaluated
28	*Ficus minahassae* (Teijsm. & de Vriese) Miq.	Moraceae	Least Concern
29	*Ficus nota* (Blanco) Merr.	Moraceae	Least Concern
30	*Ficus pseudopalma* Blanco	Moraceae	Not Evaluated
31	*Ficus ruficaulis* Merr.	Moraceae	Least Concern
32	*Ficus septica* Burm.f.	Moraceae	Least Concern
33	*Glochidion luzonense* Elmer	Phyllanthaceae	Not Evaluated
34	*Gmelina arborea* Roxb. ex Sm.	Lamiaceae	Least Concern
35	*Guioa acuminata* Radlk.	Sapindaceae	Endangered
36	*Harpullia arborea* (Blanco) Radlk.	Sapindaceae	Least Concern
37	*Heptapleurum blancoi* (Merr.) Lowry & G.M.Plunkett	Araliaceae	Not Evaluated
38	*Heptapleurum* Gaertn.	Araliaceae	Near Threatened
39	*Homalanthus macradenius* Pax & K.Hoffm.	Euphorbiaceae	Least Concern
40	*Ilex crenata* Thunb.	Aquifoliaceae	Not Evaluated
41	*Itea macrophylla* Wall.	Iteaceae	Not Evaluated
42	*Kanapia monstrosa* (A.Rich.) Arriola & Alejandro	Rubiaceae	Not Evaluated
43	*Leea philippinensis* Merr.	Vitaceae	Near Threatened
44	*Leucaena leucocephala* Lamk	Fabaceae	Not Evaluated
45	*Leucosyke capitellata* (Poir.) Wedd.	Urticaceae	Not Evaluated
46	*Leucosyke* Zoll. & Moritzi	Urticaceae	Least Concern
47	*Lithocarpus jordanae* (Laguna) Rehder	Fagaceae	Vulnerable
48	*Lithocarpus woodii* (Hance) A.Camus	Fagaceae	Vulnerable
49	*Litsea cordata* (Jack) Hook.f.	Lauraceae	Least Concern
50	*Maesa indica* (Roxb.) Sweet	Primulaceae	Least Concern
51	*Mallotus mollissimus* (Geiseler) Airy Shaw	Euphorbiaceae	Least Concern
52	*Melicope* sp1 J.R.Forst. & G.Forst.	Rutaceae	Not Evaluated
53	*Meliosma* Blume	Sabiaceae	Not Evaluated
54	*Meliosma* Blume	Sabiaceae	Not Evaluated
55	*Melodinus* J.R.Forst. & G.Forst.	Apocynaceae	Not Evaluated
56	*Micromelum minutum* (G.Forst.) Wight & Arn.	Rutaceae	Least Concern
57	*Mussaenda benguetensis* Elmer	Rubiaceae	Near Threatened
58	*Neolitsea microphylla* Merr.	Lauraceae	Vulnerable
59	*Neonauclea* Merr.	Rubiaceae	Not Evaluated
60	*Neonauclea reticulata* (Havil.) Merr.	Rubiaceae	Least Concern
61	*Pinus kesiya* Royle ex Gordon	Pinaceae	Least Concern
62	*Pipturus asper* Wedd.	Urticaceae	Least Concern
63	*Pittosporum moluccanum* (Lam.) Miq.	Pittosporaceae	Least Concern
64	*Pittosporum pentandrum* (Blanco) Merr.	Pittosporaceae	Least Concern
65	*Pittosporum ramosii* Merr.	Pittosporaceae	Other Threatened

	Scientific name	Family Name	Conservation status
66	*Premna oblongata* Miq.	Lamiaceae	Least Concern
67	*Prunus* L.	Rosaceae	Not Evaluated
68	*Psidium guajava* L.	Myrtaceae	Least Concern
69	*Pterocarpus indicus* Willd.	Fabaceae	Endangered
70	*Pterophylla* D.Don	Cunoniaceae	Not Evaluated
71	*Saurauia bontocensis* Merr.	Actinidiaceae	Vulnerable
72	*Solanum lasiocarpum* Dunal	Solanaceae	Not Evaluated
73	*Tarennoidea wallichii* (Hook.f.) Tirveng. & Sastre	Rubiaceae	Not Evaluated
74	*Toona calantas* Merr. & Rolfe	Meliaceae	Vulnerable
75	*Turpinia sphaerocarpa* Hassk.	Staphyleaceae	Least Concern
76	*Vaccinium barandanum* S.Vidal	Ericaceae	Not Evaluated
77	*Vaccinium benguetense* S.Vidal	Ericaceae	Not Evaluated
78	*Viburnum luzonicum* Rolfe	Viburnaceae	Least Concern
79	*Viburnum odoratissimum* Ker Gawl.	Viburnaceae	Least Concern
80	*Wendlandia luzoniensis* DC.	Rubiaceae	Least Concern
81	*Wendlandia philippinensis* Cowan	Rubiaceae	Endangered

Table 2. Top 15 species with the highest importance value (IV).

Scientific name	Relative Density	Relative Frequency	Relative Dominance	Importance Value
Pinus kesiya Royle ex Gordon	42.94	7.76	60.68	111.38
Ficus benguetensis Merr.	3.36	4.57	2.06	9.99
Pipturus asper Wedd.	2.91	2.74	2.54	8.19
Eurya coriacea Merr.	4.04	1.83	1.99	7.85
Toona calantas Merr. & Rolfe	1.57	2.28	3.33	7.18
Alnus japonica (Thunb.) Steud.	2.13	3.20	1.85	7.17
Homalanthus macradenius Pax & K.Hoffm.	2.69	2.74	1.69	7.12
Ficus ampelos Burm.f.	3.25	2.28	1.18	6.71
Ficus septica Burm.f.	1.57	3.65	1.41	6.63
Gmelina arborea Roxb. ex Sm.	2.02	2.28	1.90	6.20
Kanapia monstrosa (A.Rich.) Arriola & Alejandro	2.35	2.28	1.20	5.84
Heptapleurum blancoi (Merr.) Lowry & G.M.Plunkett	1.57	2.28	1.82	5.68
Wendlandia luzoniensis DC.	1.46	3.20	0.81	5.47
Euonymus cochinchinensis Pierre	1.35	2.74	1.03	5.12
Acalypha angatensis Blanco	1.79	2.28	1.03	5.11

include *Lithocarpus jordanae* (Laguna) Rehder, (1919), *Lithocarpus woodii* (Hance) A. Camus, (1931), *Neolitsea microphylla* Merr., (1906), *Saurauia bontocensis* Merr., (1915), and *Toona calantas* Merr. & Rolfe, (1908). These species play critical ecological roles and require habitat protection and regeneration efforts.

Figure 4 shows the proportion of species by distribution status in the study area. The results indicated that 60.49% of the species were indigenous, reflecting the watershed's rich native biodiversity. The presence of 14.81% endemic species could indicate the role of the area as a habitat for species found only in the Philippines. The remaining species were introduced.

Species were recorded based on use categories (Figure 4). Ecological use was noted in 87.65% of species. Multipurpose use was noted in 8.64%, and economic use in 3.70%. A few were recorded with cultural use such as *P. kesiya* and *T. calantas*, but they were counted under multipurpose use. Which explain why the cultural use category is 0%.

Spatial Database of Woody Species

The spatial database developed through GIS was used for the mapping of woody species within the established quadrats. Each point represents a species and contains information such as scientific name, family name, height, diameter at breast height (DBH), information about its endemism or status, and uses (Figure 5). These maps highlight the geographic distribution of species, particularly those that are threatened and can be used in monitoring their population in the future.

DISCUSSION

Woody Species Composition and Diversity

The presence of 81 species reflects the existing vegetation structure in the watershed. The dominance of *Ficus* species shows their ecological importance, as they contribute to vegetation structure and provide food for animals (Berg and Corner 2005). Being pioneer plants, they indicate constant exposures to disturbance. *Ficus* species can grow as epiphytes, hemi-epiphytes, or trees, allowing them to adapt to different environments. These characteristics make them a key component of the watershed vegetation.

The genera *Pittosporum* and *Ardisia* also contribute to the biodiversity of the watershed. These plants are valued for their medicinal properties and contain phytochemicals that have potential applications (Kobayashi and De Mejia 2005; Linh et al. 2024). However, difficulty in identifying some species and limited studies on their uses have led to their underutilization.

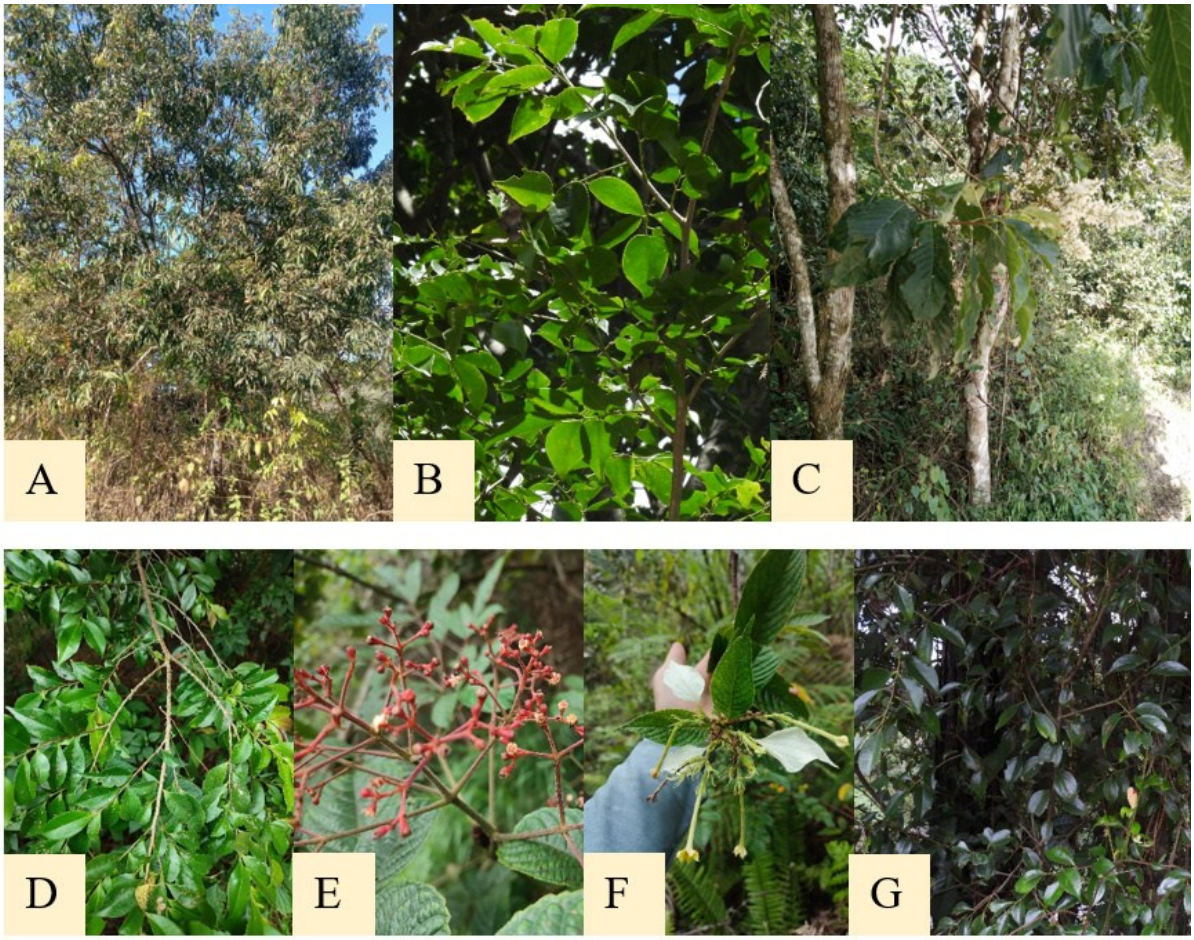


Figure 3. Threatened species of Dugo Watershed (A) *Guioa acuminata* Radlk, (B) *Pterocarpus indicus* Willd., (C) *Wendlandia philippinensis* Cowan, (D) *Eurya coriacea* Merr., 1910, (E) *Leea philippinensis* Merr., 1906, (F) *Mussaenda benguetensis* Elmer, 1906, and (G) *Vaccinium benguetense* S.Vidal, 1886.

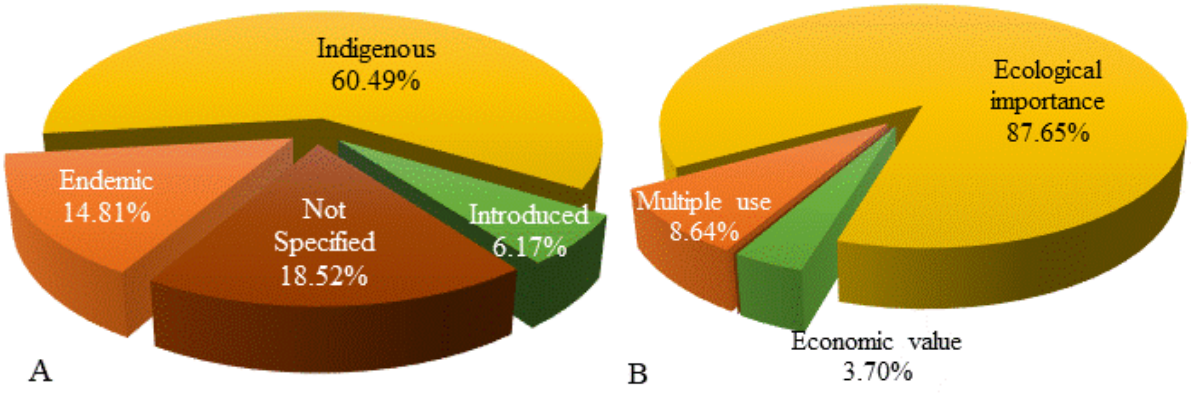
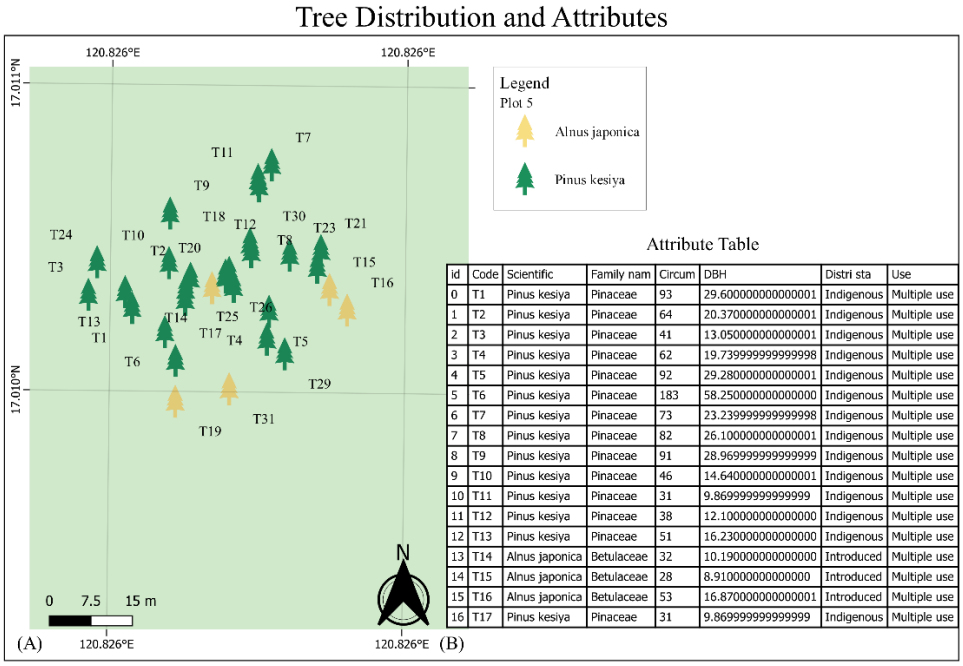


Figure 4. Proportion of species by distribution status (A), and distribution of species by their uses B.



Attribute Table

id	Code	Scientific	Family nam	Circum	DBH	Distri sta	Use
0	T1	Pinus kesiya	Pinaceae	93	29.600000000000001	Indigenous	Multiple use
1	T2	Pinus kesiya	Pinaceae	64	20.370000000000001	Indigenous	Multiple use
2	T3	Pinus kesiya	Pinaceae	41	13.050000000000001	Indigenous	Multiple use
3	T4	Pinus kesiya	Pinaceae	62	19.739999999999998	Indigenous	Multiple use
4	T5	Pinus kesiya	Pinaceae	92	29.280000000000001	Indigenous	Multiple use
5	T6	Pinus kesiya	Pinaceae	183	58.250000000000000	Indigenous	Multiple use
6	T7	Pinus kesiya	Pinaceae	73	23.239999999999998	Indigenous	Multiple use
7	T8	Pinus kesiya	Pinaceae	82	26.100000000000001	Indigenous	Multiple use
8	T9	Pinus kesiya	Pinaceae	91	28.969999999999999	Indigenous	Multiple use
9	T10	Pinus kesiya	Pinaceae	46	14.640000000000001	Indigenous	Multiple use
10	T11	Pinus kesiya	Pinaceae	31	9.869999999999999	Indigenous	Multiple use
11	T12	Pinus kesiya	Pinaceae	38	12.100000000000000	Indigenous	Multiple use
12	T13	Pinus kesiya	Pinaceae	51	16.230000000000000	Indigenous	Multiple use
13	T14	Alnus japonica	Betulaceae	32	10.190000000000000	Introduced	Multiple use
14	T15	Alnus japonica	Betulaceae	28	8.910000000000000	Introduced	Multiple use
15	T16	Alnus japonica	Betulaceae	53	16.870000000000001	Introduced	Multiple use
16	T17	Pinus kesiya	Pinaceae	31	9.869999999999999	Indigenous	Multiple use

Figure 5. Distribution of woody species within the sampling area (A) Tree distribution map of Plot 5 as sample; (B) Attribute table showing species information.

The families Moraceae, Rubiaceae, and Euphorbiaceae are the most dominant in the watershed. These families are recognized as pantropical rainforest families and indicators of tropical conditions (Wurdack et al. 2005; Corlett and Primack 2011; Delprete and Jardim 2012). Although a few species that belongs to these families are endemic to the area.

The watershed is dominated by Benguet pine. This species is dominant in the whole Cordillera Administrative Region (CAR) in the Northern Philippines. It is considered as one of the most important trees in CAR, both economically and culturally (Lumbres and Lee 2014). Its dominance is partly due to local preference for the wood of the species being termite resistant and the frequent forest fires, which hinder the growth of broad-leaf species, thus maintaining pine prevalence. In the Dugo watershed, forest fires occur almost every two years due to human activities. Community initiatives and united efforts locally known as "galatis", such as establishing fire lines help mitigate these fires. The species also grows well in soils with low fertility (Galindo-Jaimes et al. 2002). It also occupies extensive forest area in the Cordillera Mountain Range (Clemente 2024), highlighting the resilience and adaptability of *P. kesiya*.

The computed diversity indices reflect a community with moderate species diversity and balanced representation. Key species such as *P. kesiya* and *F. benguetensis* contribute to ecological stability, and the Simpson's Dominance Index indicates dominance of these two species. Maintaining diverse plant species is essential for supporting the ecological functions and resilience of the watershed (Walker et al. 1999). Studies of other areas in Benguet, such as the Alno communal mixed forest and Talinguroy Research Station, show higher diversity indices (Lumbres et al. 2014; Guron et al. 2019). Mt. Ulap in Benguet also shows varied but generally higher diversity (Guron et al. 2022). Difference in biodiversity indices can be due to various factors like climatic conditions, soil characteristics, forest management practices, disturbance regimes, historical land use, and elevation, which can influence species diversity (Kumar and Ram 2005). Effective management in Mt. Ulap leads to higher biodiversity, with these areas undergoing secondary succession towards broadleaf forests (Guron et al. 2022).

Frequent forest fires caused by human activities remain a major challenge in the watershed. These fires favor *P. kesiya* and limit the growth of broadleaf species. While occasional fires can help regenerate fire-adapted species, frequent and intense fires cause habitat loss, species decline, and changes in soil and water conditions (Shivaprasad et al. 2025). Managing these fires is a major challenge for watershed conservation. Future research should focus on how ecosystems recover and build resilience after forest fires, examining vegetation, soil, and biodiversity changes while considering climate, topography, and human interventions to guide sustainable conservation and management practices (Nolan et al. 2021; Walia et al. 2025).

Limited information on underutilized species like *Pittosporum* and *Ardisia* also poses a challenge. Although these species are present in the watershed, their ecological roles, uses and conservation status are

still poorly documented. Studies show that neglected and underutilized species can support sustainable food security because of their resilience, nutritional value, and socio-economic benefits (Knez et al. 2024; Onawo and Egboduku 2025). Further research is therefore needed to document these species and assess their potential value so they can be included in conservation and management planning for the watershed.

Conservation Status, Biogeography and Use Value

The presence of threatened woody species in the watershed highlights its importance for biodiversity conservation. *Guioa acuminata* classified as Endangered and *S. bontocensis* classified as Vulnerable were observed to be restricted to limited areas within the watershed. In particular, *S. bontocensis* was recorded in locations near streams, indicating an association with water-available sites. These species thrive only in specific habitats (POWO 2024), making them highly sensitive to disturbance. As mentioned by Manes et al. (2021), the restricted ranges of species mean that they are often at greater risk of extinction because threats such as climate change, and habitat loss can affect their population. This pattern of threatened and endemic species concentrated in specific habitats reinforces the need for targeted conservation (Arzaga and Banaticla-Hilario 2025).

The documentation of endemic species further supports the conservation value of the watershed for species found only in the Philippines. In contrast, several introduced species were also recorded, including *Alnus japonica* (Thunb.) Steud., (1840), *Gmelina arborea* Roxb. ex Sm., (1810), and *Psidium guajava* L., (1753) are introduced. *Alnus japonica* was introduced in the locality for its nitrogen-fixing capabilities and as a source of organic fertilizer or compost. Unfortunately, it became invasive in other areas (Paltiyan-Bugtong et al. 2022). The two other species, *G. arborea* and *P. guajava,* were also introduced. *Gmelina arborea* has become popular in the country as a source of timber. It has been a common species being planted in reforestation efforts, yet it is known to be exotic. Well-intended reforestation using non-native trees can harm native biodiversity (van Beijnen and Jose 2020). *Psidium guajava*, also not native to the country, is believed to have originated elsewhere and was introduced by the Spaniards. The adaptability of the guava tree to various soils and climates has enabled its naturalization in tropical and sub-tropical regions worldwide (Singh 2011).

Most species of the watershed were identified for their ecological roles. Most of these species are native, and the local community recognizes the importance of these species in preventing soil erosion, improving water retention, providing shelter to habitats. Species with multiple uses include *A. japonica* (shade for coffee, dried leaves as mulch, firewood), *Bischofia javanica* Blume, (1827) (timber, used to cure ulcer but is not widely known in the community), and *P. kesiya* (timber, firewood, house construction, cultural value). Species with economic value include *G. arborea* (timber), *P. indicus* (timber), and *P. guajava* (food, edible). Examples of species contributing to ecological functions include *Crypteronia paniculata* Blume, (1827) (helps prevent soil erosion) and *F. benguetensis* (food for animals). Species identified with cultural value include *P. kesiya* and *T. calantas* (for woodcraft). *Pinus kesiya* is widely used in traditional practices, such as house and coffin construction, firewood for household use and community gatherings and celebration. Its collection from the "Batangan" (forest land) is managed by the community in accordance with customary laws, reflecting an existing community-based management system that supports sustainable resource use. Similar practices have been documented among other indigenous groups (Bauyot et al. 2024).

Given their threatened status and limited distribution, species such as *G. acuminata* and *S. bontocensis*, should be formally included in local protection policies. The LGU can enact rules limiting their collection while promoting their propagation in community nurseries. Similarly, underutilized species like *Pittosporum* and *Ardisia* can be integrated into small-scale restoration and agroforestry programs, providing both ecological benefits and potential economic use. These measures can be supported through targeted community workshops and monitoring programs.

Spatial Database of Woody Species

The GIS database combines geographic location with specific species attributes, aiding informed decisions in forest management and conservation efforts. The use of GIS technology provided the LGU a valuable tool for assessing and monitoring the forested area within the watershed.

Areas dominated especially of threatened species should be prioritized for protection. Areas dominated by *P. kesiya* and those frequently affected by fires are suitable for restoration through enrichment planting of native broadleaf species. Continuous establishment of fire lines, regular patrolling, and targeted enrichment planting can be implemented to enhance biodiversity and conserve the watershed.

The findings emphasize the need for targeted conservation actions to protect high-priority species like *G. acuminata* and *S. bontocensis*. These species need immediate attention due to their endemism and specific habitat requirements, which makes them vulnerable to disturbance. Efforts should focus on habitat protection and restoration for species with limited distribution. This will help prevent further

habitat loss and promote natural regeneration. Community-based conservation initiatives should be prioritized. Expanding programs like "galatis" can strengthen fire prevention and engage locals in addressing threats like forest fires, illegal logging and other unsustainable land-use practices. In addition, regular monitoring and further research on threatened species and underutilized plants, such as *Pittosporum* and *Ardisia*, are essential. This will help safeguard their ecological and economic importance and support informed conservation strategies.

Overall, the study underscores the importance of maintaining biodiversity and ecological stability in the Dugo Watershed. Strengthening these aspects will ensure the watershed's resilience and provisions of continued ecosystem services and resources for surrounding communities. The spatial database created through GIS technology also provided a valuable tool for mapping and managing the woody species, and supports effective forest management and conservation planning.

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GENERATIVE AI STATEMENT

This manuscript used Grammarly tool to assist in language refinement and clarity improvement.

ETHICAL CONSIDERATIONS

All activities ensure minimal disturbance to natural habitat and complied the local permissions.

DECLARATION OF COMPETING INTEREST

The authors declare that there is no competing interests to any authors.

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Segmental disruptions in rice value chains during the COVID-19 pandemic: A systematic review and policy implications in Asia

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ABSTRACT

The COVID-19 pandemic affected several elements of the rice industry. Many rice value chains (RVCs) were harmed by preventative measures, threatening food security worldwide. This systematic review examines how the COVID-19 pandemic affected Asian RVCs. Harzing's Publish or Perish program was used to include peer-reviewed journals from 2020–2022 indexed in Scopus, Google Scholar, PubMed, and Clarivate Analytics. The upstream segment was the least affected, mainly because farmers stored agricultural inputs in preparation for lockdowns. However, domestic and household workers replaced migratory labor, reducing the availability of agricultural and production labor. In Myanmar, this labor shift contributed to a 28% reduction in overall rice production during the pandemic. The midstream segment, being the most affected, had to operate with fewer resources and longer shifts for post-harvest activities, resulting in disrupted transportation and distribution. For example, in Indonesia, logistical delays in Jakarta were 35% higher compared to Semarang, significantly impacting rice delivery times. This disruption affected the final component of the RVC, where rice scarcity in a state increases retail prices, while abundance lowers them. Retail rice prices in scarcity-affected regions rose by an average of 22%, while surplus areas experienced price drops of up to 18%, forcing farmers to consume unsold crops or sell them at low prices. Governments and farmers should collaborate on strategic planning and response, farmer support, and harvest-to-market operations to maintain market flow.

Keywords: COVID-19, food security, midstream segment, systematic review, upstream segment, value chain disruptions

INTRODUCTION

The COVID-19 pandemic triggered a global food price crisis, emphasizing the critical role of resilient domestic food systems in ensuring food security and combating malnutrition in developing countries (WHO 2021). Approximately 663 million people worldwide suffer from undernourishment,

reflecting persistent challenges in meeting basic caloric needs (Roser and Ritchie 2019; Goeb et al. 2022). When the pandemic emerged in late 2019, its widespread impact intensified global hunger, particularly in regions already vulnerable to food insecurity (Arouna et al. 2020). Nearly half of the population in Asia lives in poverty, where hunger levels in low-income households doubled during lockdowns, underscoring the fragility of food systems under crisis conditions (Pandey and Bhandari 2018; Laurent et al. 2025; Sanusi et al. 2025). While existing literature extensively addresses global food security challenges, there is limited empirical focus on how systemic shocks specifically affect staple crop value chains in Asia. This gap is critical because rice remains the cornerstone of food security for billions in the region, and understanding its value chain resilience under pandemic conditions provides actionable insights for future crisis preparedness. These realities highlight the urgent need to examine staple food systems such as rice value chains, as their resilience is fundamental to mitigating the effects of future global shocks.

The pandemic disrupted agricultural supply chains, affecting both farmers and consumers (Zahrah et al. 2021). Border closures, transportation restrictions, and market shutdowns hindered the movement of goods and labor, creating bottlenecks in food distribution (Belton et al. 2021). These disruptions not only reduced food availability but also jeopardized the livelihoods of farmers and fishers (OECD 2020). Rice value chains (RVCs), which were among the most affected during the pandemic, encompass the interconnected stages of rice production, processing, distribution, and consumption, forming the backbone of food security in Asia. These chains involve upstream activities such as input supply and farming, midstream processes like milling and marketing, and downstream retail and consumption. Their efficiency and resilience are critical because rice is the primary staple for billions of people in the region. Despite rice being a staple food for billions in Asia, there is limited understanding of how COVID-19 specifically impacted RVCs compared to other crops. This lack of clarity poses challenges for designing targeted interventions to strengthen food system resilience.

Rice-producing countries in Asia faced unique challenges during the pandemic (Mobarok et al. 2021). Lockdowns and mobility restrictions forced nations to rely heavily on domestic rice systems due to limited imports (Olvian et al. 2022). This reliance exposed structural weaknesses in local production, processing, and distribution networks. However, existing literature primarily addresses general agricultural impacts or food security trends, leaving a research gap in analyzing the differential effects of COVID-19 across upstream (input supply and production), midstream (processing and marketing), and downstream (retail and consumption) segments of RVCs. Few studies provide comparative insights across countries, making it difficult to identify which stages were most vulnerable and why. The core problem lies in the absence of comprehensive evidence on how COVID-19 disrupted RVCs in Asia and the extent to which these disruptions affected food security, pricing, and farmer livelihoods. Without such knowledge, policymakers and stakeholders lack the data needed to develop effective strategies for mitigating future shocks. Understanding these dynamics is essential for building resilient food systems capable of withstanding global crises.

Addressing these gaps, this study aims to conduct a systematic review of peer-reviewed journals published between 2020 and 2022, indexed in Scopus, Google Scholar, PubMed, and Clarivate Analytics. It also aims to synthesize policy, management, and scientific literature to rigorously identify and compare how COVID-19 disrupted the RVC across upstream (input supply and production), midstream (processing and marketing), and downstream (retail and consumption) stages. Although previous studies and reviews have examined COVID-19's impact on agriculture and global food systems, these analyses often lack a focused synthesis on staple crop value chains in Asia. This gap necessitates a systematic review to consolidate fragmented evidence, identify patterns across upstream, midstream, and downstream segments, and provide actionable insights for resilience-building. In particular, this study aims to (1) identify and analyze the disruptions caused by COVID-19 in the upstream segment of the RVC, including input supply and production; (2) examine the effects of the pandemic on midstream activities such as processing, transportation, and marketing; (3) assess the consequences of COVID-19 on downstream components, particularly retail distribution and consumer access; and (4) synthesize findings and propose recommendations for strengthening RVC resilience against future global shocks. The review aims to examine the pandemic's impact on the RVC across its three major segments: upstream (input supply and production), midstream (processing and marketing), and downstream (retail and consumption). By synthesizing evidence from multiple sources, this research seeks to identify patterns of disruption, quantify their effects where possible, and propose actionable recommendations for enhancing resilience in Asian rice systems.

This study seeks to address the following questions:

1. How did COVID-19–related disruptions (e.g., mobility restrictions, labor shortages, and logistics bottlenecks) affect input supply and farm-level rice production in terms of the availability and cost of inputs, production

volumes, yields, and production costs across different geographies and time periods?

2. What were the effects of the pandemic on rice processing and marketing— including milling throughput, storage, quality, wholesale prices, market access, and trader behavior— and through which mechanisms (e.g., supply chain interruptions, credit constraints, policy measures) were these impacts mediated?
3. How did COVID-19 influence retail availability, consumer prices, purchasing patterns, dietary substitution, and household food security for rice, and how did these outcomes vary by country income level, urban–rural setting, and the phases of the pandemic? Which adaptations or policies (e-commerce, trade facilitation, social protection) mitigated adverse effects?

STUDY DESIGN

This research employed a systematic review approach to synthesize evidence on the impacts of the COVID-19 pandemic on rice value chains (RVCs) in Asia. The review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure transparency and rigor.

SEARCH STRATEGY

A comprehensive literature search was conducted across four major databases using the Harzing's Publish or Perish 7 software program. The researchers conducted a cross-database search (Scopus, Google Scholar, PubMed, and Clarivate Analytics), employed keywords and Boolean operators, and ran searches to extract articles using the following search terms: ("rice" AND "COVID-19"), ("pandemic impact"), ("rice production AND Asia"). The search covered peer-reviewed articles published between January 2020 and December 2022. A manual search of journal articles was also conducted following the Publish or Perish search, using the terms ("rice" AND "production" AND "COVID-19"). Filters were applied, such as the English language, full-text availability, and relevance to RVCs.

ELIGIBILITY CRITERIA

The eligibility criteria for this review were established to ensure the inclusion of relevant and high-quality studies. Only peer-reviewed articles published between 2020 and 2022 were considered, as these years capture the peak period of COVID-19 impacts on rice value chains. Studies were required to focus specifically on disruptions in rice production, processing, marketing, or consumption within Asian countries, given the region's heavy reliance on rice as a staple food. Sources such as blogs, opinion pieces, and non-peer-reviewed materials were excluded to maintain academic rigor and reliability. Additionally, studies unrelated to rice or those that did not address any component of the rice value chain were omitted to keep the review aligned with its core objectives.

STUDY SELECTION

The screening and selection of studies for this systematic review were conducted by a single reviewer, who applied the predefined inclusion and exclusion criteria during both the title and abstract screening and full-text review stages. To ensure consistency and minimize bias, the reviewer followed a structured protocol and documented all decisions using a PRISMA flow diagram. While multiple reviewers are often recommended for systematic reviews, this study relied on one reviewer due to resource constraints, with careful adherence to standardized procedures to maintain rigor and reliability.

To ensure accuracy and consistency, data extraction was validated through a double-checking process, in which all extracted information was cross-referenced with the original articles before final inclusion. Each paper underwent a two-stage review process, beginning with title and abstract screening followed by full-text assessment based on predefined inclusion and exclusion criteria. During extraction, key details such as study characteristics, methodological approaches, and rice value chain segments were verified against the source material to minimize errors. Any ambiguities or missing data were resolved through a follow-up review of the original publication. This systematic approach ensured that only high-quality, relevant studies were included in the final synthesis. Refer to Figure 1 for the PRISMA flowchart used in this study.

A total of 1,000 records were identified through database searching, and 23 additional records were retrieved from other sources. After removing duplicates, 996 records remained for screening, of which 978 were excluded: 955 were unrelated to the effects of the pandemic on the rice value chain, 19 were not focused on Asia, and 4 were not journal articles. Finally, 18 full-text articles were assessed for eligibility, and 3 were excluded (2 were not journal articles and 1 lacked references), resulting in 15 studies included in the systematic review.

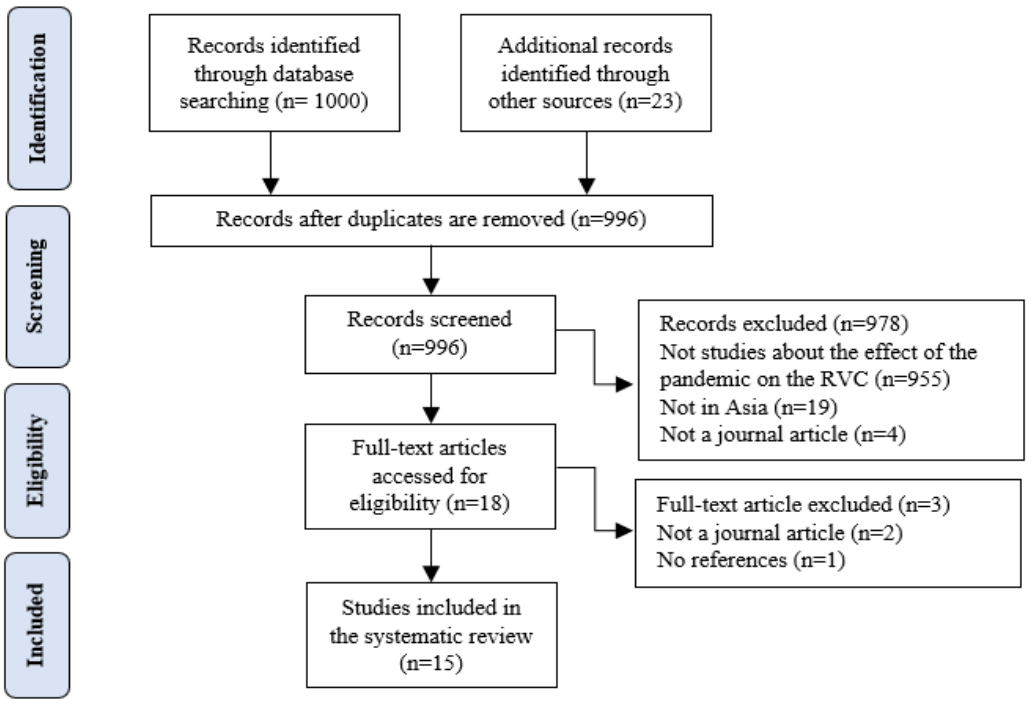

Figure 1. PRISMA Flowchart.

DATA EXTRACTION

The following data were extracted from each study: study characteristics (title, authors, authors' institution/s, database sources, research theme, research type, funding sources, and study location), study overview, and the RVC segment/s discussed in each paper. In addition, information on the year of publication, methodological approach, and key findings related to upstream, midstream, and downstream disruptions was recorded to ensure comprehensive coverage of the value chain impacts. A follow-up search was also conducted to identify the economic status of each country and whether it is rice-producing or non-rice-producing. Data were organized using a standardized extraction form in Microsoft Excel, allowing for systematic categorization and comparison across studies. Where available, quantitative indicators such as percentage changes in production, price fluctuations, and labor shortages were also documented to strengthen the analysis.

DATA ANALYSIS

This study conducted a systematic review through rice value chain analysis of each Asian country represented in the selected studies. A descriptive analysis was then performed to examine the extracted data by summarizing key points and identifying variables, actors, and activities contributing to understanding the effects of the pandemic on each rice value chain segment. Data were extracted using a standardized form that captured study characteristics, methodological approaches, and quantitative indicators such as production changes and price fluctuations. Findings were synthesized narratively and organized by upstream, midstream, and downstream segments to highlight patterns of disruption and resilience strategies across countries.

RICE VALUE CHAIN SITUATION IN SOUTHEAST ASIAN COUNTRIES

This paper reviewed fifteen (15) journal articles, examining the effect of the present COVID-19 pandemic on the three segments of Asian RVCs (see Table 1). Fourteen out of fifteen (93%) papers explored the impact of the pandemic on the input supply and production in the upstream segment of the RVC. Conversely, only six papers (40%) analyzed the condition of the constituent elements of the midstream segment, namely the processing, marketing, and logistics. Regarding downstream consumption and retail, ten papers (66%) examined the significant effects of the pandemic on these elements. This review also aligns with global assessments by FAO and IRRI, which emphasize that pandemic-related disruptions were uneven across regions, highlighting systemic vulnerabilities in RVCs (Wilson et al. 2015).

Table 1. Summary of studies meeting the inclusion criteria for the present systematic review.

Title	Published Date and study location	Authors and Author's institution	Types of Databases	Research Theme and type	Funding Resources
Reviewing Rice Tariffication in the Time of COVID-19: Rationale and Road to Rice Self-Sufficiency in the Philippines	26 April 2021- Philippines	San Juan DM. (De La Salle University, Philippines)	Social Science Research Network (SSRN)	Socio-Ecological Aspect; descriptive survey research	De La Salle University-Manila
Monitoring the impact of COVID-19 in Myanmar: Rice millers – July 2020 survey round	July 2020- Myanmar	Goeb J. et al. (Department of Agricultural, Food, and Resource Economics of Michigan State University Department of Economics, Harvard University Development Strategy and Governance Division of the International Food Policy Research Institute)	International Food Policy Research Institute	Socio-Ecological Aspect; descriptive survey research	CGIAR Research Program on Policies, Institutions, and Markets US Agency of International Development Livelihoods and Food Security Fund International Growth Center
Big Boro rice harvest during COVID-19 pandemic: an optimism for resilient rice system in Bangladesh	02 November 2021- Bangladesh	Jamal MR. (School of Environmental and Rural Science, University of New England, Australia Department of Agriculture Extension, Ministry of Agriculture, Bangladesh)	Journal of Bioscience and Agriculture Research	Socio-Ecological Aspect; descriptive survey research	School of Environmental and Rural Science & Department of Agriculture Extension
Effect of COVID-19 on Logistics of Thai Rice Export	10 October 2021- Thailand	Songsang JK. et al.	International Journal of Current Science Research and Review	Socio-Ecological Aspect; descriptive survey research	Chulalongkorn University
COVID-19 and Policy Impacts on the Bangladeshi Rice Market and Food Security	26 May 2021- Bangladesh	Mobarok MH. et al.	Multidisciplinary Digital Publishing Institute	Socio-Ecological Aspect; descriptive survey research	
Multi-faceted impact and outcome of COVID-19 on smallholder agricultural systems: Integrating qualitative research and fuzzy cognitive mapping to explore resilient strategies	29 December 2021- Eastern India (Sunandar areas)	Goswami R. et al. (Integrated Rural Development and Management Faculty Center, Ramakrishna Mission Vivekananda Educational and Research Institute, India African Plant Nutrition Institute, Polytechnic University, Morocco CSIRO Land and Water. Black Mountain Science and Innovation Park, Australia Global Evergreening Alliance, Melbourne, and Institute for Study and Development Worldwide, Australia)	Scopus	Socio Ecological Aspect; qualitative research	
How COVID 19 effect Malaysian paddy industry? Adoption of green fertilizer a potential resolution	30 September 2020-Malaysia	Adnan N and Nordin SM. (Universiti Teknologi Petronas, Seri Iskandar, Malaysia)	Scopus	Socio Ecological and Policy Aspect; quantitative and descriptive research	

Title	Published Date and study location	Authors and Author's institution	Types of Databases	Research Theme and type	Funding Resources
The Impact of COVID-19 pandemic on food sufficiency in Bantul Yogyakarta – Indonesia	February 2021-Indonesia	Fitriana L. et al. (Education and Training Human Resources Agency of Kutai Timur Kawasan Perkantoran Bukit Pelangi, Indonesia Department of Agricultural and Biosystem Engineering Faculty of Agricultural Technology Universitas Gadjah Mada, Indonesia)	Scopus	Socio Ecological Aspect; quantitative and descriptive research	Rekognisi Tugas Akhir (RTA) Grant of Universitas Gadjah Mada
Agricultural labor, COVID-19, and potential implications for food security and air quality in the breadbasket of India	21 September 2020-India	Singh B. et al. (International Maize and Wheat Improvement Center (CIMMYT) CGIAR Research Program on Climate Change, Agriculture & Food Security (CCAFS) Soil and Crop Sciences Section, School of Integrative Plant Science IRRI South Asia Regional Center (ISARC) International Rice Research Institute (IRRI) Indian Council of Agricultural Research (ICAR) CAR-Central Soil Salinity Research Institute (CSSRI) ICAR-Agriculture Technology Applications Research Institute (ATARI))	Scopus	Socio Ecological Aspect; quantitative and descriptive research	Indian Council of Agricultural Research (ICAR) Government of India CGIAR Research Programs (CRPs) on Climate Change Agriculture and Food Security (CCAFS) Wheat Agri-Food Systems (WHEAT)
Does the COVID-19 pandemic affect the social-ecological resilience of the organic rice production system in Chiang Mai Province, Thailand?	23 June 2021-Thailand (rural area)	Panpakdee C and Palinthorn F. (Department of Agricultural Extension and System Approaches, Faculty of Agriculture, Khon Kaen University)	Forest and Society-Journal Article	Socio Ecological Aspect; descriptive survey research	Research Administration Division of Khon Kaen University
Impact of COVID-19 Pandemic On Local Rice Supply Chain FlowPatterns In Kapuas Regency, Central Kalimantan, Indonesia	June 2021-Indonesia	Erlina Y and Elbaar EF. (Agribusiness, Faculty of Agriculture, Palangka Raya University)9 Indonesian Agricultural Technology Assessment and Development South Kalimantan, Indonesia Indonesian Center for Agricultural Technology Assessment and Development, Indonesia)	ResearchGate	Socio Ecological Aspect; descriptive survey research	Institute for Research and Community Service
Marketing analysis of "Siam" local rice in South Kalimantan during the pandemic of COVID-19	24 September 2021- Indonesia	Sabur A. et al. (Indonesian Agricultural Technology Assessment and Development South Kalimantan, Indonesia Indonesian Center for Agricultural Technology Assessment and Development, Indonesia)	ResearchGate	Socio Ecological Aspect; quantitative and descriptive research	
Increasing rice production: a proposed strategy during and after COVID-19 pandemics	August 2021-Indonesia	Sunandar N. et al. (Indonesia Center for Agricultural Technology Assessment and Development)	IOP Conf. Series: Earth and Environmental Science 803	Socio Economic Aspect; quantitative and descriptive research	

Title	Published Date and study location	Authors and Author's institution	Types of Databases	Research Theme and type	Funding Resources
The Effect of Labor Utilization and Rice Farming Income Due to COVID-19 in Kendal Regency	07 May 2018- Indonesia (Kendal Regency, Central Java)	Aldillah R. et al. (Indonesia Center for Socio Economy and Policy Studies)	Socio Economy and Policy Studies (SEPS)	Socio Economic Aspect; quantitative and descriptive research	
The implications of the COVID-19 pandemic on rice market performance in Java, Indonesia	2021- Indonesia (Jakarta, Semarang, & Surabaya)	Nasir MA. et al. (Department of Agricultural Socioeconomics Faculty of Agriculture, Universitas Gadjah Mada, Indonesia)	IOP Conf. Series: Earth and Environmental Science 637	Socio Economic Aspect; quantitative and descriptive research	Ministry of Research and Technology / National Agency for Research and Innovation

In terms of study location, seven Asian countries were covered by this pool of papers. This included four lower-middle-income countries namely, the Philippines, Myanmar, Bangladesh, and India, together with three upper-middle-income countries specifically Thailand, Malaysia, and Indonesia. Following the first confirmed COVID-19 case on December 31, 2019, these countries reported their first cases in the first quarter of 2020 (Adnan and Nordin 2020). This was followed by the announcement of lockdowns in each country as preventive measures. All seven countries, except Thailand and Indonesia, imposed lockdowns as early as March 2020. These two countries declared lockdowns a month later. Thus, all the papers were conducted and published between April 2020 and November 2021. As of May 2022, these countries were reported by the WHO Coronavirus (2019) dashboard as having a PHSM Severity Index (Public Health and Social Measures) of "Severe" to "Most Severe." Bangladesh and Thailand had a PHSM Severity Index of "Severe." On the other hand, "Severe to Most Severe" characterized the Philippines, India, and Indonesia, while Myanmar and Malaysia had a "Most Severe" PHSM Severity Index. To examine how the severity of public health and social measures impacted the RVC, the methodologies used in these studies included descriptive survey research, qualitative research, and quantitative research. These journal articles examined the complex interplay between ecology (in terms of rice as a natural resource), social actors and processes, and economic activities, during this global health issue. Cross-country differences were evident, as nations with stronger local input systems (e.g., Thailand) showed greater resilience compared to those reliant on imports (e.g., Bangladesh). These variations underscore the role of governance and pre-existing infrastructure in mitigating shocks.

IMPACTS OF COVID-19 ON UPSTREAM SEGREMENT OF THE RVC

The analysis of the papers yielded diverse findings. However, it was determined that the COVID-19 pandemic had a substantial impact on the input supply and output in the upstream portion of the RVC in several ways. Table 2 presents the results of the scholarly literature regarding the influence of COVID-19 on the upstream segment of the RVC. Only individuals engaged in discussions pertaining to the upstream segment were included in this section. The findings were categorized based on the actors and activities in the input supply and production, which are the two constituents of the upstream segment. Regarding input supply, there was only a small impact observed on the actors and activities involved, particularly in terms of seed and machinery availability, as well as fertilizer costs. Conversely, the pandemic significantly disrupted labor patterns. Nevertheless, with improved governance coupled with government assistance, several regions demonstrated successful adaptation to these issues.

In a study authored by Goswami et al. (2021), it was observed that most farmers in the Sundarbans Areas in eastern India were not challenged in securing inputs such as seeds and fertilizers or obtaining them at high prices because they had kept them in reserve in preparation for expected lockdowns and societal restrictions. This is in line with the availability of their machinery. Aside from the fact that tractors and other machinery were not constantly used by marginal farmers, some were already available locally, such as power tillers. Thus, the only challenge for them was the increasing fuel prices; however, it was observed that there were no changes in the imposed charges by operators. Erlina and Elbaar (2021) also reported similar results. Farmers in Indonesia, specifically in Kapuas Regency, Central Kalimantan, had also stocked sufficient seeds for one planting season. Additionally, the transportation of inputs during the pandemic was noted to remain stable. In Chiang Mai Province in Thailand, as reported by Panpakdee and Palinthorn (2021), the stable supply of farming inputs was mainly due to self-dependency on local organic rice systems, using locally available inputs. In contrast, dependency on exported fertilizers, such as in Thailand, as stated by Songsang and

Suthiwartnarueput (2021), resulted in higher input costs.

As regards labor patterns, the pandemic has greatly disrupted farmer labor patterns due to restrictions. According to a descriptive survey conducted by Goeb et al. (2020), there was a decrease in the number of workers in rice paddies in Myanmar by 28%. This was further aggravated by the implementation of health protocols that rice millers needed to follow, such as hygienic practices (four-fifths of rice millers) and temperature screening (one-third of rice millers) (Borras et al. 2017). Similarly, a reduction of workers was also noted in Kendal Regency in Indonesia. As explained by Aldillah et al. (2021), paddies were normally maintained by non-family members. However, during the pandemic, non-family labor decreased to 69%, mainly due to precautions against virus transmission and the scarcity of resources that would otherwise be used as wages for workers within the family. In two other studies focusing on India, authored by Singh et al. (2020) and Goswami et al. (2020), the pandemic was shown to affect migratory labor. Before the pandemic, labor during both planting and harvesting stages mainly relied on migratory workers. Thus, due to ongoing societal restrictions, the workforce in these areas was challenged.

In terms of production, as one of the components of the upstream segment of the RVC, most of the papers reported positive results despite the ongoing health crisis. According to Erlina and Elbaar (2021) and Sabur et al. (2021), who studied rice paddies in Indonesia, production continued in the central and southern areas of Kalimantan. In two separate studies, production was found to be supported by adaptive strategies implemented by farmers. In the case of the study presented by Jamal (2021), the production of Boro rice in Bangladesh in 2021 recorded the highest yield ever (4.29 t/ha), which was 6% higher than the previous year, due to better farm management and government support. Similarly, according to Panpakdee and Palinthorn (2021), an increase in production was also observed in Chiang Mai Province in Thailand, attributed to farmers exploring adaptive strategies and increased autonomy in managing local food supply.

Table 2. Impact of COVID-19 on the actors and activities in the input supply and production of the upstream segment. (Note: [1] = raw materials, labor, and goods for rice cultivation; actors: seeds, fertilizers, machinery, farmers, suppliers; millers activities: seed collection, seed supply, fertilizer supply; [2] = series of activities and processes in cultivation, pre-harvesting, and harvesting; actors: yield, farmers, millers, mills; activities: growing, harvesting, threshing, drying).

References	Upstream Segment			
	Input Supply[1]		Production[2]	
	ACTORS	ACTIVITIES	ACTORS	ACTIVITIES
Goswami et.al. (2020)	(-) fertilizer (imported) (+) seed & fertilizer (+) machinery (-) farmers (-) millers	(+) seed collection (+) seed supply (+) fertilizer supply	(+) farmers (family labor) (-) farmers (hired labor)	(-) growing
Erlina and Elbar (2021)	(+) seed & fertilizer	(+) seed collection (+) seed supply		(+) growing (+) harvesting
Palinthorn and Panpakdee (2021)	(+) seed & fertilizer (+) machinery (-) farmers (-) millers	(+) seed supply (+) fertilizer supply		(+) growing (+) harvesting
Songsang and Suthiwartnarueput (2021)	(-) seed & fertilizer	(-) fertilizer supply		
Goeb et al. (2020)	(-) millers			
Aldillah et al. (2021)	(-) farmers (-) millers			
Singh et al. (2020)	(-) farmers			(-) growing (-) harvesting
Sabur et al. (2021)	(+) seed & fertilizer	(+) seed supply (+) fertilizer supply		(+) growing (+) harvesting (+) threshing (+) drying
Jamal (2021)	(+) seed & fertilizer (+) farmers (+) millers	(+) seed supply (+) fertilizer supply		(+) growing (+) harvesting

One common variable among the papers analyzed is that the countries are all categorized as rice-producing countries. Therefore, these countries theoretically have strong knowledge of the processes and components of the rice value chain. In the paper of Goswami et al. (2020), the study location is in the Sundarbans areas of east India, which belong to West Bengal— a state that has consistently been among the top rice-producing regions in the country (Pathak et al. 2020). According to key biophysical factors (temperature, rainfall, and soil type), rice farms in West Bengal are categorized as suitable to very suitable zones for rice production. From 1990 to 2018, despite a decreased in farming land, the region showed positive changes in rice production and productivity (Pathak et al. 2020). Therefore, as a major rice production area contributing about 15% of India's output (National Food Security Mission, 2016), it was expected to have sufficient reserves of seeds and fertilizers, thereby contributing to resiliene in input supply. Also, the presence of tractors, local power tillers, and other machinery was of no surprise because India has been implementing this development called Farm Mechanization for its agricultural production, with a goal to achieve a sustainable increase in both farm yield and income (Bhattarai et al. 2018; Shukla et al. 2019; Gulati et al. 2020). The same applies to a rice estate in Kapuas Regency, Central Kalimantan, Indonesia where seeds stocked for another planting season (Erlina and Elbaar 2021). Historically, Central Kalimantan was a part of the failed Mega Rice Project in 1995, where converted peatlands were found unsuitable for major crop production (IPS 2020). However, more recent findings (Wardie & Sintha, 2018) indicate that, at the household level, farming systems in the region have become highly sustainable. This suggest that the local rice system in the regency is self-sufficient, explaining the minimal negative effect of the pandemic on their RVC. Self-sufficiency and local dependency also contributed to Thailand's stable rice production during the pandemic. Thailand is one of the largest rice producers (FAO 2019) and the second-largest exporter after India (Asia Pacific Foundation of Canada 2021). As a result, there is a well-established domestic market for farming inputs, particularly in Chiang Mai Province. Several suppliers of fertilizers and seeds are located within the area, and irrigation concerns are minimal due to presence of the Ping River, a major tributary.

As agriculture is a principal sector in many Asian economies (APFC 2021), ensuring safety during food processing became vital. This created additional challenges for worksites as the pandemic emerged, and therefore restrictions were implemented. In Southeast Asia alone, there was an estimated 116 million workers affiliated with the agriculture and food industry, with 10 million being migrant workers (APFC 2021). Therefore, agriculture was not only a source of food for these countries, but it also served as the driving force for employment and their respective economies. The availability of labor for agricultural activities varied from place to place. The labor workforce available for rice fields in Myanmar, Indonesia, and India was found to be challenged by fewer workers available for rice production (Goeb et al. 2020; Singh et al. 2020; Aldillah et al. 2021; Goswami et al. 2021). India announced its first COVID cases in late January 2020, Indonesia in the first week of March 2020, and Myanmar in the last week of March 2020. Since then, necessary restrictions were implemented across Asian countries to ease the transmission of the virus. The leading cause for this decrease in workers was the health protocols mandated by the government, most notably physical distancing. In a report on Myanmar migrant workers, 66% of the interviewees either lost their jobs due to decreased operations at their worksites or left voluntarily due to the threat of the virus. From this, it can be inferred that migrant workers from other countries likely faced similar situations and therefore reduced the workforce in their previous jobs. Some workers engaged in agriculture left their jobs not only due to the virus but also because of decreasing wages and increasing living costs, which left them unable to support their families and forced them to return to their respective countries (ILO 2021). Therefore, rice field owners who were dependent on migrant workers shifted to local household laborers, which sometimes resulted in reduce or earlier rice production operations. This was the case in Bangladesh (Jamal 2021). The country shifted to local village laborers since the mobility for city farm laborers was reduced. However, this benefited them, as some workers returned home, leading to an increased farm workforce.

Even with the challenges to labor availability the rice sector across different Asian countries, it was notable that production not only maintained its rate but, in some cases, even surpassed pre-pandemic levels. Indonesia was still able to sustain its rice production amid COVID-19 stresses (Erlina and lbaar 2021; Sabur et al. 2021). This is an expected outcome, as the country is one of the top rice-producing nations in the world. Among ASEAN countries, Indonesia has the highest employment related to the agriculture and food industry (APFC 2021). Therefore, although affected by a decrease in workforce, the country still had sufficient local labor. Additionally, since Indonesia has local farming input reserves such as seeds, rice production did not cease. In Bangladesh, rice production not only continued but also reached the highest yield of Boro rice in history (Jamal 2021). One factor contributing to this increase was the rise in market prices of rice, which motivated farmers, supported by government subsidies such as farming inputs and machinery. Thailand also showed resilience

to COVID-19 restrictions on rice production (Panpakdee and Palinthorn 2021), mainly due to its autonomy in farm inputs and reliance on local supply. Farmers also implemented adaptive strategies during pandemic rice production stresses, such as adjusting harvesting dates based on available resources. In conclusion, Asian countries were able to cope with and continue rice production during the pandemic. It has been reported that despite various agricultural stresses, the food supply chain, including the RVC, remained robust and resilient (CGIAR 2020). On the other hand, while the agricultural sector has been less affected by the pandemic, the greater challenge lies in how this produce reaches consumers (FAO 2019).

The study of Goeb et al. (2020) revealed that due to pandemic restrictions, there was a reduction in operating hours in 56% of mills, while 36% stopped milling activities for at least one week. Similarly, post-harvest processing of rice was reduced in Chiang Mai Province, Thailand due to an inadequate workforce (Panpakdee and Palinthorn 2021).

As regards other actors in the midstream segment, specifically the traders and wholesalers, most of them resorted to buying directly from farmers, which, aside from faster commerce, resulted in lower costs in buying and selling their produce. As stated by Erlina and Elbaar (2021), rice milling owners in Central Kalimantan bought unmilled rice directly from farmers and paid them immediately (Sabur et al. 2021). On the contrary, farmers in Sunandar areas in eastern India are reported to be challenged by disruptions in marketing and logistics. Unlike farmers from Indonesia, they are forced to sell their produce to local and neighboring markets at lower prices because no wholesalers buy their produce.

Another activity in the midstream segment that was found to be greatly affected was the transport of rice produce, both within and outside the areas of study. In a study by Goswami et al. (2020), it was reported that farmers resorted to using bicycles to transport rice produce in small quantities. Some used rickshaws and motor vans, which were observed to be more costly. Restrictions in trading from country to country have also caused delays in transport. As explained by Songsang and Suthiwartnarueput (2021), since Chinese traders reduced the number of containers for transport, there was an increase in sea freight. As a result, local traders waited in the hope of lower sea freight, delaying the transport and storage of rice produce, which resulted in a shortage of space for the new harvest. In parallel with these delays, Erlina and Elbaar (2021) reported that this caused wholesalers and retailers alike to experience inaccurate transport schedules.

The COVID-19 pandemic intensified concerns about the security of food supplies in the Asian and Pacific countries. As health risks increased, travel restrictions also increased. With the introduction of unanticipated stresses, it created immediate challenges for individuals wordwide. According to Kim et al. (2020), the disruptions mentioned in the upstreamsegment delayed and even postponed many processes in the midstream-processing, logistics, and marketing. According to the OECD, the global health crisis undoubtedly disrupted processes in food industries (CGIAR 2020). Due to illness and lockdown measures, there was a workforce shortage and an inadequate number of workers. In the case of vegetable and meat processing facilities, social

Table 3. Impact of COVID-19 on the actors and activities in the processing, marketing, and logistics in the midstream segment. (Note: [1] = series of post-harvesting activities and processes; actors: millers, collectors, traders, wholesalers, milling; activities: milling, paddy collection, drying, trading, wholesaling. [2] =delivery and transport of harvest from paddy to market; actors: traders, transporters, importers, exporters; activities: trading, transport, import, export).

References	Midstream Segment			
	Processing[1]		Marketing & Logistics[2]	
	ACTORS	ACTIVITIES	ACTORS	ACTIVITIES
Goeb et al. (2020)	(-) millers	(-) milling		
Panpakdee and Palinthorn (2021)	(-) millers	(-) paddy collection (-) paddy selling (-) drying (-) milling		
Erlina and Elbaar (2021)	(+) collectors (+) millers	(+) paddy collection (+) paddy selling (+) milling	(-) traders	(-) transport
Sabur et al. (2021)	(+) collectors	(+) paddy collection (+) paddy selling (+) drying (+) milling		(+) trading (+) selling
Goswami et al. (2020)				(-) transport (-) trading (-) selling

Table 4. Impact of COVID-19 on the actors and activities in retail and consumption in the downstream segment. (Note: [1]=selling of rice produce to consumers; actors: rice produce, price, wholesalers, retailers, consumers; activities: wholesaling, retailing, selling, buying; [2] =use and value of goods to consumers or households; actors: price, demand, buyers, suppliers; activities: supply, demand.)

References	Downstream Segment			
	Retail[1]		Consumption[2]	
	ACTORS	ACTIVITIES	ACTORS	ACTIVITIES
Goswami et al. (2020)	(-) wholesalers (-) retailers (+) consumers	(-) wholesaling (-) retailing	(+) consumers (-) suppliers	(-) supply (+) demand
Nasir et al. (2021)				(-) supply (rice deficit areas) (+) supply (riced surplus areas)
San Juan (2021)	(-) wholesalers (-) retailers (-) consumers	(-) wholesaling (-) retailing	(-) consumers (-) suppliers	
Goeb et al. (2020)	(-) wholesalers (-) retailers (-) consumers	(-) wholesaling (-) retailing	(-) suppliers	(-) demand
Fitriana et al. (2021)			(+) consumers (-) suppliers	(-) supply (+) demand

distancing measures had to be applied to protect employees from the virus. In relation to this, processing and logistics operations were disrupted due to high rates of work absences. In a study by the ICLEI Southeast Asia Secretariat (2020), countries in Southeast Asia were found to have experienced major adverse effects of the pandemic. In the Philippines, the transportation of goods suffered delays due to strict inspections and border restrictions. Due to disruptions in transportation, a large volume of fresh produce became oversupplied, and to prevent waste, farmers decided to lower prices. Countries like Indonesia and the Philippines developed online delivery services that greatly helped in transporting supplies without risking lives.

In the study by Sabur et al. (2021), social constraints due to COVID-19 led to significant disruptions in the logistics and marketing sectors in South Kalimantan, Indonesia. The respondents of the study were individuals who experienced rice marketing firsthand--local traders, wholesalers, and farmers. Based on the result, the marketing of Siam local rice remained efficient despite the pandemic. In another study by Erlina and Elbaar (2021), the focus was on the impacts of COVID-19 on the local rice supply chain in Central Kalimantan, Indonesia. The study indicated that farming activities were not significantly affected during the pandemic. However, COVID-19 had noticeable effects on the rice industry, particularly in the distribution process. In addition, the likelihood of incorrect product delivery due to restrictions and delays was high. In the study conducted by Goeb et al. (2020) in Myanmar, 60% of rice mills were expected to experience a revenue drop compared to 2019. The results showed that almost all mills considered sales as beneficial to business, and approximately half reported no changes in byproduct prices, especially compared to 2019. Therefore, mills producing large volumes of high-quality rice may have experienced negative effects on lower-value outputs.

Following these adverse effects, another study by Panpakdee and Palinthorn (2021) investigated the effects of rice production systems in four districts in Chiang Mai, Thailand. The study found that the pandemic did not significantly influence organic rice production in terms of social-ecological resilience. Even with labor conflicts due to COVID-19 restrictions, the impact on the midstream segment was minimal. In conclusion, the impacts of COVID-19 on the midstream varied by location and country resilience. Some countries adapted effectively, while others struggled.

In a study by Goswami et.al. (2020), it was revealed that although farmers in the Sunandar areas resorted to selling their produce at low prices, some produce remained unsold. Farmers brought this unsold produce back to their homes; some were consumed, while some were sold to private intermediaries, thereby incurring losses.

Several studies reported that increases in rice prices were largely influenced by the pandemic. In a study by Nasir et al. (2021), rice prices in Indonesia increased in deficit areas such as Jakarta, while they decreased in surplus areas like Semarang and Surabaya. A similar pattern was observed in Eastern India, where surplus supply combined with low demand forced rice prices to decrease (Goswami et al. 2021). In another study by San Juan (2021), the price increase of exported rice in the Philippines was attributed to the imposition of tariffs on imported rice.

Finally, since the pandemic brought undesirable effects to the upstream and the midstream segments of the RVC, consequently, it also affected the endpoint of the RVC--retail and consumption. In a survey by Goeb et al. (2020), rice prices in Myanmar increased in 2020 compared to 2019, mainly due to reduced milling operations. In contrast, prices

declined in Sunandar areas in Eastern India. According to Goswami et al. (2020), limited market hours and an excess of sellers over buyers led to lower prices, with some produce retained for household consumption. While this benefited consumers, it was unfavorable for farmers. This finding is consistent with a study by Fitriana et al. (2021), which examined rice supply and demand in Bantul Regency, Indonesia. In 2020, the production was 115,988.47 tons, while demand rose to 145,131.25 tons, resulting in a supply deficit.

The Philippines also experienced a price increase during the pandemic; however, this was mainly due to tariffs on imported rice (San Juan 2020). Myanmar also reported higher rice prices in 2020 compared to 2019 (Goeb et al. 2020), largely due to the challenges faced by milling companies, some of which were forced to close due to health restrictions. Rice millers had already anticipated a decrease in revenue, especially for those small milling company owners.

Therefore, it is important to note that the effects of the pandemic on the downstream segment of the RVC varried by location, depending on whether a country had enough rice production and effective market flow. Although global food prices remained relatively stable, exceptions occurred, especially for rice produce, as Thailand—a major rice exporter—experienced drought, which reduced production (Papademetriou et al. 2020; Songsang and Suthiwartnarueput 2021). Nevertheless, international market flows continued, supported by commitments from countries such as Myanmar, Brunei, and Singapore (Mobarok et al. 2021). This ensured that rice was successfully transported and delivered to consumers (Mobarok et al. 2021; Bhandari 2020). However, these findings should be interpreted with caution due to limitations: the evidence base is small, only English-language journals were included, and gray literature was excluded, which may omit critical local insights.

CONCLUSION

The COVID-19 pandemic remarkably influenced Asian rice value chains (RVC). Overall, the analysis reveals significant disruptions concentrated in midstream and downstream activities, while upstream operations demonstrated relative stability. This contrast emphasizes the critical role of preparedness and adaptability in agricultural systems. Global health issues such as COVID-19 affect not only public health, but also the economy. The findings underscore the need for integrated strategies that combine government support, private sector engagement, and farmer resilience to maintain continuity in production and trade. Collaboration between farmers and government is essential to reduce costs and strengthen local rice systems. Future efforts should focus on policy innovation, infrastructure development, and digital solutions to enhance connectivity and efficiency. In addition, tariffs on imported goods may help protect the domestic market, while generating government revenue that can be used to subsidize farmers. Ultimately, these measures can stabilize the rice value chain and build long-term resilience against future global disruptions.

Only the midstream and the downstream segments were severely affected. Evidence shows mixed outcomes in input supply and production, with stability in some contexts and declines or cost increases in others; results vary by country, pandemic phase, and policy environment. Therefore, collaboration between the farmers and government remains critical. In times of crisis, government and private sectors should enhance support systems for farmers and ensure continuous market flow.

Across studies, upstream outcomes were heterogeneous: some contexts reported stable input access due to logistics exemptions and domestic sourcing, while others experienced higher input prices, delivery delays, and labor shortages. Midstream processing and marketing showed mixed throughput changes, influenced by mobility restrictions, working capital constraints, and market access, with price transmission varying by policy regime and market structure. Downstream retail and consumption effects ranged from temporary availability gaps and price volatility to rapid shifts toward e-commerce and smaller-package purchases, with stronger household food security impacts in lower-income, urban, or import-dependent settings. Where comparable quantitative indicators were available, we applied random-effects models, finding substantial heterogeneity (I^2/τ^2) and moderation by pandemic stringency, trade dependence, and digital readiness. These findings underscore that RVC outcomes are context-specific, and policy effectiveness depends on baseline capacities, timing, and complementarities.

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Generative AI tool (i.e., ChatGPT) was used exclusively for grammar checking and language refinement during the preparation of this manuscript.

All ideas, analyses, data interpretations, and written content were originally produced by the authors without any assistance in generating or developing the paper's substance. No generative AI was used to create, expand, or modify the intellectual content, findings, or conclusions presented in this work.

ETHICAL CONSIDERATIONS

This study was reviewed by the Cebu Normal University-Ethics Research Committee. This study was exempted from review because it was a systematic review paper which did not involve human participants.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper. All authors confirm that no conflicts of interest exist and that the research was conducted independently.

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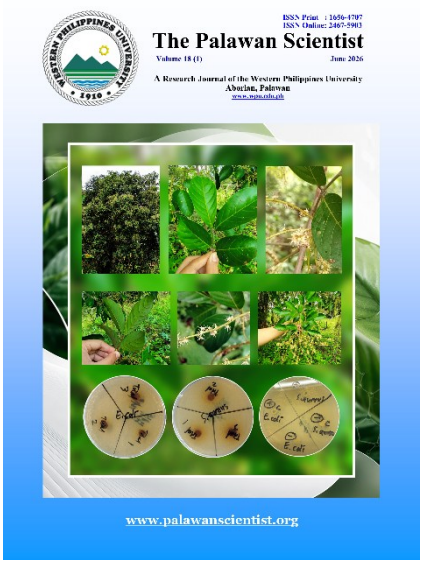

Effects of fermented plant juice (FPJ) concentration and application frequency on the growth and flowering of *Ruellia simplex* C. Wright 'Katie Pink'

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ABSTRACT

The increasing popularity of organic farming and home gardening in the Philippines has heightened interest in sustainable alternatives to synthetic fertilizers. Fermented plant juice (FPJ), a liquid organic supplement from plant materials and molasses, shows potential for promoting plant growth and development but remains understudied in ornamental species. This study evaluated the effects of different FPJ concentrations and application frequencies on the growth and flowering responses of *Ruellia simplex* C. Wright 'Katie Pink', a widely cultivated ornamental plant. The experiment followed a completely randomized design with eight treatments, including different FPJ concentrations (1.5%, 3%, 6%) and application frequencies (once, twice, four times, and eight times per month), along with ammonium sulfate (21-0-0) and a control (T1 - no fertilizer application). Results showed that application of ammonium sulfate (21-0-0) (T8) significantly enhanced all growth parameters and flowering traits, including plant height, leaf number, chlorophyll content, and flower production. Among the FPJ treatments, 6% FPJ applied eight times per month (T7) demonstrated the best performance in both vegetative and reproductive development, characterized by early bud emergence, the highest bud count, and high root volume. However, its flower conversion rate (52.4%) was lower than that of T8 (86.6%), although the difference was not statistically significant. Treatments with lower FPJ concentrations or reduced application frequency resulted in delayed or absent flowering but still performed better than the control (T1). These findings highlight the importance of optimizing both FPJ concentrations and application frequency. High-concentration and high-frequency FPJ applications can enhance plant growth and reproductive performance, making FPJ a viable and sustainable alternative to chemical fertilizers when properly managed.

Keywords: ammonium sulfate, concentration, frequency, organic fertilizer, ornamental plant

INTRODUCTION

In the Philippines, home gardening became an internet sensation during the COVID-19 pandemic, with the terms "plantito" and "plantita" emerging on social media to refer to individuals who developed an interest in growing plants, particularly ornamental plants at home (Sunga and Advincula 2021).

Following the popularity of home gardening, people began using fertilizers indiscriminately, driven by the belief that higher dosages promote better plant growth. In principle, fertilizer application is intended to optimize growth and yield for maximum profit and to increase production to meet the demands of a growing population. The introduction of synthetic fertilizers during the Green Revolution has led to an exponential increase in the yield of most crops (Paull 2009; Maaz 2025; Sharafi et al. 2025). However, the continuous overapplication of fertilizers has led to various environmental issues, including soil degradation, water pollution, and ecosystem imbalance (Li et al. 2018; Gaytancıoğlu and Yılmaz 2024). In response to these issues, there has been growing advocacy for sustainable agriculture.

Sustainable agriculture is a holistic farming system that focuses on increasing agricultural productivity while considering the short- and long-term effects of farm practices on the environment and human health (Velten et al. 2015; Hiywotu 2025; Singh et al. 2025). One of the key sustainable agricultural practices is organic farming, which prohibits or limits the use of synthetic fertilizers and pesticides. Instead, organic agriculture relies on natural alternatives such as organic concoctions or plant-based supplements (Reganold and Wachter 2016; Seufert and Ramankutty 2017; Caipang and Avillanosa 2019; Chojnacka et al. 2020). These inputs are derived from locally available natural materials and include common types such as indigenous microorganisms (IMO), fermented fruit juice (FFJ), fermented plant juice (FPJ), fish amino acid (FAA), lactic acid bacteria serum (LABS), and natural indigenous activator (NIA) (BAFS 2014; Keliikuli et al. 2019).

According to the Bureau of Agriculture and Fisheries Standards (BAFS, 2014), these organic plant supplements are compounds of organic origin in liquid or solid form that, in low concentration, promote or modify physiological processes in plants. Recent studies have shown that organic and biologically derived inputs applied at low rates can influence plant physiological functions by enhancing nutrient use efficiency, stress tolerance, and overall growth performance, even without serving as direct nutrient sources (du Jardin 2015; Rouphael and Colla 2020; Mannino et al. 2025). Moreover, these supplements should contain a total nitrogen, phosphorus, and potassium (NPK) content of not less than 0.5% and not more than 2.5%. They may also include beneficial microorganisms, micronutrients, and naturally occurring plant growth regulators, which contribute to improved plant physiological responses under sustainable and organic farming systems (Silva et al. 2025; Win et al. 2025).

Fermented plant juice is an organic plant supplement made up of plant extracts and molasses. The current recommendation of the Agricultural Training Institute for preparing FPJ is a 1:1:1 ratio of chopped plant materials, molasses or crude sugar, and clean water. It has been applied to a wide range of crops, including organic rice, corn, fruit trees, and vegetables (ATI-DA 2022). The use of FPJ and related organic concoctions has been found to have positive effects on plant growth, yield, and soil biological and chemical properties when applied as foliar or soil amendments (Sakimin et al. 2017; Sulok et al. 2021; Taer 2025). Another important benefit of using organic fertilizers such as FPJ is their potential to recycle food waste by utilizing large volumes of discarded fruits and vegetables resulting from poor marketability, quality rejection, and overproduction of perishable crops.

Several studies have shown that the application of FPJ significantly enhances various aspects of crop production. For instance, Anuada et al. (2021) reported that FPJ induced early flowering and significantly increased the number of flowers and fruits per plant in cherry tomato. Sakimin et al. (2017) observed that the application of FPJ increased plant height, induced early flowering and fruiting, and enhanced photosynthetic rates in tomato plants. Other studies have also noted improvements in reproductive and yield parameters, such as reduced days to fruit setting in pepper, increased fruit number and weight in eggplant, and higher yield per hectare in cowpea and eggplant (Pagluanan and Anical 2010). Denona et al. (2020) observed increased productivity in leaf mustard, while Lorio and De Asis (2021) reported improved fruit production in hot pepper (*Capsicum frutescens* L.). Similarly, Alam (2021) and Tagotong and Corpuz (2015) found that FPJ enhanced both the growth and yield of lettuce (*Lactuca sativa* L.) and pechay (*Brassica pekinensis* L.), respectively.

Although FPJ and other organic fertilizers are often considered environmentally friendly, excessive application can still have negative effects beyond economic considerations. High application rates of organic fertilizers can compromise soil physical and chemical properties, including salinization, clay dispersion, and reduced soil function, ultimately affecting plant growth and soil ecosystem services (Silva et al. 2024). In addition, increased use of organic fertilizers, such as manure and compost, can lead to higher nitrogen and phosphorus loads in surface waters, raising concerns about nutrient runoff and water quality degradation (Lisenbee et al. 2024). Fertilizer inputs also interact with broader environmental systems and can contribute to contamination of soil, air, and water if not properly managed (Tagkas et al. 2024). These considerations underscore that appropriate fertilizer rates and sustainable management are crucial not only for economic efficiency but also for mitigating potential environmental impacts.

While several studies have explored the use of FPJ on fruit and vegetable crops, there is limited research on its effects on ornamental plants. Unlike food crops, ornamental plants are primarily cultivated for aesthetic traits, such as flowering intensity, plant form, and visual quality, rather than for yield or biomass. The nutritional requirements of ornamental species are often less well established than those of major food crops, and imbalanced fertilization can lead to poor ornamental quality or increased production costs (Khoddamzadeh et al. 2025; Furtini Neto et al. 2015). In ornamentals, proper nutrient balance, particularly of macronutrients such as nitrogen and potassium, can enhance quality traits, including plant height, flower formation, and postharvest performance (Bashaboina et al. 2025; Joiner et al. 1983). Studies on biostimulants, including organic inputs, have shown significant effects on growth, nutrient uptake, and quality traits in ornamental plants (Tütüncü et al. 2024). Because ornamental plants differ from food crops in production goals and physiological priorities, responses to organic inputs such as FPJ may not be directly comparable across plant types, underscoring the need for focused research on ornamental species and caution in extrapolating results to other crops.

In this study, *Ruellia simplex* C. Wright 'Katie Pink', a perennial herbaceous flowering plant, was used. It belongs to the family Acanthaceae, a large family of angiosperms that is widely distributed across tropical, subtropical, temperate, and subtemperate regions. This species is widely cultivated as an ornamental ground cover or border in various landscapes due to its adaptability to diverse environmental conditions, minimal cultural requirements, and attractive, brightly colored flowers. It is easily propagated through cuttings or seeds and is tolerant of drought, soil acidity, and salinity, although it grows best in well-drained soils. The plant can thrive under full sun or partial shade and flowers throughout the year, particularly during the summer season (Wilson et al. 2020; Gilman 1999). Because of its rapid growth, consistent flowering response, and widespread use in ornamental landscaping, *R. simplex* was selected as a model ornamental species to evaluate the effects of FPJ on vegetative growth and flowering. While responses to FPJ may vary among ornamental species, this study provides baseline information that may guide future research on other ornamentals with similar growth habits.

This study focused on the growth and flowering responses of *R. simplex* to different concentrations and application frequencies of FPJ, compared with a synthetic fertilizer applied at the recommended rate. Specifically, this study aimed to: (1) identify the optimal frequency of FPJ application at a fixed concentration, (2) determine the most effective FPJ concentration when applied at a consistent frequency, and (3) assess the overall effectiveness of FPJ in comparison with ammonium sulfate application.

METHODS

Plant Material and Experiment Treatments

The experiment was conducted from March to June 2023 at the Fruit Crops Nursery, University of the Philippines Los Baños (UPLB), under full sunlight conditions. Uniform juvenile-stage *R. simplex* plants were obtained from a local nursery in Bay, Laguna, and transplanted into 4-inch plastic pots filled with a mixture of garden soil, coir dust, compost, and carbonized rice hull in a 3:1:1:1 ratio. After a week of acclimatization (root establishment period), fertilizer treatments were applied. Soil pH was monitored weekly using a soil pH tester (AgraTronix^{TM}, USA).

The experiment was laid out in a completely randomized design (CRD) with eight treatments and three replicates per treatment. The treatments were as follows: (T1) control (no fertilizer); (T2) 3% FPJ applied once a month; (T3) 3% FPJ applied twice monthly (every other week); (T4) 3% FPJ applied four times a month (once a week); (T5) 3% FPJ, (T6) 1.5% FPJ, and (T7) 6% FPJ – all applied eight times per month (twice a week); and (T8) 0.4% ammonium sulfate (21-0-0) (w/v) applied twice monthly. All treatments were summarized in Table 1.

The FPJ treatments were based on either increasing or decreasing the general recommendation of the Department of Agriculture – Agricultural Training Institute (ATI-DA 2022), which prescribes two tablespoons (approximately 15 mL) of concentrated FPJ per liter of water. In contrast, the rate of ammonium sulfate (T8) followed common nursery practice at the source of the planting material. Each plant received 300 mL of treatment solution per application for six weeks. Commercially available FPJ and ammonium sulfate were used to prepare the fertilizer treatments.

The commercial FPJ used in the study was sourced from a local manufacturer (Natural Organic Fertilizer) and was formulated from a 1:1 mixture of molasses and fresh malunggay (*Moringa oleifera* Lam.) and oregano (*Origanum vulgare* L.) leaves, fermented for two weeks as specified on the product label. Chemical analysis of the FPJ was conducted at the Division of Soil Science Laboratory, Agricultural Systems Institute, College of Agriculture and Food Science, University of the Philippines Los Baños, to verify its nutrient composition.

Table 1. Summary of fertilizer treatments with corresponding classification, concentration, and application frequency. *Based on the type of fertilizer input.

Treatment			Fermented Plant Juice (FPJ) Concentration (%)	Frequency of Application (per month)
Code	Description	Classification*		
T1	Control	None	0.0	0.0
T2	FPJ (2 tbsp/L)	Organic	3.0	1.0
T3	FPJ (2 tbsp/L)	Organic	3.0	2.0
T4	FPJ (2 tbsp/L)	Organic	3.0	4.0
T5	FPJ (2 tbsp/L)	Organic	3.0	8.0
T6	FPJ (1 tbsp/L)	Organic	1.5	8.0
T7	FPJ (4 tbsp/L)	Organic	6.0	8.0
T8	Ammonium sulfate (21-0-0) (0.25 tbsp/L)	Inorganic	0.0	2.0

Plant Growth and Reproductive Parameters

Growth parameters were measured at designated intervals throughout the experiment. At the onset, plant height (cm) was measured from the base of the stem to the highest leaf. Leaf area (cm²) was estimated non-destructively using linear leaf dimensions, where maximum leaf length (L) and width (W) were measured with a ruler, and leaf area was calculated as:

$$\text{Leaf area} = L \times W \times 0.75$$

The correction factor (0.75) accounts for the non-rectangular shape of most leaves and is commonly used in simplified leaf-area models based on length and width measurements (Stickler et al. 1961). Recent work also supports the use of shape-based correction factors for estimating leaf area from length and width (Schrader et al. 2021). The total number of leaves and chlorophyll content (SPAD value) were recorded weekly, with SPAD readings taken in the morning from three leaves per plant using a SPAD meter (Minolta SPAD-502Plus, Singapore).

At termination, plant height and leaf area were re-measured. Stem diameter (mm) was measured at mid-height using a digital vernier caliper (HilkaTools, China), and root volume (mL) was determined using the water displacement method. The samples were then harvested and air-dried for one week, after which dry weight (g) was measured using a digital weighing scale.

Flowering response was assessed by recording four key parameters: (1) days to first floral bud emergence, defined as the number of days from transplanting to the emergence of the first visible floral bud; (2) days to first anthesis, measured as the number of days from transplanting to the full opening of the first flower; (3) total floral bud count per plant, including both open and unopened buds; and (4) total number of flowers that reached anthesis per plant. Data were collected on individual plants and averaged per treatment. Plants that did not exhibit flowering were recorded as non-flowering and excluded from analyses involving flowering time and flower counts.

Statistical Analysis

Analysis of variance (ANOVA) and Tukey's HSD (Honestly Significant Difference) test were performed using Jamovi *version 2.3.28.0* (Jamovi 2024).

RESULTS

Optimal Frequency of FPJ Application

The application of 3% FPJ at varying frequencies (T2-T5) showed no statistically significant differences compared with the control (T1), although some increase in vegetative growth parameters was observed. Application at four times per month (T4) resulted in the tallest plants (16.37 cm), followed by T5 (14.97 cm), but neither differed significantly from T1 (13.47 cm) (Table 2). Chlorophyll content also tended to increase with frequency, with T5 (40 SPAD units) recording higher values than T2-T4 and the control (35.30 SPAD). Similarly, leaf area peaked in T5 (13.47 cm²), with all FPJ treatments exceeding T1 (11.01 cm²). Root volume and dry biomass were also enhanced, especially in T4 and T5 (7 mL and 6.67–4 g, respectively), compared with T1 (3.33 mL and 2 g), but the differences were not significant.

Table 2. Effects of fermented plant juice (FPJ) and ammonium sulfate (21-0-0) applications on the growth of *Ruellia simplex* C. Wright 'Katie Pink'. Values (Mean ± SE; n = 3) within each column followed by the same letter are not significantly different using Tukey's HSD test at $P \leq 0.05$.

Treatment		Plant Height (cm)	Stem Diameter (mm)	Chlorophyll Content (SPAD value)	No. of Leaves	Leaf Area (cm^2)	Root Volume (ml)	Dry Weight (g)
T1	Control	13.47 ± 0.44^{b}	16.90 ± 0.83^{a}	35.30 ± 1.25^{b}	17.00 ± 3.06^{b}	11.01 ± 2.00^{ab}	3.33 ± 0.33^{b}	2.00 ± 0.00^{b}
T2	3.0% FPJ (1x/month)	13.60 ± 2.02^{b}	15.20 ± 0.68^{a}	36.97 ± 4.43^{b}	38.00 ± 12.5^{ab}	9.27 ± 0.65^{b}	5.67 ± 1.76^{ab}	2.30 ± 0.33^{b}
T3	3.0% FPJ (2x/month)	14.27 ± 0.72^{ab}	16.47 ± 0.81^{a}	36.20 ± 4.48^{b}	34.00 ± 8.08^{ab}	11.09 ± 0.31^{ab}	4.67 ± 0.67^{b}	7.00 ± 1.15^{ab}
T4	3.0% FPJ (4x/month)	16.37 ± 1.35^{ab}	16.80 ± 0.70^{a}	37.20 ± 3.65^{b}	53.00 ± 9.29^{ab}	11.16 ± 0.44^{ab}	7.00 ± 0.58^{ab}	6.67 ± 1.76^{ab}
T5	3.0% FPJ (8x/month)	14.97 ± 1.49^{ab}	14.13 ± 0.46^{a}	40.00 ± 2.10^{b}	36.67 ± 1.67^{ab}	13.47 ± 1.95^{ab}	7.00 ± 1.15^{ab}	4.00 ± 1.00^{b}
T6	1.5% FPJ (8x/month)	13.50 ± 0.76^{b}	12.13 ± 0.57^{a}	36.90 ± 1.56^{b}	26.67 ± 1.76^{ab}	8.94 ± 0.17^{b}	3.33 ± 0.88^{b}	2.67 ± 1.20^{b}
T7	6.0% FPJ (8x/month)	17.07 ± 1.45^{ab}	15.83 ± 0.72^{a}	45.70 ± 3.20^{ab}	39.33 ± 9.4^{ab}	11.46 ± 0.28^{ab}	11.00 ± 2.65^{a}	3.67 ± 0.88^{b}
T8	0.4% Ammonium sulfate (2x/month)	19.97 ± 1.16^{a}	12.97 ± 0.86^{a}	55.93 ± 2.36^{a}	57.33 ± 12.6^{a}	15.23 ± 1.11^{a}	7.00 ± 0.58^{ab}	10.67 ± 0.67^{a}

Leaf production trends over time (Figure 1) showed a rapid increase in T4 and T5 starting at week 2, with T4 maintaining a consistent lead from weeks 4 to 6, outperforming T1 throughout. As shown in Figure 2, T4 increased plant height by approximately 21.5%, leaf number by 211.8%, and dry weight by 233.5% relative to T1, while T5 increased leaf area by 22.3%, SPAD value by 13.3%, and root volume by 110.1% compared with the control. These results indicated that more frequent applications promoted more robust growth, particularly in leaf development and root expansion.

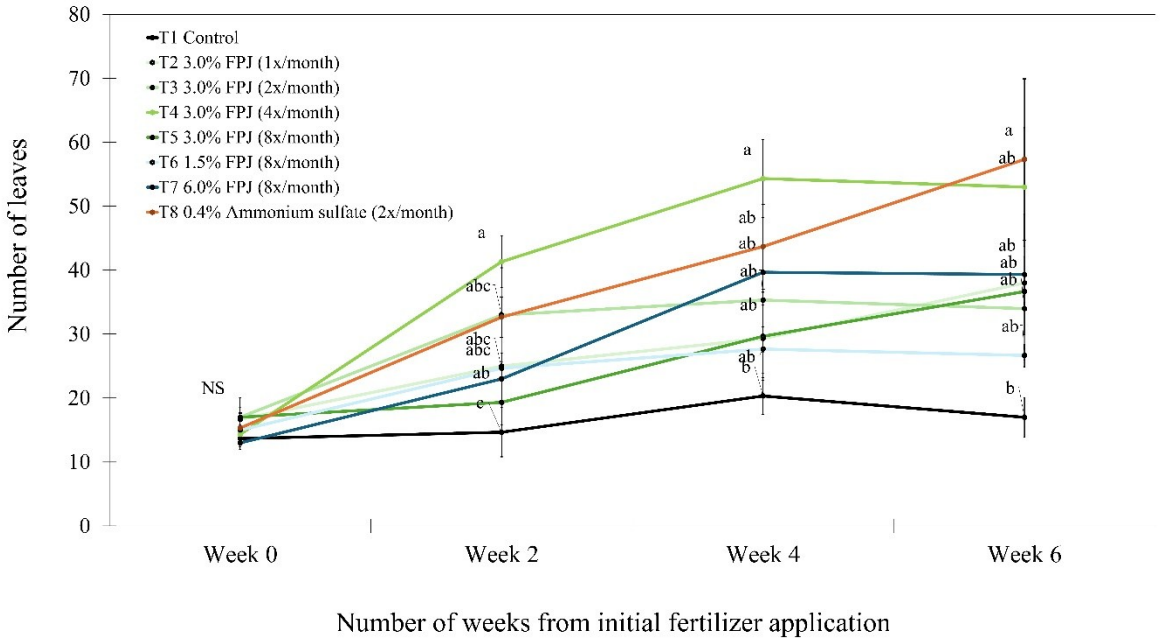

Figure 1. Leaf production in *Ruellia simplex* C. Wright 'Katie Pink' over six weeks as affected by different concentrations and application frequencies of fermented plant juice (FPJ) and ammonium sulfate (21-0-0). Means within each week with the same letter are not significantly different using Tukey's HSD test at $P \leq 0.05$. Error bars indicate ± SE; n = 3.

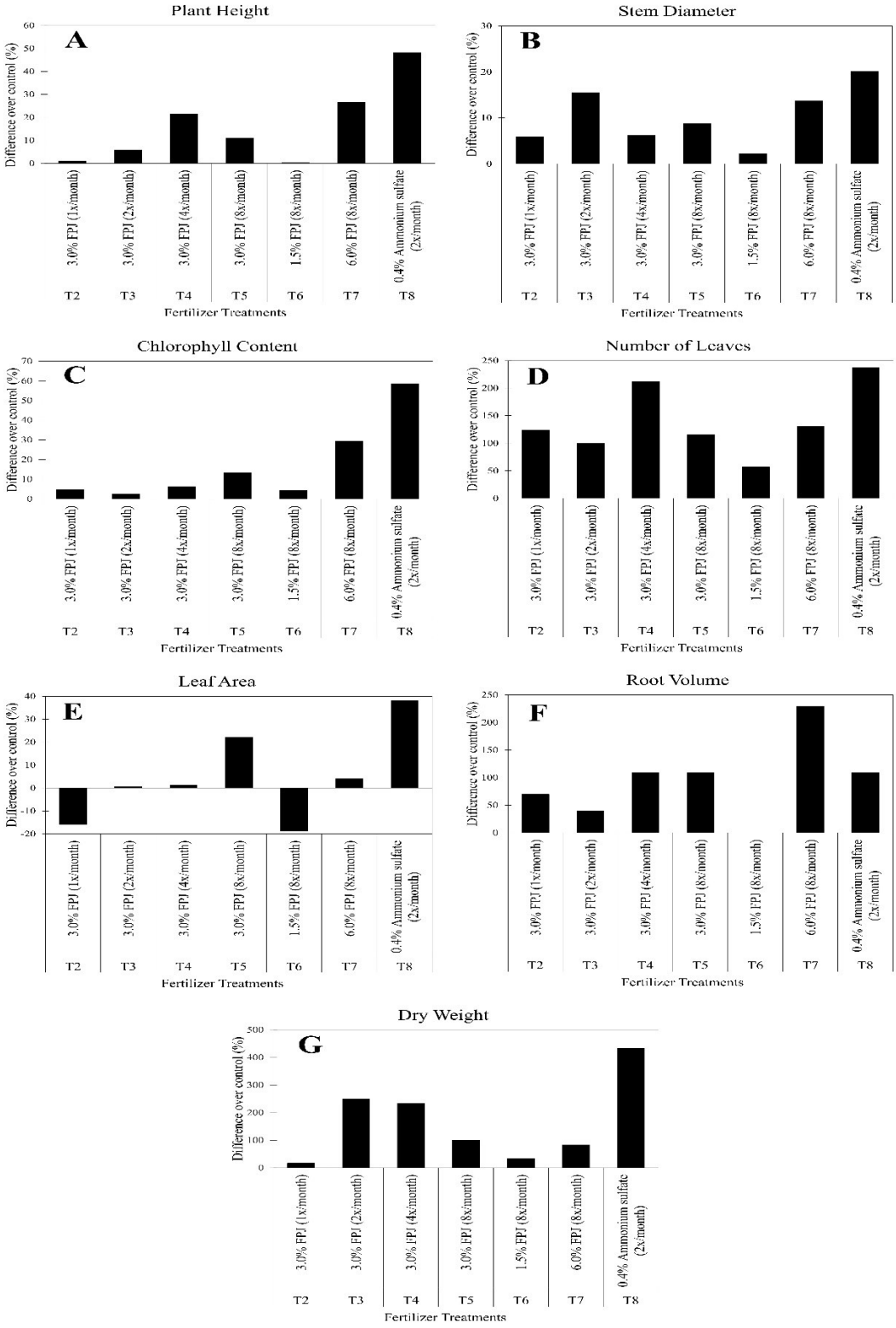

Figure 2. Percent difference from control in various growth parameters of Rohelia (*Ruellia simplex* C. Wright 'Katie Pink') in response to concentrations and application frequencies of fermented plant juice (FPJ) and ammonium sulfate (21-0-0). Parameters measured include (A) plant height, (B) stem diameter, (C) chlorophyll content (SPAD), (D) number of leaves, (E) leaf area, (F) root volume, and (G) dry weight. All values are expressed as percentage differences relative to the untreated control (T1).

In terms of reproductive development, all FPJ frequency treatments (T2–T5) induced bud formation, while T1 remained non-flowering. Treatments T4 and T5 showed earlier bud emergence (26–28 days) than T2 (42.33 days) and produced 1.33–2 floral buds per plant (Figure 3). However, all frequency treatments produced only one flower per plant, with moderate conversion rates (50–75%) (Figure 4). Among the frequency treatments, four applications per month (T4) were identified as the optimal frequency, although the results also suggested that frequency alone was insufficient to significantly enhance floral yield.

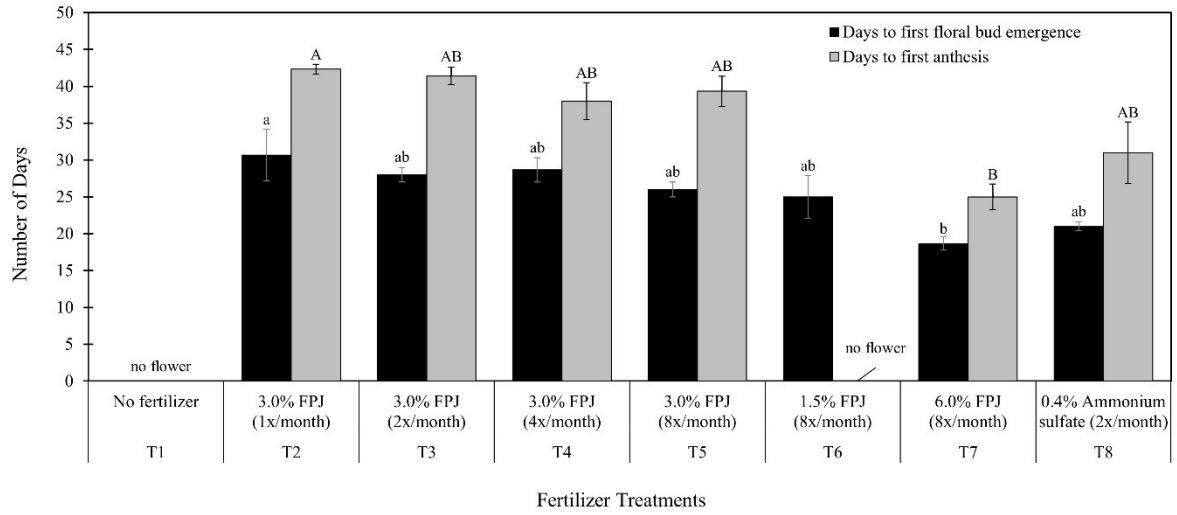

Figure 3. Effect of different concentrations and application frequencies of fermented plant juice (FPJ) and ammonium sulfate (21-0-0) on days to first floral bud emergence (black bars) and days to first anthesis (gray bars) in *Ruellia simplex* C. Wright 'Katie Pink'. Bars with the same letter are not significantly different based on Tukey's HSD test at $P \leq 0.05$. Error bars indicate ± SE; n = 3. Lowercase letters denote significant differences in bud emergence; uppercase letters denote significant differences in anthesis. No flowering was observed in T1 and T6.

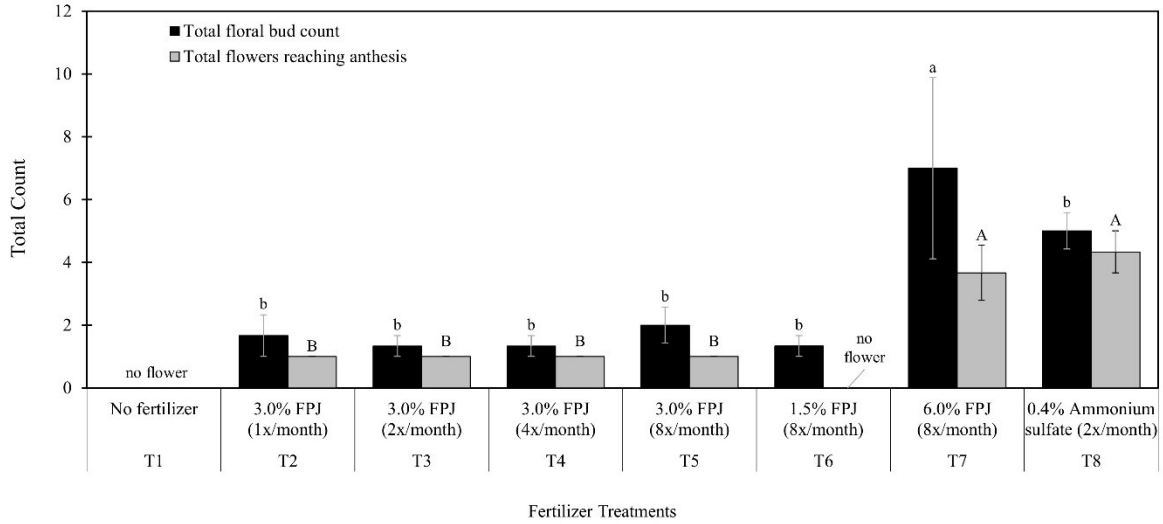

Figure 4. Effect of different concentrations and application frequencies of fermented plant juice (FPJ) and ammonium sulfate (21-0-0) on total floral bud count (black bars) and total flowers reaching anthesis (gray bars) in *Ruellia simplex* C. Wright 'Katie Pink'. Bars with the same letter are not significantly different based on Tukey's HSD test at $P \leq 0.05$. Error bars indicate ± SE; n = 3. Lowercase letters denote significant differences in bud count; uppercase letters denote significant differences in flower count. No flowering was observed in T1 and T6.

Most Effective FPJ Concentration

With frequency fixed at eight applications per month, increasing FPJ concentration resulted in an apparent dose-dependent improvement in both vegetative and reproductive performance. The application of 6% FPJ (T7) produced the tallest plants (17.07 cm), outperforming both 3% (T5; 14.97 cm) and 1.5% (T6; 13.50 cm), the latter being statistically similar to the control (T1; 13.47 cm) (Table 2). Chlorophyll content peaked in T7 (45.70 SPAD), significantly exceeding values recorded in other FPJ treatments and the control (35.30 SPAD). Root volume was also greatest in T7 (11.00 mL), compared with 7.00 mL in T5 and only 3.33 mL in both T6 and T1. Leaf area was highest in T5 (13.47 cm^2), followed by T7 (11.46 cm^2), with both treatments surpassing T1 (11.01 cm^2). Dry weight in T7 (3.67 g) was slightly lower than T5 (4 g) but remained markedly higher than T6 and T1.

Leaf production was notably higher in T7 (39.33) and T5 (36.67) than in T1 (17.00), as shown in Figure 1. The percent difference analysis revealed that T7 increased plant height by 26.7%, SPAD value by 29.5%, and root volume by 230.0% relative to T1 (Figure 2A, 2C, 2F). Leaf number increased by 131.4%, while dry weight increased by 83.5% (Figure 2G). In contrast, T6 showed minimal improvements over the control, with only a 0.2% gain in height and no change in root volume, indicating that 1.5% FPJ was ineffective even at high frequency. Treatment T5 provided moderate gains, including a 21.9% increase in SPAD and a 100.3% increase in leaf area, supporting the interpretation that concentration had a more substantial impact than frequency when held constant.

Reproductive performance was most prominent in T7, which produced 7 floral buds and 3.67 open flowers per plant, with a flower conversion rate of 52.4% (Figures 3 and 4). In comparison, T5 produced only one flower from fewer buds (1–2), while T6 and T1 remained non-flowering throughout the experiment. These findings indicated that a higher FPJ concentration was essential to support floral development when frequency is controlled.

Among the concentration treatments, 6% FPJ (T7) was identified as the optimal concentration at a fixed frequency of eight applications per month, producing the most consistent improvements in both vegetative and flowering stages.

Overall Effectiveness of FPJ and Comparison with Ammonium Sulfate

Across all FPJ treatments (T2–T7), application resulted in consistent improvements in vegetative growth parameters compared to the control (T1). The FPJ treatments demonstrated significant percent gains: plant height increased by up to 26.7% (T7), SPAD value by 29.5% (T7), number of leaves by 211.8% (T4), and dry weight by 233.5% (T4) (Figure 2A-G). Reproductive success also improved, with T5 and T7 producing up to 2 and 7 floral buds, respectively, whereas T1 produced none (Figures 3 and 4). The highest number of flowers among FPJ treatments was observed in T7 (3.67), corresponding to a conversion rate of 52.4% (Figure 4).

When compared with ammonium sulfate (T8), however, FPJ treatments remained inferior in most parameters. T8 produced the tallest plants (19.97 cm), the highest SPAD reading (55.93), the greatest number of leaves (57.33), and the largest leaf area (15.23 cm^2), all of which exceeded the top-performing FPJ treatments (Table 2; Figure 2). Treatment T8 also yielded the highest dry weight (10.67 g), representing a 433.5% increase over T1 and substantially exceeding all FPJ treatment. Root volume in T8 reached 7 mL, which, although higher than the control (+110.1%), was still lower than T7 (11 mL), indicating that FPJ may provide a specific advantage in root enhancement (Figure 2F).

In terms of flowering, ammonium sulfate outperformed all FPJ treatments. Treatment T8 initiated bud emergence earliest (21 days), produced 5 buds and 4 flowers per plant, and achieved the highest conversion rate (86.6%) (Figures 3 and 4). Although T7 produced more buds (7), it yielded fewer flowers (3.67) due to its lower conversion efficiency, reflecting a gap in bud-to-flower transition under organic treatment (Figure 4).

Among the FPJ treatments, T7 (6% concentration applied eight times per month) emerged as the most effective, achieving the highest gains in height, SPAD value, root volume, and flowering capacity. Despite not outperforming ammonium sulfate, T7 demonstrated substantial improvements over the control.

DISCUSSION

Optimal Frequency of FPJ Application

The application frequency of FPJ significantly influenced the vegetative and reproductive development of *R. simplex*. Moderate treatments (T2–T5) resulted in enhanced plant height, leaf number, and chlorophyll content, with the most notable improvements observed under four (T4) and eight (T5) applications per month. These findings suggest that more frequent applications support sustained nutrient availability and possibly stimulate rhizosphere microbial activity, thereby promoting vegetative growth.

All frequency-based FPJ treatments induced flowering, though floral output was modest and generally limited to one flower per plant. This limitation may be associated with insufficient phosphorus levels. The FPJ used in this study was analyzed and found to contain 0.14% nitrogen (N), 0.04% phosphorus (P_2O_5), and 0.52% potassium (K_2O)—a relatively low macronutrient profile. This composition may explain the limited reproductive response observed, despite the enhancements in vegetative traits. The absence of flowering in the control (T1) and the restricted performance under moderate FPJ frequencies (T2–T5) are consistent with reports that phosphorus deficiency delays floral initiation and reduces flower production, as phosphorus plays a key role in energy transfer and reproductive development (Malhotra et al. 2018; Heriansyah et al. 2025). Recent studies indicate that macronutrient availability regulates flowering time through integrated physiological and molecular signaling pathways, and inadequate nutrient supply can constrain floral development (Baek et al. 2026). Similarly, potassium has been shown to enhance flower development and reproductive performance by facilitating carbohydrate transport and cellular regulation, suggesting that limited potassium availability may further restrict floral output (Ye et al. 2019; Biswas et al. 2025).

These results indicate that while frequent application enhances growth and induces bud initiation, it alone is insufficient for maximizing flower production. Frequent application increases the cumulative supply of nutrients and organic compounds, enhances nutrient availability in the rhizosphere, and may stimulate microbial activity that supports vegetative growth and early reproductive signaling. However, increased application frequency cannot compensate for deficiencies in essential macronutrients required for sustained flower development. Adequate phosphorus and potassium are critical for floral initiation, energy transfer, carbohydrate transport, and flower formation, while nitrogen primarily supports vegetative growth (Baek et al. 2026; Malhotra et al. 2018). Thus, frequency amplifies nutrient delivery and microbial effects but does not offset limitations imposed by low concentrations of key macronutrients.

Most Effective FPJ Concentration

At a fixed frequency of eight applications per month, increasing FPJ concentration produced a clear dose response trend. The 6% treatment (T7) resulted in the most pronounced gains in plant height, SPAD value, and root volume. The relatively low nutrient content of the FPJ used in this study suggests that its effectiveness may be attributed to microbial and organic constituents rather than direct nutrient supply. Sulok et al. (2021) described fermented inputs as rich in beneficial microorganisms capable of solubilizing phosphorus and potassium, which can enhance nutrient availability and soil health. This microbial action, likely compounded by frequent application, may account for the improved leaf development, chlorophyll content, and floral bud formation observed in this treatment.

In terms of reproductive response, the 6% FPJ concentration also produced the highest number of floral buds and flowers among FPJ treatments. However, the flower conversion rate remained lower than that achieved with ammonium sulfate. This may be explained by the limited availability of nitrogen and phosphorus, as emphasized by Ruamrungsri et al. (2021), who noted that nutrient-deficient ornamental plants tend to exhibit delayed flowering and reduced floral quality.

Overall Effectiveness of FPJ and Comparison with Ammonium Sulfate

Among all FPJ treatments, the 6% concentration applied eight times monthly (T7) produced the most consistent improvements in both vegetative and reproductive parameters. These outcomes—increased plant height, SPAD value, leaf number, root volume, and floral bud production—can be attributed to the cumulative effects of bioactive stimulation and potential microbial synergy resulting from sustained application. Despite its low nitrogen and phosphorus content, the FPJ used in this study demonstrated the capacity to support moderate growth and floral initiation when applied intensively.

However, ammonium sulfate (T8) delivered superior results across nearly all parameters. As a synthetic fertilizer with readily available nitrogen (21-0-0), it promoted vigorous vegetative growth, enhanced chlorophyll biosynthesis, and supported higher dry biomass accumulation. These findings align with previous reports on nitrogen-driven increases in chlorophyll content and biomass. Recent studies have documented that increased nitrogen availability enhances chlorophyll concentration and photosynthetic capacity, leading to greater vegetative growth and dry matter accumulation (Zhang et al. 2021; Oner et al. 2024). Ammonium-based fertilizers have also been shown to alter leaf nutrient composition and promote growth when applied at appropriate rates (Al-Dosary et al. 2022). That said, the type and rate of nitrogen source can influence outcomes; excessive or imbalanced nitrogen application may reduce growth or cause physiological stress in some cases (Gülüt and Şentürk 2024).

In reproductive development, T8 achieved the highest number of flowers per plant (4.33), along

with the earliest bud emergence and most efficient flower conversion rate (86.6%). While the 6% FPJ treatment initiated more floral buds (7), fewer were successfully converted to flowers, underscoring a limitation in nutrient sufficiency for completing the reproductive cycle. These results are consistent with the findings of Malhotra et al. (2018) and Ruamrungsri et al. (2021), who highlighted phosphorus and nitrogen limitations as constraints on flowering potential.

Despite this, all FPJ treatments—particularly the high-concentration, high-frequency group—outperformed the control and demonstrated strong potential as organic alternatives. Moderate treatments (T2–T5) also led to measurable vegetative improvements and occasional flowering, validating the utility of FPJ in low-input systems. Prior studies support the effectiveness of FPJ in enhancing plant growth across various crops, although responses vary depending on species and application strategies. For instance, FPJ increased the number of leaves and the mean weight per hill in lettuce (Alam 2021). Different FPJ concentrations also influenced mustard growth, where 2 tbsp/L produced lower fresh weight than 1.5 and 2.5 tbsp/L (Denona et al. 2020). Weekly FPJ application increased plant height in hot pepper but had limited effects on overall yield (Lorio and De Asis 2021). Similarly, a one-time FPJ application at 2 tbsp/L significantly increased plant height, leaf number, and yield in pechay (Tagotong and Corpuz 2015).

Overall, the 6% FPJ applied eight times monthly (T7) showed strong potential in promoting plant vigor, root development, chlorophyll content, and floral initiation in *R. simplex*. Despite its low nutrient content, this optimized treatment outperformed the control and lower FPJ rates, highlighting its value as a sustainable input. While ammonium sulfate application remained superior in overall growth and flowering, FPJ presents a viable organic alternative for reducing reliance on synthetic fertilizers in ornamental crop production, particularly when applied at optimized concentrations and frequencies.

Based on the findings of this study, the application of FPJ at a 6% concentration with a high application frequency (8 times per month) may be considered the most effective FPJ-based input for improving vegetative growth and initiating flowering in *R. simplex.* This treatment can be recommended for growers practicing organic or low-input ornamental production systems where synthetic fertilizers are limited or undesirable. However, given the relatively low macronutrient content of FPJ, particularly phosphorus and nitrogen, supplementation with other organic nutrient sources may be necessary to achieve optimal flowering performance and improve flower conversion rates.

Future studies may explore integrating FPJ with other organic nutrient inputs or biofertilizers to enhance nutrient balance and reproductive development in ornamental crops. Further investigation into nutrient dynamics, rhizosphere microbial activity, and the long-term effects of repeated FPJ application under different environmental conditions would provide deeper insights into optimizing its use in sustainable ornamental plant production.

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GENERATIVE AI STATEMENT

A generative AI tool, specifically Grammarly (Grammarly, Inc., web-based version, accessed in 2026), was used solely for language improvement and editing purposes. The authors take full responsibility for the content of the manuscript.

ETHICAL CONSIDERATIONS

This study did not involve human participants or animals; therefore, ethical approval was not required.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests among the authors.

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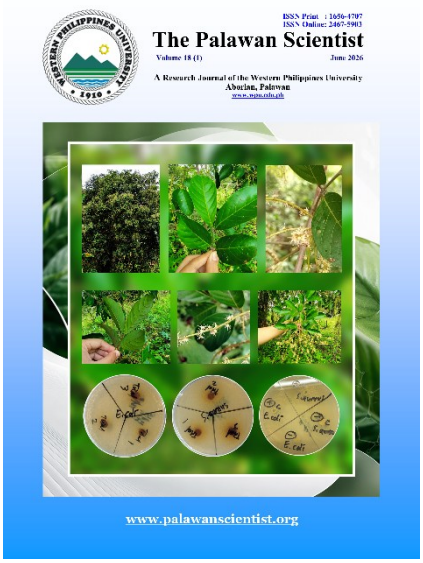

Notes on tick infestation in free-ranging Philippine Pangolins (*Manis culionensis*)

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ABSTRACT

The article provides quantitative and qualitative data on the infestation of wild Philippine Pangolin with the tick species *Amblyomma javanense* (Supino, 1897). Samples were gathered during routine health checkup of 71 pangolins when these were turned over or rescued between 2015 and 2025. Prevalence with *A. javanense* was 67.6%. The mean abundance of ticks was 8.1 and the mean intensity was 12.0.

Keywords: ectoparasites, Palawan Pangolin, *Amblyomma javanense*

Pangolins host different endo- and ectoparasites. The most conspicuous are ticks. The Philippine Pangolin *Manis culionensis* (de Elera, 1915), that is endemic to the Province of Palawan, is known to host the tick species *Amblyomma javanense* (Supino, 1897) (Carpos-Raros 1993; Jaffar et al. 2018). Information on the infestation is limited to a statement by Schoppe et al. (2020), who found a prevalence of 86% for 14 wild *M. culionensis*.

Katala Foundation Incorporated (KFI), a non-profit NGO working on highly threatened wildlife in the Province of Palawan, adopted the Philippine Pangolin as one of its flagship species and implements a comprehensive, holistic conservation program for the species since 2007. Part of KFI's health protocol is the examination for ticks, hence information on tick infestation was gathered from 71 free-ranging *M. culionensis* that were either caught during populations surveys (Figure 1) or turned over or rescued, and from one confiscated and repatriated individual. Of these 71 pangolins, 43 (60.6%) are male and 28 (39.4%) are female; 40 (56.3%) are adults (25♂ and 15♀) and 31 (43.7%) are juveniles (18♂ and 13♀). Ticks were removed with forceps and preserved in 75% denatured alcohol. Species identification was based on morphological characteristics using Voltzit and Keirans (2002). Identification, assignment of life history stages and counting required the magnification under a stereo microscope.

Amblyomma javanense was the only ectoparasite species detected on the Philippine Pangolin (Figure 1). Prevalence with *A. javanense* was 67.6% (48 out of 71 were infested). More males (62.5%) than females (37.5%) were among the infested pangolins (Figure 2). Most of the ticks were found under the scales and very few only on the unprotected belly.

Figure 1. A pangolin in its natural habitat and the ticks concealed beneath its scales (inset).

The 48 infested pangolins were almost equally composed of mature (52.1%) and immature (47.9%) individuals (Figure 2). Among the adults infested, more male (64.0%) than female (36.0%) pangolins had ticks (Figure 3). The infestation rate by municipality and by year was compared. In all except one municipality, the infestation was higher than 50% (Figure 4). Over the years, the percentage of infested pangolins was lowest in 2020 (33%) and highest in 2022 and 2024 (both 100%), with 5 and 8 pangolins recorded, respectively (Figure 4).

For 31 of the 48 infested pangolins, quantitative data on the tick are available. A total of 577 ticks were collected. The number of ticks per pangolin ranged from 1-92 (median = 12.0; mean = 18.6 ± sd 22.8) (Figure 5). The mean abundance (MA) of ticks, that is the total number of ectoparasites divided by the total number of pangolins, was 8.1. The mean intensity (MI), that is the total number of ectoparasites divided by the number of infested pangolins, was 12.0.

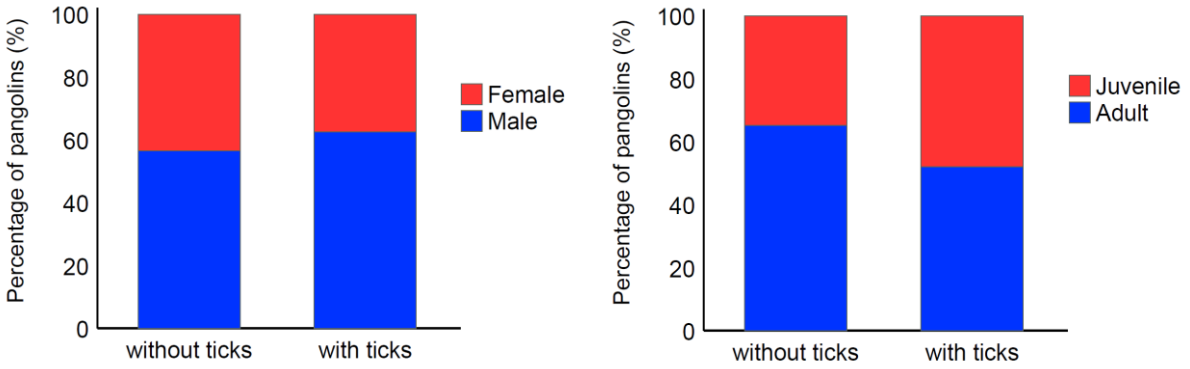

Figure 2. Percentage of female and male pangolins (Left) and percentage of juvenile and adult pangolins (Right) that were infested (n = 48) or tick-free (n = 23).

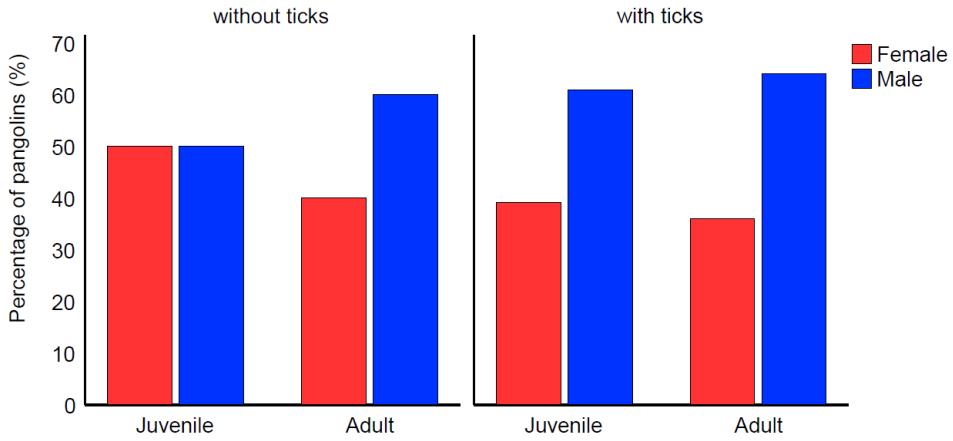

Figure 3. Sex composition (%) of juvenile and adult pangolins with and without ticks. Percentages were calculated within each life stage and tick-status category (each category sums to 100%).

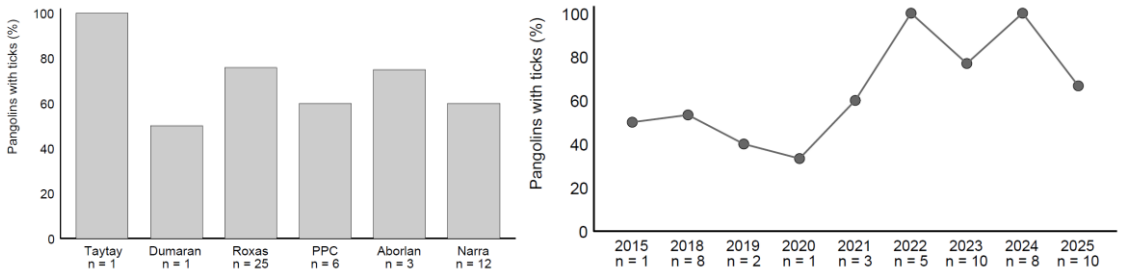

Figure 4. Percentage of pangolins infested with ticks (n = 47) by municipality (Left) and percentage of pangolins infested (n = 48) with ticks by year (Right). The reduced number per municipality was because one was repatriated and, hence, could not be attributed to a municipality.

For 19 pangolins we have information on the life history stages of the ticks. There were 375 ticks collected: 53.2% were adults while 15.5% were nymphs and 31.3% larvae (Table 1). Adult ticks were dominated by males (88.9%).

The presented tick infestation of *M. culionensis* was similar to the one of 16 confiscated Sunda Pangolin with *A. javanese* prevalence of 68.8% (Hassan et al. 2013) but lower than the one for 21 free-ranging Sunda Pangolins (100%) studied by Chong et al. (2023). Hassan et al. (2013) had found more juveniles (100%) than adults (63.6%) *M. javanica* infested, and adult males seemed more susceptible than adult females (Hassan et al., 2013). The same trend was found for *M. culionensis*. Likewise, Hassan et al. (2013) had also found more adult than immature ticks, and more male (81.7) than female ticks.

There was no information on the infestation rate of Philippine Pangolins that were confiscated from trade. Access was available to only one individual, which had been repatriated to Palawan and arrived without ticks; however, it is unknown whether ticks had been removed prior to repatriation. Likewise, there is no published information on the tick-borne diseases of *M. culionensis.*

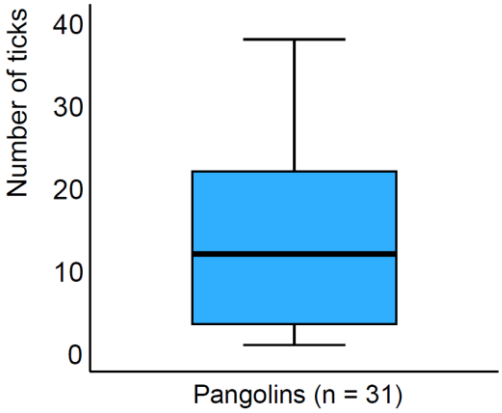

Figure 5. Tick abundance on infested pangolins (n = 31).

Table 1: Life history stages of *Amblyomma javanense* collected from free-ranging *Manis culionensis* (n=19).

Pangolin ID	Larva	Nymph	Adult female	Adult male	Total
1	0	2	2	1	**5**
2	1	2	0	14	**17**
3	12	0	1	0	**13**
4	0	4	5	47	**56**
5	0	2	0	1	**3**
6	9	0	0	10	**19**
7	0	0	0	1	**1**
8	0	1	0	9	**10**
9	0	26	0	12	**38**
10	0	1	1	2	**4**
11	92	0	0	0	**92**
12	0	0	2	0	**2**
13	0	3	0	28	**31**

Pangolin ID	Larva	Nymph	Adult female	Adult male	Total
14	1	3	0	11	**15**
15	0	1	2	11	**14**
16	2	8	2	4	**16**
17	0	2	6	17	**25**
18	0	1	0	5	**6**
19	0	2	1	4	**7**
Total	**117**	**58**	**22**	**177**	**374**
%	**31.3**	**15.5**	**5.9**	**47.3**	

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GENERATIVE AI STATEMENT

The concepts, results, and discussion within this paper are the authors' original work and no AI was used.

ETHICAL CONSIDERATIONS

The handling of pangolins was permitted under MOA between KFI and PCSDS, and gratuitous permits. The study followed all institutional and national ethical guidelines for the care and use of animals.

DECLARATION OF COMPETING INTEREST

The authors declare no competing interests.

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Leveraging artificial intelligence as a Vygotskian tool to enhance Filipino university students' language learning and critical thinking skills

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ABSTRACT

Drawing on Vygotsky's Sociocultural Theory, this study investigated how artificial intelligence (AI) functions as a "more knowledgeable other" in enhancing language proficiency and critical thinking skills. Employing a within-subjects quasi-experimental repeated-measures design, 175 Filipino university freshmen participated in five sequential Contextual Writing Assignments (CWAs) integrated with ChatGPT feedback. Results revealed statistically significant improvements in both English language proficiency and critical thinking skills over time ($P < 0.05$). The AI provided adaptive scaffolding within the students' Zone of Proximal Development, creating a psychologically safe environment that lowered affective barriers and reduced the fear of making mistakes. Furthermore, a moderate positive correlation was found between linguistic gains and analytical competencies. The study concludes that pedagogically structured AI integration—utilizing a draft-feedback-reflection-revision cycle—fosters an inclusive environment and empowers students to become independent and critical thinkers. This research provides an evidence-based model for leveraging generative AI to bridge the gap between workforce demands and graduate skills.

Keywords: academic writing, analytical skills, language proficiency, large language models, negotiation of meaning, scaffolding

INTRODUCTION

The increasing use of generative artificial intelligence (AI) promises a new economic paradigm. Projections suggest that it can contribute trillions to the global economy by boosting productivity and fostering innovation (Corvello 2025). Businesses are adopting these tools to streamline operations and gain a competitive advantage (Vărzaru and Bocean 2024). This rapid acceptance of AI technology, however, stands in stark contrast to the fear prevalent within academic communities, where apprehensions about academic integrity and the loss of foundational learning skills dominate the discourse (Bin-Nashwan et al. 2023; Rodrigues et al. 2025). This contrast poses a critical challenge. If the AI-driven economy

increasingly demands advanced communication and critical thinking skills, then the academe risks failing students by avoiding these powerful tools, potentially increasing the gap between workforce demands and graduate skills.

This challenge is serious in the Philippines, especially in the context of second language acquisition. The Philippines has encountered significant challenges in English literacy, as evidenced by its low ranking in the 2022 Programme for International Student Assessment at 76th out of 81 participating countries (OECD 2023). This ranking is alarming because deficiencies in comprehension skills can hinder students from processing diverse information (Butterfuss et al. 2020), which is essential for fostering critical thinking and creativity for lifelong learning (Paige et al. 2024). The World Bank emphasizes the importance of learning skills, noting that these skills drive innovation, productivity, and long-term economic growth (Liang and Chen 2013; Ansari and Amegah 2025). However, this national struggle is evident in classroom contexts, where empirical studies document that Senior High School students struggle to recognize textual patterns, evaluate coherence, and possess limited vocabulary knowledge (Urbano et al. 2021). These challenges also indicate that students' English language proficiency is declining (Gustilo 2016; Altınmakas and Bayyurt 2019; Islam and Stapa 2021; Portillo-San Miguel 2021; Chemir and Kitila 2022; Lobo 2023; Marmita et al. 2023; Pangket et al. 2023; Baracheta 2024; Hua and Huynh 2024; Ky Nhan 2024 May 31; Muniruzzaman and Afrin 2024; Zheng et al. 2024; Chavez et al. 2025). This culminates in a pervasive lack of confidence and hesitancy to use English in class discussions among university freshmen (White 2011), severely limiting their cognitive development.

This learning shortfall is not just a skill deficit but is deeply rooted in the affective dimensions of learning (Ben-Eliyahu 2019; Huang and Lajoie 2023). The apprehension of committing mistakes and the anxiety of being judged by peers create a high affective filter (Krashen 1982; Krashen 1995), a psychological barrier that hampers language acquisition by discouraging interactions essential for developing practical communication skills. When this anxiety becomes severe, it creates an environment that discourages immersive and safe learning environments (Córdova et al. 2023) to develop language proficiency and critical thinking.

To challenge this dilemma, an immersive and adaptive pedagogical approach is needed that simultaneously addresses affective barriers and cognitive development. Vygotsky (1987) argued that learning is a social process in which a more knowledgeable other (MKO) provides support or scaffolding within the learner's zone of proximal development (ZPD). Accordingly, the ZPD refers to the space between what the students can do independently and what they can achieve with guidance (Marginson and Dang 2017; Tzuriel 2021). In this study, conversational tools like ChatGPT operationalize aspects of the MKO. It has a capacity for conversational interaction and personalized feedback (Lester et al. 2024; Al-Obaydi et al. 2025; Aslan et al. 2025). Likewise, it provides students with opportunities to practice vocabulary in real-life contexts without fear of making mistakes (Alharbi and Khalil 2023; Hsu et al. 2023; Huang and Wang 2023; Oktadela et al. 2023; Aleena Taj et al. 2025). This promising AI-scaffolded student interaction can be applied in Philippine language classrooms.

While several studies have effectively identified the efficacy and impact of AI in students' language enhancement, few have empirically tested innovative solutions that address both cognitive and affective barriers. Likewise, no studies have yet examined the mechanisms underlying how and why AI, such as ChatGPT, improves language learning performance and critical thinking skills. Drawing from this context, this study addresses this gap by employing a rigorous repeated-measures design, grounded explicitly in Vygotsky's Sociocultural Framework, to explore the underlying mechanisms of this process. This study is guided by the following research questions: (1) What is the effect of AI-scaffolded conversational interaction on students' English language proficiency over time? (2) What is the effect of AI-scaffolded conversational interaction on students' critical thinking skills over time? (3) Is there a significant relationship between students' English language proficiency and their critical thinking skills following the AI-scaffolded intervention?

This study moves beyond theoretical speculation to provide an evidence-based model for integrating AI to create a dynamic, interactive, and psychologically safe environment for second language acquisition and the development of critical thinking.

METHODS

Study Design

This study used a within-subjects quasi-experimental repeated-measures design, wherein the participants' performances were compared to their own previous performances. These were the same people who provided data for all writing conditions. The researchers preferred a within-subjects design because it reduces individual differences in the research (Birnbaum 1999; Kim 2010). Since participants vary significantly in their starting language skills and critical thinking abilities, this method controls the participants' individual differences because each student has own control. By

comparing the students' performance with their previous performances, it was easier for researchers to determine the effect of ChatGPT as an intervening treatment on students' English language skills and critical thinking abilities.

During the first week of the study, students were given a comprehensive orientation session to familiarize themselves with the course content and procedure, including the configuration and utilization of generative artificial intelligence.

Participants and Sampling Procedure

Employing population sampling, this study investigated a sample of 175 freshmen students taking up Bachelor of Science in Criminology. They were native Filipino speakers who learned English as a second language. The sample represented the entire cohort of first-year students in the program, ensuring comprehensive coverage of the target population.

Instruments and Validation

To assess student outcomes, two primary instruments were used. First, an analytical scoring rubric was used to evaluate students' English language proficiency based on the use of vocabulary, organization, grammar, and coherence in their written incident reports. Second, a revised holistic critical thinking scoring rubric (Facione and Facione 1994) was employed to assess the quality of students' analytical thinking in their written outputs. To ensure scoring reliability, two independent raters assessed a random sample of 20% of the writing tasks, and inter-rater reliability was calculated using Cohen's Kappa, achieving a coefficient of $k = 0.84$, indicating strong agreement. Discrepancies were resolved through discussions to maintain consistency across all ratings.

Data Gathering Procedure

They were provided with writing journals at the beginning of the semester. The journal consisted of four (4) parts, namely: original draft, ChatGPT correction (Model), ChatGPT feedback, and the student's reflection (Figure 1). There were five (5) contextualized writing activities. The first writing task served as the baseline "Beginner", the pre-test for all comparisons, and the succeeding tasks represented specific cases. The students were given a topic that centered around a crime scene analysis. They were tasked to watch five (5) videos depicting a specific case or crime for their writing tasks. These tasks represented five different experimental cases for interacting with ChatGPT.

After watching the assigned video for each task, the students were given prompts that were analytical and expository. The same prompts were used for all students to maintain consistency across variables (same prompt as the controlled variable). On one hand, the expository aspect determined the students' language proficiency, which in this study was operationalized as academic writing performance. On the other hand, the analytical aspect measured the students' critical thinking. These prompts require them to describe events objectively, analyze actions and potential evidence, and construct a coherent and logical narrative from their observations. An example of an expository prompt for Task 1 (Baseline) stated: "Describe the crime scene objectively: location, time, people present, physical evidence, and chronological sequence of events." The analytical prompt for Task 2 stated: "What potential evidence would you collect to verify or disprove the claims made?"

After watching a video depicting a specific crime scene, the students wrote a formal incident report. The report was assessed based on the clarity of its organization, the quality of its language, and the strength of its analytical thinking. The report described the nature of the incident and summarized the account provided by the witness or victim, including the key details from the witness or victim's statement that could be considered as evidence. After writing the first draft, the students opened their generative AI tool and used a prompt, instructing the AI to act as a writing tutor by correcting grammar and vocabulary use and explaining the reasons for the most important corrections.

Statistical Analysis

The statistical analysis followed a systematic process. First, the data were subjected to descriptive statistics, including the computation of means and standard deviations, to provide an overview of the students' performance and to determine the initial and subsequent levels of enhancement in their English and critical thinking skills (Scale: 1.00 – 1.49 – very low; 1.50 – 2.49 – low; 2.50 – 3.49 – high; and 3.50 – 4.00 – very high) (Pimentel 2019).

A Paired Sample t-test was used to confirm students' progress from pre-test to post-test scores. This test was appropriate for determining the improvement of skills after the intervention. A Repeated Measures MANOVA was employed to confirm an overall effect of the task sequence, given that the same participants were assessed at different points in time, allowing researchers to track changes in their English proficiency and critical thinking skills under similar conditions. This design was chosen to control for individual variability and to ensure that differences were duly observed through the intervention. The Friedman Test was used to rank the progressive improvement. This step enabled a more comprehensive assessment of whether the observed improvements in one domain were associated with changes in the other. Finally, Pearson's Correlation was used to establish the relationship between English proficiency and critical thinking skills.

All statistical computations were performed using the Statistical Package for the Social Sciences (SPSS) version 25, with the level of significance set at $P < 0.05$ to determine the statistical reliability of the findings.

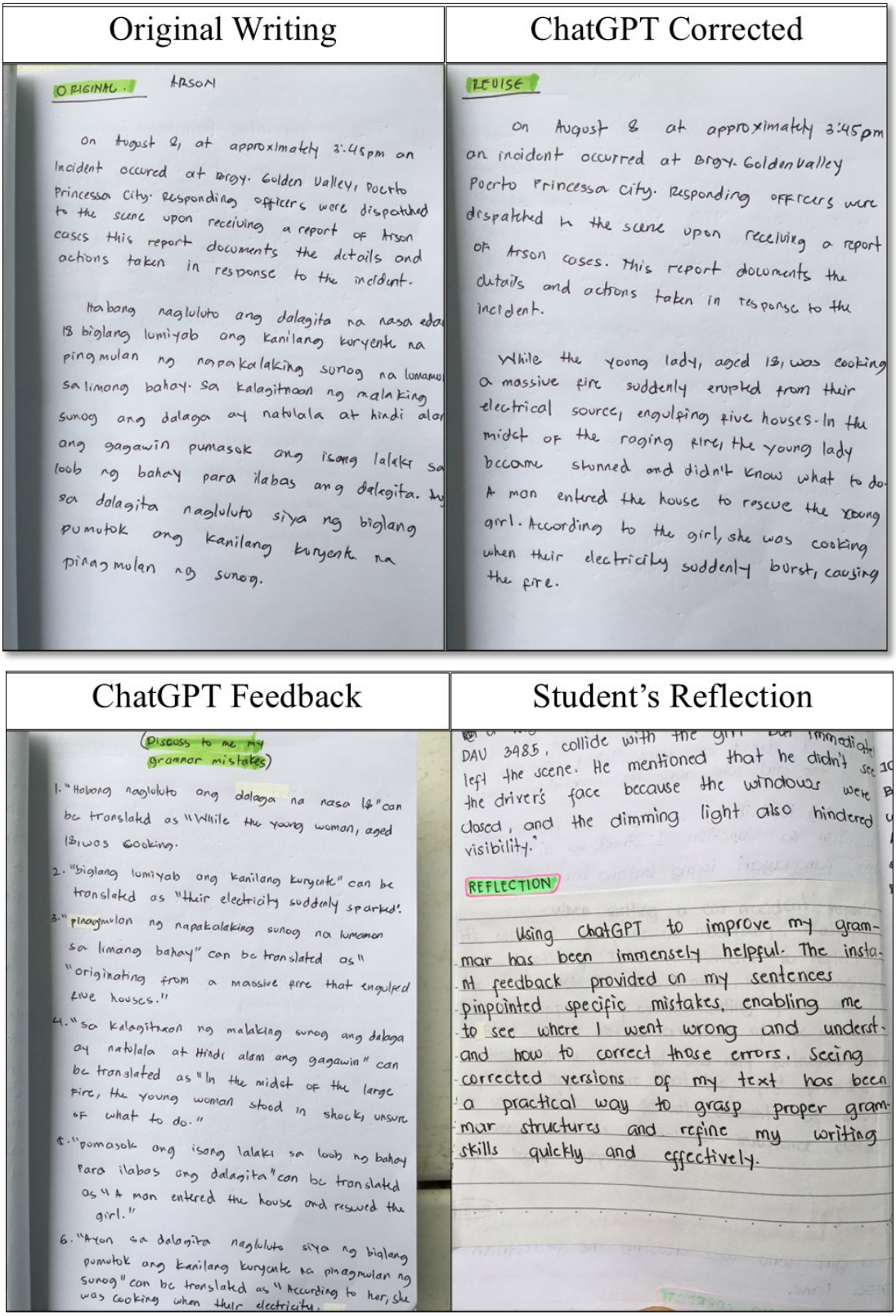

Figure 1. Parts of students' writing journal.

RESULTS

Influence of AI-Powered Conversational Interactions on Students' Language Proficiency

The students' baseline performance and the learning trajectory. To track the students' performance across five (5) sequential writing tasks over the AI-assisted intervention, descriptive statistics were employed. The results in Table 1 show that the students achieved a good level of proficiency in English (2.43 ± 0.34) as indicated by the "high" qualitative descriptor and the associated scale across all tasks of the AI-assisted writing activities. Students started with a skill level that required improvement in Task 1 (1.75 ± 0.31), which served as the baseline pre-test. A significant qualitative shift occurred as students' language proficiency in Task 2 (2.13 ± 0.41) increased in Task 3 (2.45 ± 0.33). Likewise, the highest level of enhancement was achieved in the final task (Task 5) (3.04 ± 0.37). Through repeated feedback and practice, students' language skills have progressed to a good level of proficiency (M = 2.43).

Comparative analysis of students' language proficiency enhancement. To examine whether there was a significant difference in performance compared to the initial stage of the experiment, the Paired Sample t-test was conducted. The data revealed a statistically significant improvement in students' English language skills from Task 1 (1.82 ± 0.32) to the fifth and final task (3.12 ± 0.48), $t(174) = -58.883$, $P < 0.05$ (Table 2). Each successive writing task demonstrated a highly significant result for every pair ($P < 0.05$). The magnitude of improvement was reflected in the constant increase in post-test means, which rose from 2.01 in Task 2 to 3.12 in Task 5. The final mean for Task 5 represents an improvement of over 1.3 points on the scale from the baseline pre-test mean of 1.82 in Task 1.

Table 1. Level of enhancement of students' English language and critical thinking skills.

Cases	English Language Proficiency			Critical Thinking Skills		
	Mean±SD	Qualitative Descriptor	Interpretation	Mean	Qualitative Description	Interpretation
Task 1 (Baseline)	1.75±0.31	Low	Needs improvement	1.85 ± 0.33	Low	Below basic
Task 2	2.13±0.41	Low	Needs improvement	2.00 ± 0.42	Low	Below basic
Task 3	2.45±0.33	Low	Needs improvement	2.38 ± 0.36	Low	Below basic
Task 4	2.68±0.34	High	Good	2.79 ± 0.43	High	Proficient
Task 5	3.04±0.37	High	Good	3.21 ± 0.49	High	Proficient
Overall	2.43±0.34	High	Good	2.59 ± 0.39	High	Proficient

Table 2. Comparative analysis of the enhancement of students' English language and critical thinking skills. n=175, Sig. (2-tailed) = *$P < 0.05$, **$P < 0.01$.

Pair	Cases	English Language Proficiency		Critical Thinking Skills	
		Mean ± SD	*t*	Mean ± SD	*t*
Pair 1	Task 1 (Baseline) Pre-test	1.82 ± 0.32	-8.90**	1.85 ± 0.32	-9.41**
	Task 2 Post-test	2.01 ± 0.40		1.99 ± 0.42	
Pair 2	Task 1 (Baseline) Pre-test	1.82 ± 0.32	-35.99**	1.85 ± 0.32	-36.99**
	Task 3 Post-test	2.48 ± 0.35		2.38 ± 0.36	
Pair 3	Task 1 (Baseline) Pre-test	1.82 ± 0.32	-45.22**	1.85 ± 0.33	-48.23**
	Task 4 Post-test	2.99 ± 0.43		2.79 ± 0.43	
Pair 4	Task 1 (Baseline) Pre-test	1.82 ± 0.20	-58.88**	1.85 ± 0.33	-60.88**
	Task 5 Post-test	3.12 ± 0.48		3.21±0.49	
Pair 5	Task 1 (Baseline) Pre-test	1.82 ± 0.32	-53.67**	1.85 ± 0.33	-54.67**
	Overall	2.49 ± 0.37		2.59 ± 0.39	

Ranking of Students' English language learning and overall effect of the intervention. After the Paired Sample t-test provided compelling evidence that there was a significant difference in every writing activity (Table 2), a multivariate test (Table 3) was conducted to collectively assess the impact of the sequential series of AI-assisted writing (Factor 1) on students' English language skills enhancement (Table 3). The Friedman Test confirmed the constant and cumulative improvement in students' English language skills throughout the study (Table 4). It demonstrated the exact sequence of learning. The students began with the lowest performance level (1.82 ± 0.31), and with each subsequent AI-assisted writing task, the mean scores showed a substantial and monotonic increase from Task 1 with a mean of 1.82 (Rank 5) to Task 5 with a mean of 3.10 (Rank 1). The rank progression was systematically enhanced and improved predictably in every successive AI-assisted writing task, with the final task yielding the highest independent performance of the student's language skills.

A repeated-measures MANOVA revealed that there was a statistically significant overall effect of the writing task sequence through a series of sequential AI-assisted writing (Factor 1) on the combined measures of English language skills, with an effect size of Pillai's Trace = 0.942, $F(4,171) = 737.914$, $P < 0.05$ (Table 5). This test was used to determine if there were statistically significant changes in multiple related measures over time. All four standard multivariate test statistics: Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root, yielded an identical F-value of 737.914 and a significance value of $P < 0.05$ (Table 5), indicating that the possibility of statistical ambiguity in the interpretation of this study was minimal. The within-subjects factor represented the sequence of tasks (Task 1 through Task 5), where students wrote, received AI feedback, reflected and revised. The F-value of 737.914 denoted that the differences in English language scores between the writing cases were substantial and systematic, far exceeding what could be expected from random variation. The highly significant p-value ($P < 0.05$) indicates overwhelming statistical evidence that the students' performance changed significantly across the different tasks.

Table 3. Multiple comparison test of the students' English language enhancement (Repeated Measures MANOVA).

Multivariate Tests[a]						
Effect		**Value**	***F***	**Hypothesis df**	**Error df**	**Sig.**
Factor 1 (ChatGPT)	Pillai's Trace	0.942	737.914b	4	171	0
	Wilks' Lambda	0.022	737.914b	4	171	0
	Hotelling's Trace	20.671	737.914b	4	171	0
	Roy's Largest Root	20.671	737.914b	4	171	0
a. Design: Intercept Within Subjects Design: Factor1						
b. Exact statistic						
c. Computed using alpha = 0.05						

Table 4. Ranking of the enhancement of the students' English language proficiency and critical thinking skills (Friedman Test).

Cases	**English Language Proficiency**		**Critical Thinking Skills**		**Rank**
	Mean ± SD	**Mean Rank**	**Mean ± SD**	**Mean Rank**	
Task 1 (Baseline) Pretest	1.82 ± 0.31	1.24	1.85 ± 0.33	1.28	5
Task 2	1.90 ± 0.40	1.65	2.00 ± 0.42	1.79	4
Task 3	2.31 ± 0.34	2.72	2.38 ± 0.36	2.99	3
Task 4	2.69 ± 0.42	3.89	2.79 ± 0.43	4.01	2
Task 5	3.10 ± 0.49	4.69	3.21 ± 0.49	4.94	1

Table 5. Multiple comparison test of the students' English language and critical thinking skills enhancement (Repeated Measures Manova).

Multivariate Tests[a]						
Effect		Value	*F*	Hypothesis df	Error df	Sig.
Factor 1	Pillai's Trace	0.956	933.658b	4	171	0
	Wilks' Lambda	0.044	933.658b	4	171	0
	Hotelling's Trace	21.84	933.658b	4	171	0
	Roy's Largest Root	21.84	933.658b	4	171	0
a. Design: Intercept Within Subjects Design: Factor 1						
b. Exact statistic						
c. Computed using alpha = 0.05						

The Dynamic Nature of AI-Driven Conversations Fosters Students' Critical Thinking Skills

Level of enhancement of students' critical thinking skills. The data indicate that the students achieved a highly proficient level of critical thinking skills (2.59 ± 0.39) across all AI-assisted writing activities (Table 1). Students' critical thinking skills showed gradual improvement from Tasks 1 and 2. A significant improvement that reached the proficiency threshold was observed in Task 3 (2.79±0.43), which continued in the final task (Task 5) (3.21±0.49). The result implies significant improvement in students' analytical and evaluative skills.

Evidence of improvement of students' critical thinking skills. Students' critical thinking skills showed significant improvement across different cases and time points (Table 2). Every iterative writing task showed highly significant results ($P < 0.05$) in the Paired Sample t-tests for every pair. The extent of improvement was shown in the steadily increasing post-test means (from 1.99 in Task 2 to 3.21 in Task 5). On the one hand, the mean for Task 5 is critical, as it represents an improvement of over 1.36 points on the scale from the baseline pretest mean of 1.85 in Task 1. On the other hand, the *t*-values progressing from pair 1 (-9.41) to pair 4 (-60.88) demonstrated that the efficacy of the AI-assisted intervention in developing critical thinking skills substantially increased with each successive writing task.

Order of the students' critical thinking skills and overall effect of writing sequence. Likewise, the Repeated Measures MANOVA revealed a statistically significant overall effect of the writing sequence on students' critical thinking skills (Table 3). To rank the effectiveness of the different cases, a Friedman Test was used (Table 4). The data confirmed the steady and cumulative improvement in students' critical thinking capabilities throughout the study. The students started at the lowest performance level (1.85±0.33) (see Table 1) and, in subsequent writing performances, showed increasing mean scores from the Task 1 mean of 1.85 (Rank 5) to Task 5 with a mean of 3.21 (Rank 1; Table 4). The results of the ranking affirmed that the students' critical thinking capabilities were systematically improved with each AI-assisted writing task. The results imply that the analytical and evaluative capabilities required for critical thinking were progressively developed through repeated engagement with the AI's feedback cycle. This was shown by the various multivariate test statistics (Pillai's Trace, Wilks' Lambda, Hotelling's Trace, Roy's Largest Root) that yielded an identical *F*-value of 933.658 and a significance value of $P < 0.05$ (Table 5). A remarkably large *F*-value signifies that the differences in critical thinking scores between the writing cases were substantial and systematic, outpacing what could be expected from random variation. Likewise, the exceedingly significant p-value ($P < 0.05$) suggests compelling statistical evidence that the students' critical thinking performance changed significantly across the different tasks as a direct result of the AI-assisted intervention.

Relationship between Students' English Language Skills and Critical Thinking Skills

The data present a moderate positive correlation between students' English language proficiency and their critical thinking skills. The results yielded a Pearson correlation coefficient of $r = 0.536$ and a p-value of 0.006 ($P < 0.05$). On one hand, the significance level of 0.006 suggests that the observed correlation between critical thinking and English language skills is unlikely to have occurred by random chance alone, providing evidence that there is a real relationship between these variables in the population of 175. On the other hand, the positive correlation indicates that as scores on critical thinking increase, the scores on English language skills also increase. This suggests that AI-assisted writing activities fostered a synergistic effect in the

development of students' linguistic and analytical competencies. This further implies that the improvement in English language proficiency provided the tools for clearer expression and analysis; at the same time, the use of critical thinking most likely contributed to the more complex use of the English language.

DISCUSSION

The Influence of AI-Powered Conversational Interactions on Students' Language Proficiency

This study explored how ChatGPT, as an AI-assisted writing tool, influenced the development of English as a second language. The results of the descriptive analysis indicate a statistically significant improvement in students' English proficiency levels. This progression suggests that the AI-supported feedback cycle promotes heightened noticing and facilitates language development. The consistent negative *t*-values and significant p-values across comparisons further substantiate the presence of meaningful learning gains. These improvements can be interpreted through established frameworks in second language acquisition (Krashen 1982; Vygotsky 1987; Schmidt 1990; Long 1996). In particular, the findings align with Vygotsky's Sociocultural Theory, as the AI appears to function as an adaptive scaffold that supports learners in producing language slightly beyond their current level of independent performance. Rather than treating theoretical constructs separately, the results point to an integrated process in which scaffolding, noticing, and interaction collectively contribute to language development within the AI-mediated environment. Learning occurs most effectively in the ZPD, the distance between the actual development level and the level of potential development under the guidance of an adult. In this study, AI serves as the dynamic MKO, providing precise assistance within the students' ZPD, the space between what the student can do alone in writing and what they can do with AI's help. The constant increase in mean scores from the initial writing task to the final task suggests a direct effect of the scaffolded support of the AI-assisted writing (Katona and Gyonyoru 2025). This is evident in pair 4 (Table 2), where students significantly improved after the AI's scaffolding. AI provides answers and explanations to the students' language errors, the proper process, and language models. The initial smaller jump in Table 2 (pair 1) denotes AI providing basic and foundational scaffolding. However, as the tasks progressed, the scaffolding adopted a more complex process compared to the natural variation in scores. Moreover, the result that each subsequent mean is higher than the last indicates that the students' ZPD was not static. With each cycle of supported practice, the student's independent abilities grew, and a new higher ZPD was established (Ferguson et al. 2022). The result implies that the effect of AI-assisted writing was not just consistent but became progressively stronger. AI's consistent just-in-time scaffolding bridges the ever-advancing learning of students in the English language. The sequence of writing tasks successfully navigated the students' ZPD.

Likewise, the cumulative improvement in language proficiency (Table 4) demonstrates that with each cycle of AI-generated feedback and student reflection, learners gradually internalize the vocabulary and language rules from previous writing tasks, allowing them to perform at a higher level of independent competence (Cavalcanti et al. 2021; Tağa and Kalenderoğlu 2022). The result indicates that the iterative cycle of interacting with the AI supports the development of systematic cognitive change. The students were not only memorizing the corrections, but they were also internalizing the underlying language system. This finding is critical, as it signifies that the more activities students completed with feedback from the AI, the more their language learning was reinforced and solidified (Ekizer 2025; Liu 2025). It implies that the teachers' creativity in approaches and strategies for employing repeated, scaffolded writing tasks was instrumental in the students' development. The language teaching strategies contributed to the development of the students' English language skills. These findings further illustrate that the adoption of Artificial Intelligence in classroom instruction is beneficial in second language learning (Almelhes 2023).

Meanwhile, as AI provides adaptive scaffolding, it is also a catalyst for lowering students' learning anxiety. The sustained and statistically significant improvement in students' English language skills in AI-assisted writing coincides with the affective filter hypothesis (Krashen 1989). It is proposed that when learners are relaxed, free from anxiety, and not preoccupied with errors or external pressure, the language input is able to reach the brain's language acquisition device (LAD), where it is processed subconsciously and integrated into the developing language system (Stander 2025). Conversely, when anxiety, stress, or self-consciousness are present, the flow of comprehensible input is hindered or blocked before it reaches the LAD, limiting the extent of language acquisition. The statistically significant improvement in students' English language skills from the initial task to the final task confirms that AI has created an environment of psychological safety that lowered the mental barriers to learning. It implies that the cumulative process of non-threatening engagement with the AI-driven writing and feedback cycle was highly effective in enhancing language proficiency. Moreover, the initial

smaller *t*-value in pair 1 and the soaring *t*-values of subsequent pairs in pair 4 indicate a massive increase in the volume and quality of engagement. This engagement signifies the low anxiety and non-judgmental environment, which was subsequently instrumental in dismantling the emotional barrier. Unlike in traditional classroom settings, wherein students' fear of making mistakes will likely suppress their willingness to practice and take risks, the AI platform provides a private, non-judgmental environment. Ultimately, the systematic reduction of anxiety in AI-assisted writing activities unlocks the students' latent potential for language proficiency.

Every student's submission initiated the cycle of Long's (1996) negotiation of meaning. Long's hypothesis posits that language acquisition is most effective when a communication breakdown occurs and is repaired through interaction. Long (1996) termed this as "negotiation of meaning". In this research, as students began to write, their outputs naturally contained errors. These errors constituted a communication breakdown. Since the students asked for an explanation of the errors in their writing, the AI's corrective feedback served as the interactive repair that made the target language input comprehensible. The initial low scores in Task 1 were a rich source that triggered negotiation. It implies that in every grammatical inaccuracy, wrong choice of words, awkward phrases, or misspelling in the students' draft was a potential learning point. The errors where the intended meaning was not correctly encoded in formal English created the essential precondition for negotiation. Thus, when AI provided the corrected version and further gave reasons for the corrections, AI was involved in recasting or repairing the students' language breakdown. This "repair" represented the total negotiation of meaning. This was evident in the escalating *t*-values across the paired tests from pair 1 (-8.900) to pair 4 (-58.883). It was a statistical signature of a fully operational, virtuous learning cycle. The results demonstrate that the efficacy of these negotiations increased in each cycle. The progression from the initial to massive *t*-values indicates that after repeated micro-negotiations in every writing task, students internalized the corrections; thereby, contributing to improved writing output. The result confirms Lyster's (2001) finding that written grammar, vocabulary, and organization benefit most from negotiation of meaning. Interestingly, the overall effect reflected in the Repeated Measures MANOVA indicates that negotiations were not random and isolated. Students formed a systematic process of pattern recognition. The result implies that as students engaged with the AI's feedback across different writing tasks, they were not just fixing individual errors, but were also learning the underlying patterns and rules of the language. These negotiations led the students to realize the language rules explicitly, moving simply from knowing that something was wrong to understanding why it was wrong. This is a moment of learning after a profound shift facilitated by the interactive repair provided by the AI. The result is consistent with Ba et al. (2025) and Mekheimer (2025), who found that AI's feedback can effectively enhance targeted learning outcomes. Ultimately, the significant progression in scores from the first to the final task is empirical evidence that over time, the repeated process of negotiation of meaning facilitates the development of students' overall proficiency.

The statistically significant progression in scores confirms that for language input to become intake, it must first be noticed. The research findings that students showed a good level of proficiency in English and statistically significant improvement are proof of how AI's corrective feedback contributes to cognitive intake. In this study, every AI's corrective feedback forcefully directed the learners' attention to the gap between their current ability and the target language. This made linguistic gaps explicitly clear, triggering "noticing". This process is consistent with Schmidt's Noticing Hypothesis (Schmidt 1990). The noticing hypothesis claims that learners cannot continue advancing their language abilities unless they consciously notice the input. The interaction between the AI and the students, or the engagement of the students with the feedback of the AI, is a tool of cognitive development that triggers conscious learning (Quan 2025; Vieriu and Petrea 2025; Zhan and Yan 2025). Since AI provides an explanation contrasting the error with the correct form, each feedback cycle forces the students to consciously notice the gap between their interlanguage and the target language. This conscious recognition of these gaps was the essential prerequisite for internalization. The significant results across all tests are evidence that the AI successfully and consistently triggered this critical cognitive process of internalization. Thus, as AI's feedback provides the correct form of the language, contrasting with the students' errors, it makes the linguistic gap impossible to ignore. Since the most significant gain occurred at the end of the process, this signals that students were not passive recipients of corrections, but were actively internalizing the rules. Moreover, moving from the low score in the initial task to having a steady and cumulative improvement in Task 4 is recorded proof of noticing events from the cycle of feedback in all writing tasks. The effect of the continuous cycle of noticing that triggers internalization was confirmed in the highly significant MANOVA result. This enormous *F*-value indicates an extremely powerful effect on the language learning of students as they progressed. They repeatedly applied and built upon previously internalized rules, compounding their knowledge with each new task. These findings suggest that internalization fosters

independent mastery and eventually reduces students' reliance on the AI's assistance. Likewise, all four standard multivariate test statistics: Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root were in perfect agreement, yielding an identical *F*-value of 737.914 and a significance value of $P < 0.000$ (Table 5), indicating a systematic, unified, and coherent learning trajectory. This result signifies that students were not just getting better in isolated, unrelated grammar points or vocabulary words in every writing task; they were internalizing a broader and more integrated language system. This indicates further that improvement in grammar, vocabulary, and organization happened in a synchronized manner, denoting that the AI-assisted process developed their proficiency as a whole. The results illustrated that students' grammar, vocabulary, and discourse competencies developed interdependently. Additionally, the substantial jump in mean rank between Task 1 and Task 2 indicates that the initial noticing with the AI feedback began to yield significant returns. This positive trajectory that continued with the largest mean score and the top rank being achieved in Task 4 signifies the point where the scaffold provided by AI had been most fully internalized. It established that the more noticing activities students completed with feedback from the AI, the more their language learning was reinforced and strengthened leading to proficient and independent work. Critically, the learning did not plateau, but accelerated, denoting that the cognitive framework built through repeated cycles is capable of supporting even advanced language application and use.

Similarly, Kim et al (2023) observed promising results and areas of improvement by using prompts in the ChatGPT-driven task-based language teaching (TBLT) classroom. Our study offers a blueprint for those improvements in Schmidt's noticing and Long's negotiation of meaning, ultimately proving that AI-student interaction is an effective form of sociocultural learning (Nguyen et al. 2024; Szcześniak 2024; Anders and Dux Speltz 2025).

This study also provides empirical proof on how adaptive guidance from AI in a discovery learning environment improves learning outcomes (Ferguson et al. 2022). Accordingly, AI automatically adapted textual instructions from humans. However, our study explains what specific cognitive actions those adaptive textual instructions should trigger to be effective for learning. It pinpoints that effective guidance works by directing students' attention to the gap between the students' current performance and the target.

Finally, whereas Fauzi et al. (2023) highlighted ChatGPT's potential to enhance student productivity, the present findings not only support its contribution but also provide insight into possible underlying mechanisms. The results suggest that it may support the development of language skills by offering a safe and non-judgmental environment that can help reduce learner anxiety. Beyond providing information and resources, ChatGPT appears to function as a cognitive partner by encouraging metacognitive processes and hypothesis testing. In this regard, the study positions AI not merely as an information tool but as a pedagogical resource with meaningful instructional potential.

The Dynamic Nature of AI-Driven Conversations Fosters Students' Critical Thinking Skills

The scaffolding nature of AI cultivates systematic cognitive development. The robust data show that the enhancement of students' critical thinking skills was directly facilitated by the dynamic and interactive nature of the AI as a Vygotskian tool. The iterative process of AI-assisted writing systematically cultivated critical thinking through iterative scaffolding, metacognition, and hypothesis testing. Across the entire empirical dataset presented in Tables 1 to 4, the findings provide compelling evidence for the efficacy of this intervention. The critical thinking enhancement is well-documented through a significant progression from "below basic" to "proficient". This trajectory is further substantiated by the step-by-step ranking in Table 4, and the steadily increasing means in Table 1, which together show a clear relationship between students' interaction with AI and the predictable and measurable increase in their critical thinking. This cumulative progression is the hallmark of a direct, causal intervention because every student's interaction with AI is appropriately challenging and is consistently working within, while expanding the students' ZPD.

The safe learning environment fosters metacognition. The initial low scores in Task 1 reflect students' lack of awareness of their own cognitive processes and strategies needed to regulate their writing. The empirical data show that the process of AI-assisted writing moves the students from unconscious incompetence to conscious proficiency. To illustrate, each of the five writing tasks was a repeated cycle of writing a draft (plan), getting AI feedback (monitor), and revising and reflecting (evaluate). Thus, when the students wrote their initial drafts, they were configuring their current cognitive understanding and their language skills. Since the draft was the product of an unreflective thought process, the AI's feedback that explicitly highlighted the gap between their thought process and the intended language use was an essential cognitive process of "learning how to think." The steady improvement in mean scores in Table 1 (Task 1: M=1.85) shows that they began to internalize this reflective process. This was the result of the research methodology, which

mandated that the students document the AI's corrections and write their reflections based on the AI's feedback. The reflections allowed the students to articulate what they learned about their own mistakes. This process allowed them to self-assess their own error patterns, thus developing strategies to avoid similar errors in the future. This observation was reflected in the significant jump in scores between Tasks 2 and 3 (Table 1). This leap to proficiency indicates that students moved from simply fixing errors to understanding their own cognitive habits.

The cycle of feedback enables hypothesis testing and analytical reasoning. The repeated cycles of writing a draft (plan), getting AI feedback (monitor), and revising and reflecting (evaluate) functioned as a powerful engine for hypothesis testing and analytical reasoning, transforming the writing process into a live, iterative laboratory for analytical reasoning. It happened every time students submitted their crime scene analysis. The students' reports that describe what happened, who did it, and why it happened, constituted their best inference because they are not just listing facts but are constructing a causal model of an event. This construction of a mental model is the product of their analytical thought. The efficacy of this structured Write – Receive AI Feedback – Reflect and Revise is evident in the dramatically escalating *t*-values in Table 3. These data signify that students became more proficient at constructing and defending their hypotheses with every writing cycle. Subsequently, this proficiency led to the strengthening of their analytical skills. This is clear in the crossing of the proficiency threshold in Task 3 (Table 1) where the mean score reached the high proficiency threshold from basic low proficiency. This suggests that after several writing cycles, the students had internalized the standards of good analysis. Zhang (2025) confirmed that engagement of students with ChatGPT enhanced students' argumentative writing more than engagement with other learners' prompts that yielded negative logic development. In short, analytical reasoning was not acquired as an abstract concept, but was embedded in the AI-assisted writing tasks that required students to practice it continuously. Similarly, studies have confirmed that AI can be a valuable tool in developing students' critical thinking skills, provided that it is properly scaffolded (Darwin et al 2024; Lawasi 2024).

In contrast, Tudy et al. (2025) claimed that students become passive and unthinking by being dependent on AI in their cognitive processes. While the study identified a crisis of fear, seeing the tool as a utility and its consequences leading to anxious uncertainty, our study offers a pedagogical pathway that addresses this uncertainty. Moreover, while ASEAN educators fear technology dependence among students (Holmes and Lee 2024), our study establishes evidence that when ChatGPT is embedded in a structured learning environment (Draft - AI Feedback - Reflection - Revision), it builds learning independence. This study refutes the claim of mindless "copy-pasting" and instead shows that the Vygotskian MKO, through mandatory reflection, facilitates metacognitive reflection, thereby building academic integrity into the writing process itself. Thus, our study demonstrates cognitive gain instead of the ethical fear raised regarding AI use.

The result of this study also explains and provides a solution to cognitive offloading identified by Gerlich (2025). While Gerlich warns of the negative effect of frequently using AI on students' critical thinking, our study demonstrates that intentionally, pedagogically designed student-AI interaction actively builds students' critical thinking. The frequent passive use of AI to get answers may harm critical thinking; however, our study shows that if it is done through active, integrative use through structured, task-specific AI interaction, the result is the opposite. This validates Gerlich's claim that unstructured frequent use of AI leads to cognitive offloading, a situation wherein the student delegates thinking to the machine, leading to weakening of their own cognitive muscles. Our research demonstrates that through Vygotskian MKO, structured scaffolding leads to cognitive enhancement, not offloading.

Relationship between Students' English Language Skills and Critical Thinking Skills

The moderate positive correlation between students' English language learning and their critical thinking skills points out that language and critical thinking are deeply intertwined and mutually reinforcing within the Vygotskian AI-assisted writing process. The AI-powered Vygotskian scaffold in writing tasks ensured that development in one domain did not happen without development in the other. It occurred when the students analyzed evidence and evaluated claims, as they first needed to encode these complex thoughts into language. On the other hand, when AI provided feedback in the form of grammar nuances, precise vocabulary for accuracy, and transitional phrases for coherence, it directly improved students' capacity to articulate their critical thoughts. Thus, this research illustrates how language serves as a tool for thinking. Language affects critical thinking, as studies have shown that the lack of significant correlations between students' language proficiency and critical thinking requires critical evaluation instruction (Manalo and Sheppard 2016), which, in this research, was operationalized through structured AI-assisted writing. Second, the said study concluded that L2 proficiency is a potential limiting factor in students' use of critical thinking and suggests that teaching strategies and appropriate classroom instruction to improve language proficiency are

beneficial. Our study empirically offers the needed strategy. The AI-assisted writing process of Draft - AI Feedback - Reflection - Revision embedded the development of a second language within critical thinking tasks. The Vygotskian feedback cycle mediated by AI became an appropriate blueprint for teaching strategies. This study demonstrates that with the right scaffold, integrated into the thinking task itself, the development of language and critical thinking becomes a mutually reinforcing process.

Although the study provides robust evidence for the efficacy of AI-assisted writing, it is still subject to some limitations. First, since the study was conducted within a single academic discipline (Criminology), this could limit the generalizability of the findings to other disciplines. Future research should replicate this design across diverse disciplinary contexts. Second, the study was situated within a specific cultural context, where AI intervention helped to mitigate affective barriers such as language anxiety and fear of peer judgment. The findings may not be directly transferable to educational settings with different linguistic landscapes. A cross-cultural comparative study would be valuable in exploring how the sociocultural context moderates the effectiveness of AI-mediated learning. Lastly, the intervention was relatively short-term, using only a single semester with five sequential tasks. It is unclear whether the long-term retention or internalization of language rules and critical thinking strategies gained by the students would persist over time or whether students would continue to apply these skills independently without the ongoing support of the AI scaffold. A longitudinal study that would track students' performance after the intervention would provide critical insights into the durability of cognitive gains from AI-facilitated learning, and whether these translate into sustained academic competencies.

In conclusion, through pedagogically structured integration, ChatGPT enhances the students' language learning experience and empowers non-linguistically inclined Filipino students to become independent and critical thinkers. Likewise, the use of ChatGPT is instrumental in creating an inclusive environment for non-linguistically inclined students. This study recommends that the components of this study be analyzed to help identify effective strategies that can be replicated in other classroom contexts. Likewise, teachers shall provide an inclusive environment where learners take more responsibility for making the best use of AI Language applications, and become conscious of their own individual needs, with emphasis on ethical use of information and technology.

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GENERATIVE AI STATEMENT

This study utilized Grammarly solely for grammar checking and minor language refinement. All ideas, analyses, and substantive content were developed by the authors.

ETHICAL CONSIDERATIONS

The researchers ensured that all ethical considerations were addressed throughout the inquiry. All participants voluntarily agreed to participate in the data gathering process.

DECLARATION OF COMPETING INTEREST

To the best of the authors' knowledge, no conflict of interest exists.

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Aloe vera (L.) Burm.f. enhances caudal fin regeneration in zebrafish (*Danio rerio*)

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ABSTRACT

Zebrafish are widely recognized for their ability to completely regrow their caudal fins following an amputation injury. This makes them an ideal model organism for investigating the wound-healing potential of various medicinal plants. In this study, the regenerative effect of *Aloe vera* (L.) Burm.f. extracts on the amputated caudal fins of zebrafish was evaluated using three extract concentrations (i.e., 0.3%, 0.6%, and 1.2%) applied per 1.3 L of water in the tank. Results showed that *A. vera* extracts contributed to the survivability of wounded zebrafish and the regeneration of their caudal fins compared with untreated wounded zebrafish. Among the extract concentrations applied, 0.6% yielded a regeneration rate of 0.19 mm/day and an endpoint caudal fin regeneration percentage of 99.17% within 14 days of application, with no recorded mortality. The results suggest that *A. vera* is a promising candidate for wound healing and the regeneration of damaged tissues in zebrafish. Furthermore, it indicates its potential use by fish pet owners and businesses as an alternative to commercial fish wound-healing products.

Keywords: medicinal plants, tissue regeneration, survivability, wound-healing

INTRODUCTION

There is increasing interest in exploring the regenerative potential of plant extracts in fishes with damaged fins due to the growing demand for sustainable solutions in the aquaculture industry. Fins, specifically the caudal fins, produce propulsive force, which is essential for locomotion, balance, and survival in fish (Liu et al. 2020). The loss of this vital part can result in decreased growth rates, reduced reproductive success, and increased mortality, thereby negatively impacting the fish industry. Existing commercial treatments for fin damage can be expensive and may have adverse environmental effects. Meanwhile, plant extracts possess potential therapeutic properties, which make them promising alternatives for improving overall fish health, increasing wound-healing capabilities, and reducing possible environmental impacts (Ayisi et al. 2025).

Traditional herbal plants are widely recognized as cost-effective wound healing treatments. *Aloe vera* (L.) Burm.f. is known to possess

antibacterial, antiseptic, and antiviral properties that serve as a basis for investigating its potential as an all-natural fish wound treatment. In addition, it has demonstrated regenerative properties. For instance, Hekmatpou et al. (2019) reported that its regenerative properties are attributed to the compound glucomannan, which is rich in polysaccharides such as mannose.

Aloe vera is a succulent plant that belongs to the family Asphodelaceae and is commonly found in arid regions of Africa, Asia, Europe, and America (Surjushe et al. 2008). The leaf of this plant is fleshy, triangular in shape, and has serrated edges. It consists of three layers. The first layer comprises an inner gelatinous liquid that contains 99% water, while the remaining 1% consists of glucomannans, amino acids, lipids, sterols, and vitamins. This layer is commonly extracted for skin and hair care applications, as it effectively cleanses the hair shaft by removing excess oil and residue from previously applied hair products. When used regularly, it may also promote hair growth and improve hair texture. The middle layer, on the other hand, consists of latex which is the bitter yellow sap rich in anthraquinones and glycosides. Lastly, the outer layer, called the rind, serves as protection for the inner layers and helps in the synthesis of carbohydrates and proteins (Jadhav et al. 2020).

In addition to the moisture and hydration effects of *A. vera* on the skin, it also provides medicinal benefits attributed to its antibacterial, antiviral, and antiseptic properties, which help treating wounds and other skin conditions. According to Surjushe et al. (2008), it contains 75 active compounds, including vitamins A, B, C, E, B12, folic acid, and choline, which function as antioxidants that neutralize free radicals. Additionally, enzymes such as alkaline phosphatase, amylase, bradykinesia, carboxypeptidase, catalase, cellulase, lipase, and peroxidase facilitates sugar and fat breakdown. Minerals such as calcium, chromium, copper, selenium, magnesium, manganese, potassium, and zinc also act as antioxidants that serve a variety of metabolic roles, specifically in various metabolic pathway.

Given the existing reports on the phytochemicals and wound-healing capacity of *A. vera*, it is hypothesized that it may also exhibit a regenerative activity essential for the recovery of fishes from caudal fin injuries. Thus, this study aimed to investigate the regenerative effect of *A. vera* in zebrafish. Specifically, it aimed to determine the survivability rate of zebrafish following exposure to varying concentrations of *A. vera* extract; to evaluate the regenerative effects of varying concentrations of *A. vera* extract in terms of morphological caudal fin changes; to determine the rate and percentage of caudal fin tissue regeneration in zebrafish exposed to varying concentrations of *A. vera* extract; and to identify the most effective concentration of *A. vera* extract for caudal fin tissue regeneration in zebrafish.

METHODS

Research Design

The testing of the sample extract was conducted using an experimental approach to compare and contrast the regenerative activity of varying *A. vera* concentrations based on caudal fin growth in zebrafish.

Seven aquarium tanks were assigned to different treatments: T_1, wounded zebrafish with 0.3% *A. vera* extract; T_2, wounded zebrafish with 0.6% *A. vera* extract; T_3, wounded zebrafish with 1.2% *A. vera* extract; T_4, wounded zebrafish with a commercial fish wound medicine (Obat Ikan); T_5, wounded zebrafish with no treatment; T_6, non-wounded zebrafish with 0.6% of *A. vera* extract; and T_7, non-wounded zebrafish with no treatment. The positive control was T_4, while the negative controls were T_5, T_6, and T_7. This study consisted of three trials, each comprising three replicates (Figure 1).

Sample Collection

Mature leaves of *A. vera* plant were obtained from a commercial garden located in Silang, Cavite. Meanwhile, adult male zebrafish measuring 3 to 4 cm and aged 12 to 18 months were purchased from a local pet supplier in the same municipality. Zebrafish are typically considered to be in optimal condition between 3 to 18 months of age (Avdesh et al. 2012). In this study, 12- to 18-month-old zebrafish were selected because they possess larger caudal fins as compared to younger ones, making them suitable for amputation procedures. Moreover, the ability of injured caudal fins to fully regenerate decreases with age (Shao et al. 2011), which was considered to ensure reliable measurement of mortality and regeneration rates. In addition, male zebrafish were used to eliminate the risk of mating and reproduction. Male zebrafish could be distinguished from females by their more slender bodies, darker coloration, and yellow anal fin (Avdesh et al. 2012).

Habitat Set Up and Maintenance

Five zebrafish were placed in each aquarium tank measuring $4.5 \times 3.0 \times 6.0$ in^3. Each tank was filled with 1.3 L of water based on standard tank volume calculations. White stones (250 g per tank) were added to each aquarium tank to help boost algal growth, which served as additional food source and helped mimic the natural habitat of zebrafish. Fish tank aerators were used to provide sufficient oxygen and were set to create a mild water current preferred by zebrafish prefer (Lawrence and Mason 2012). In addition, a centralized light system was operated for

12 h daily to maintain a good habitat and environmental conditions. The tank water was partially replaced on the second, fourth, sixth, eighth, 10th and 12th days prior to treatment application and caudal fin measurement using an aquarium siphon. During replacement, a partial water change method was employed, as it is less stressful for fish and preserves beneficial biological communities (Pelletier et al. 2020). Water with a height of one inch (i.e. 25%) was retained in the tank to ensure zebrafish survival. Stock tap water was then added to the tank as a part of the replacement procedure. Temperature and pH level were maintained at 24°C and 8.17 respectively (Brand 2014), and were monitored after each water replacement and treatment application to prevent physiological stress, reduced growth, disease, or mortality (Jorge et al. 2023).

Preparation of the *A. vera* Plant Extract

Fresh *A. vera* leaves were thoroughly washed, placed upright for 10 min to allow the yellow-tinted resin to drain, and then peeled to remove the thick outer skin. The inner gel was scooped and blended to achieve a liquid consistency (Shoemaker 2019). The mixture was strained to separate the gel and liquid extract, 55% crude yield on average. This crude gel extract was used for all treatments. In this study, concentration is referred to the proportion of crude *A. vera* extract added to the water tanks rather than the concentration of a specific isolated active compound. Although *A. vera* contains several bioactive constituents such as glucomannan, anthraquinones, and flavonoids, the present study did not quantify these individually.

***Aloe vera* Extract Application**

Twelve aquarium tanks (four per replicate) received different amounts of *A. vera* extract on the first, second, fourth, sixth, eighth, 10th and 13th days. Three concentration treatments were applied: 0.3%, 0.6%, and 1.2% (v/v), following Khanal et al. (2012). These concentrations were prepared by adding 4 mL, 8 mL, and 16 mL of crude *A. vera* extract, respectively, to 1.3 L (1,300 mL) of water per tank, corresponding to 0.3%, 0.6%, and 1.2% of the total solution volume. Additionally, 1 mL of commercial fish wound medicine, known to treat fish diseases by acting as an anti-bacterial, anti-rot, and antifungal agent, was also applied to tanks devoted to the positive control during the same application schedule.

Zebrafish Acclimation and Feeding Strategy

The floating bag acclimation method was used to acclimatize the zebrafish. The plastic bag containing the zebrafish was placed on the water for 1 h (Dhanasiri et al. 2013). After the acclimation period, zebrafish were given 24 h to adapt to their respective tanks prior to the application of the treatments.

The zebrafish in each aquarium tank were fed with Maxflo dry fish feed once a day in the morning to promote intestinal emptying and avoid the risk of overfeeding. Most fish need at least 16 h or one whole day to fully digest the food they consume (Sharpe 2024). Overfeeding the fish may result in indigestion and the uneaten food may become excess waste that can be a risk to the fish. In addition, the excess food waste may lead to higher ammonia levels in the water and may eventually lead to serious health consequences for the zebrafish.

Zebrafish Caudal Fin Amputation

Each zebrafish was placed on a Petri dish, and the caudal fin was amputated distal to the end of the bony musculature using a sterile razor blade. The cut was positioned at the terminal portion of the caudal fin, as incisions made close to the musculature may increase the risk of mortality (Haney et al. 2021).

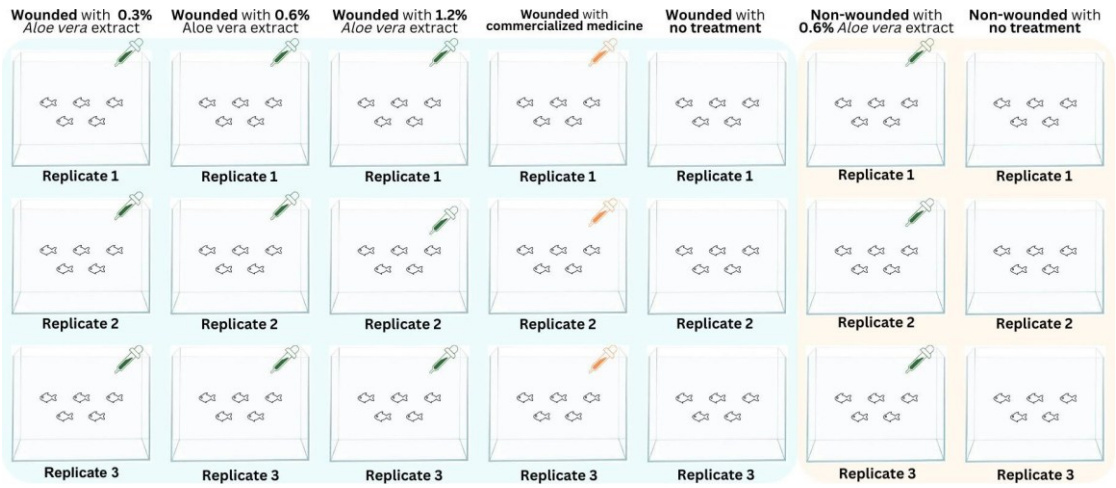

Figure 1. An illustration showing the experimental setup for each trial.

The amputation procedure for each zebrafish was completed within approximately two minutes to minimize handling time and reduce physiological stress associated with prolonged removal from water (Wallace et al. 2018). Caudal fin clipping was a commonly employed method in zebrafish regeneration studies due to the species' high regenerative capacity and the minimally invasive nature of distal tissue removal. All procedures were conducted in a Bureau of Animal Industry-accredited veterinary diagnostic laboratory under the supervision of a licensed veterinarian, ensuring compliance with animal welfare standards.

Wound Exposure to Treatments

After the amputation, the wounded zebrafish was placed back in its aquarium. Then, after 30 min, the *A. vera* extracts and the commercial fish wound medicine were administered in their specific aquarium tanks to allow the exposure of the wound to the treatments. The whole observation period for the caudal fin regeneration was conducted over 14 days (Sehring and Weidinger 2020). The zebrafish was exposed to the different *A. vera* extract concentrations and other treatments seven times throughout the observation period. During each partial water replacement, approximately 25% of the original tank water was retained, and the designated concentration of *A. vera* extract and the fish wound medicine were re-applied to the restored tank volume. This procedure approximated the target treatment concentration, although some residual extract from the previous dosing was unavoidably retained. In cases of mortality, the dead fish was isolated immediately to avoid spread of contamination.

Data Gathering Procedure

Both qualitative and quantitative data were gathered daily for 14 days. Caudal fin measurement was done during nighttime as zebrafish exhibit reduced tail mobility in the evening (Kalueff et al. 2013). The initial length of the zebrafish prior to the amputation was recorded in a data sheet. After the amputation, the initial length on the remaining tail was measured, as well as the development of their length on the third, fifth, seventh, ninth, 11^{th}, and 14^{th} days. To identify the length of the regenerated caudal fin, each zebrafish was placed on a convex Petri dish, which had an attached measuring device to measure the progress in millimeters. This procedure was also done on the first, third, fifth, seventh, ninth, 11^{th}, and 14^{th} days of the observation period.

Data Analysis and Statistical Treatment

Statistical data analysis was used to determine the survivability rate of the zebrafish after exposure to the varying concentrations of *A. vera* extract, and the percentage of caudal fin tissue regeneration in zebrafish exposed to varying concentrations of *A. vera* extract.

To determine the survivability rate, this study used frequency counts and percentages, wherein the number of live zebrafish at the end of the observation period was counted and divided by the total number of zebrafish at the beginning, then multiplied by 100. The average daily regeneration rate of the caudal fin, which was determined to assess the rate of tissue regrowth over time, was calculated by dividing the difference between the final fin length and the initial fin length by the total number of observation days minus one. This provided an estimate of the mean daily increase in fin length across the observation period. On the other hand, to determine the regeneration percentage of the caudal fin, the average final length was divided by the initial length of the caudal fin, then multiplied by 100.

The data were also analyzed using appropriate statistical tools to identify the regenerative effect of *A. vera* and provide possible recommendations. Tests for normality and homogeneity of variances were first conducted to ensure the validity of the statistical comparisons. Based on these assumptions, suitable parametric or non-parametric tests were applied. Bivariate statistics and a chi-square test for independence were employed using the Statistical Package for Social Science (SPSS) program, version 25.

RESULTS

Survivability Rate

The survivability rate of zebrafish in each treatment was determined by counting the number of surviving fish in each tank after 14 days of treatment application. As shown in Table 1, a 100% survivability rate was observed in all treatments with *A. vera* extract (T_1, T_2, T_3, and T_4), indicating the extract's positive effect on survival following caudal fin amputation. In contrast, the untreated control group (T_5) exhibited a lower survivability rate of 77.77%, suggesting that the absence of *A. vera* treatment may have increased the mortality risk in wounded zebrafish.

Table 1. Survivability of the zebrafish 14 days after exposure to different treatments.

Treatment	Survivability Rate (%)
T_1	100 ± 0.00
T_2	100 ± 0.00
T_3	100 ± 0.00
T_4	100 ± 0.00
T_5	77.77 ± 19.44
T_6	100 ± 0.00
T_7	100 ± 0.00

Regenerative Effects of *A. vera* in Zebrafish

The regenerative effects of *A. vera* extract on zebrafish caudal fins after amputation showed progressive changes over the observation period (Figures 2 and 3). On day one, images displayed caudal fins after amputation. By day three, no significant growth progression, changes in shape or size, or blastema formation were observed. However, by day five, notable changes were evident in treatments T_1, T_2, T_3, and T_4, with increased fin thickness and hardening, while T_5 showed no such changes. The caudal fins of T_1-T_4 also exhibited a color change, with the bright original color fading due to a thin white slime developing on the surface, likely contributing to pathogen protection. Additionally, a distinct blastema line appeared, demarcating old fin tissue from the regenerating areas.

On day seven, the caudal fins began to regain their original thickness, though their color appeared more faded than on day five. By day nine, the regenerating fin tips had begun to approximate the shape and size of the original fin, with the blastema line gradually fading and blending with the new tissue. By day 11, fins in T_1, T_2, and T_3 had an almost translucent appearance, indicating the continued formation of new tissue. By day 14, caudal fin regeneration in all treatments closely resembled the original fin shape, size, color, and ray formation, marking substantial recovery.

Regeneration Rate and Endpoint Regeneration Percentage in Zebrafish

The average daily regeneration rate of amputated caudal fins ranged from 0.14 to 0.19 mm/day across treatments (Table 2). Zebrafish exposed to T^2 exhibited the highest regeneration rate (0.19 ± 0.59 mm/day), while those in T^3 showed the lowest (0.14 ± 0.40 mm/day). Treatments 1, 4, and 5 recorded comparable values, ranging from 0.15 to 0.16 mm/day. These findings indicate that, although differences were relatively small, T_2 showed a slight advantage in promoting caudal fin regrowth compared with the other treatments.

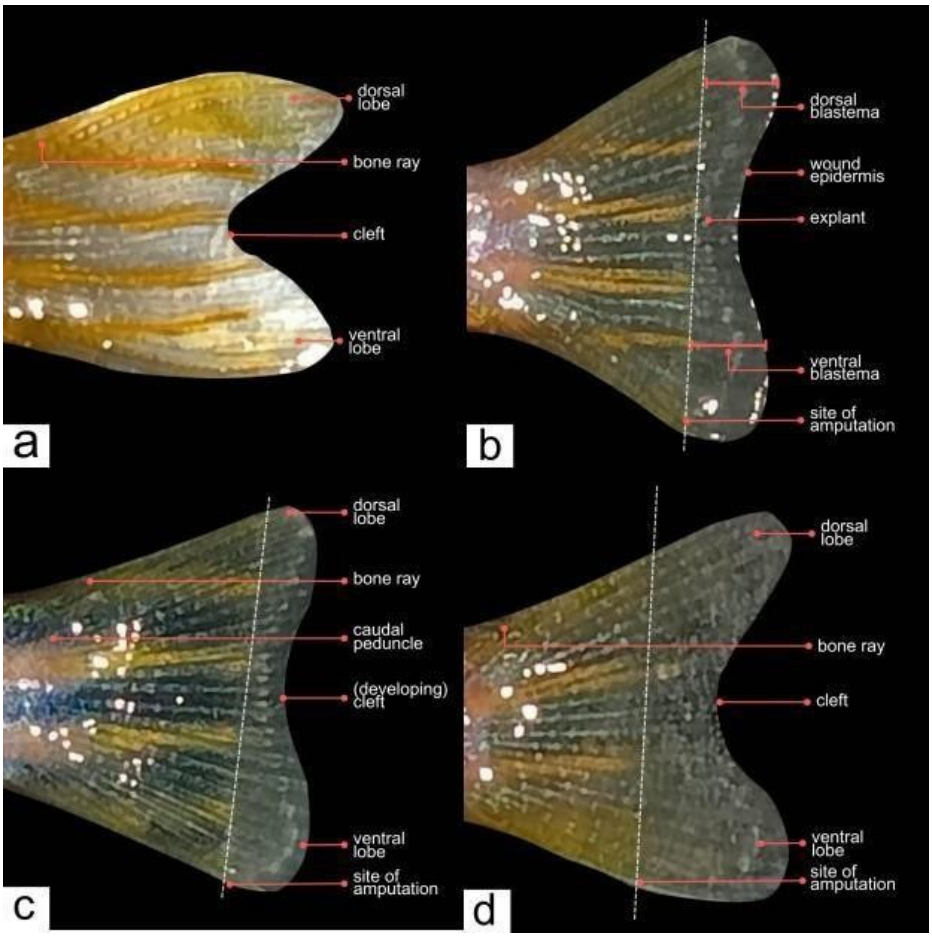

Figure 2. Morphology of (a) non-amputated caudal fin, (b) caudal fin with blastema formation, (c) regenerated caudal fin structure and (d) final regenerated caudal fin structure. Images shown are representative samples selected to highlight the morphological changes observed.

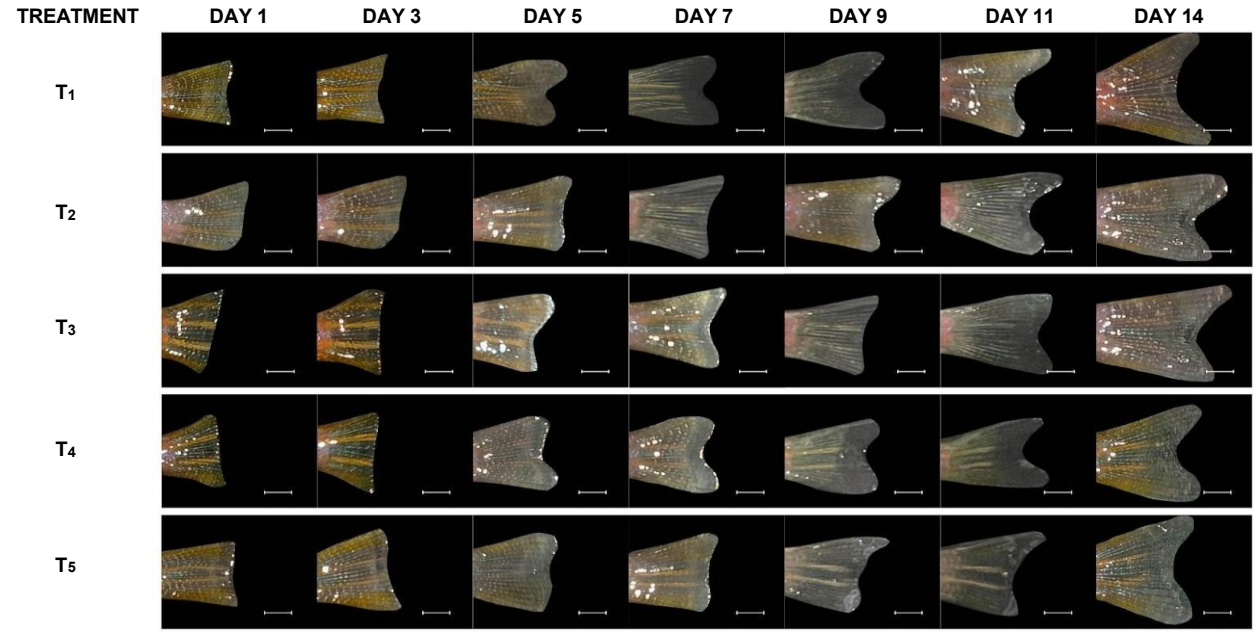

Figure 3. Regeneration progresses of amputated caudal fins within 14 days of exposure to each treatment. Reference size: 1 mm. Note: T_6 and T_7 are not shown as no amputation was done on the zebrafish. Images shown are representative samples selected to highlight the morphological changes observed.

Table 2. Average regeneration rate (mm/day) of amputated caudal fins exposed to different treatments.

Treatment	Regeneration Rate (mm/day)
T_1	0.16 ± 0.39
T_2	0.19 ± 0.59
T_3	0.14 ± 0.40
T_4	0.16 ± 0.36
T_5	0.15 ± 0.44

The caudal fin regeneration percentage was measured at the end of the observation period and compared with the fin length prior to amputation. As shown in Table 3, the highest endpoint regeneration percentage (99.17%) was observed in zebrafish treated with 0.6% *A. vera* extract (T_2), which was significantly higher than the positive control (95.39%). Zebrafish treated with 1.2% *A. vera* (T_3) also showed a notable regeneration percentage (93.24%), though this was not significantly different from the positive control, indicating its potential as an effective regenerative treatment. The commercial wound treatment (T_4) resulted in a similar regeneration percentage to the positive control, at 95.39%. In contrast, the untreated group (T_5) showed the lowest regeneration percentage (87.24%), which was not significantly different from that of zebrafish treated with 0.3% *A. vera* (T_1), which had an 85.80% regeneration percentage. These results highlighted 0.6% *A. vera* as the most effective concentration for promoting caudal fin tissue regeneration in zebrafish.

Table 3. Endpoint regeneration percentage of amputated caudal fins after 14 days of exposure to different treatments. Means followed by the same letter indicate no significant difference at 0.05 level of significance.

Treatment	Regeneration Percentage
T_1	85.80^c
T_2	99.17^a
T_3	93.24^b
T_4	95.39^b
T_5	87.24^c

DISCUSSION

Survivability Rate

The 100% survivability observed in the tanks treated with *A. vera* extract may be attributed to the diverse phytochemicals present in the plant, including saponins, anthraquinones, flavonoids, alkaloids, and phenols. These compounds collectively exhibit properties beneficial for wound healing and infection prevention. Saponins, anthraquinones, and phenols have well-documented antibacterial activities, which could have contributed to the inhibition of pathogenic bacteria in the zebrafish tanks, thus reducing infection risks associated with open wounds (Sayhan et al. 2017). Additionally, alkaloids possess analgesic and local anesthetic properties, potentially reducing pain

and stress in the injured zebrafish, which can support recovery by lowering physiological stress responses (Sayhan et al. 2017). Flavonoids, known for their role in regulating cell growth and anti-inflammatory effects, could further aid in tissue repair by modulating cellular responses around the wound site. Together, these bioactive compounds likely contributed to the 100% survivability observed, supporting the potential efficacy of *A. vera* in reducing mortality related to caudal fin injuries.

In contrast, the tanks with wounded zebrafish that received no treatment experienced mortality, indicating that *A. vera* extract may have a protective effect against mortality in injured zebrafish. These findings align with previous studies demonstrating *A. vera*'s therapeutic properties, which include wound healing and infection control in aquatic animals (Subramanian et al. 2006; Hekmatpou et al. 2019).

Regenerative Effects of *A. vera* in Zebrafish

The presence of a white slime on the regenerating caudal fin in zebrafish treated with *A. vera* extract suggests an increase in the natural slime coat. This slime coat acts as an additional protective barrier against infection, which may enhance tissue regeneration by reducing the likelihood of pathogenic invasion in the affected area (Subramanian et al. 2006). *Aloe vera* extract has also been reported to stimulate collagen synthesis and collagen cross-linking in injured tissues, accelerating wound healing and supporting the structural integrity of the new tissue (Hekmatpou et al. 2019).

Tannins, another key component of *A. vera*, are known to enhance blood clotting, which supports the formation of new tissue in the wounded area by promoting coagulation and tissue stabilization (Chokotho 2005). This could have played an essential role in accelerating caudal fin regeneration. In addition to tannins, saponins, phenols, and anthraquinones present in *A. vera* offer a range of protective properties, including anti-inflammatory, antibacterial, antifungal, and antiviral effects, which can mitigate complications and reduce the risk of infection in the regenerating fin. These combined effects likely aided cell and tissue repair processes, promoting the swift regeneration observed in the caudal fins.

Interestingly, flavonoids in *A. vera* have been noted for their stress-reducing effects due to their polyhydroxylated aromatic structures, which may have helped the zebrafish manage physiological stress during the amputation and subsequent measurement phases. The antioxidative effects of flavonoids could mitigate oxidative stress, further supporting a conducive environment for tissue regeneration (Hekmatpou et al. 2019).

Regeneration Rate and Endpoint Regeneration Percentage in Zebrafish

The regeneration rate analysis supports the potential of *A. vera* in enhancing fin regrowth, as zebrafish treated with 0.6% extract (T_2) exhibited the fastest daily regeneration compared with the other treatments. Although the differences across treatments were relatively small, this trend highlights that *A. vera* can accelerate the pace of tissue repair, not only increasing the extent of regeneration but also reducing the time required for recovery. This suggests that the previously mentioned phytochemical constituents of *A. vera* may act to stimulate cellular processes associated with rapid wound closure and tissue regrowth. The slower regeneration observed in the 1.2% group (T_3) reinforces the idea that an optimal concentration threshold exists, beyond which the stimulatory effects may be diminished.

In terms of the endpoint regeneration percentage, the observation that T_3 (a higher concentration of *A. vera*) exhibited a lower regeneration percentage than T_2 may be due to variations in tank pH levels. Treatment 3 tanks had a slightly acidic mean pH of 7.84, while T_2 tanks maintained a more basic pH of 8.32. Since pH can influence enzyme activity and nutrient absorption in zebrafish, a more acidic environment may have hindered optimal conditions for tissue regeneration. Furthermore, the performance of T_1, which resulted in a regeneration rate comparable to untreated wounded zebrafish, may indicate that this concentration of *A. vera* was insufficient to exert its phytochemical effects fully. Research has demonstrated that higher concentrations of bioactive compounds improve absorption and efficacy at wound sites, supporting the notion that a threshold concentration may be necessary for effective treatment (Soni et al. 2019).

By identifying the treatment with the highest caudal fin regeneration percentage, fastest regeneration rate, and survivability over the 14-day observation period, the most effective concentration of *A. vera* was determined. T_2, with a 0.6% *A. vera* concentration, resulted in the highest regenerated caudal fin tissue, significantly surpassing the positive control. Additionally, T_2 achieved a 100% survivability rate, making it the optimal concentration for both survival and tissue regeneration in wounded zebrafish. These results highlight the potential of using a 0.6% *A. vera* concentration as an effective, natural therapeutic agent for promoting caudal fin regeneration and overall survivability in zebrafish.

In conclusion, *A. vera* extract demonstrated potential in enhancing survivability and promoting tissue regeneration in zebrafish with amputated caudal fins. Tissue regeneration began with blastema formation, and the presence of a white slime coat by day five suggested an enhanced barrier against pathogens due to *A. vera*'s natural protective

properties. Among the tested concentrations, 0.6% was the most effective, yielding the highest caudal fin regeneration rate and surpassing the outcomes of commercial fish wound treatments, as confirmed by post-hoc analysis. These findings support the potential use of *A. vera* extract, particularly at 0.6%, as an effective natural remedy to enhance wound healing and tissue regeneration in zebrafish. Although the phytochemicals of *A. vera* are well studied, future research should focus on quantifying these constituents in crude extracts to improve reproducibility and enable more precise comparisons across studies.

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GENERATIVE AI STATEMENT

During the preparation of this work, the authors used ChatGPT-4 to improve language and grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of this publication.

ETHICAL CONSIDERATIONS

This study was conducted in accordance with ethical guidelines for the care and use of animals in research. It has undergone review and approval by the Ethics Review Board of Cavite State University. To meet the recommendations set by the Institutional Animal Care and Use Committee (IACUC), the study was conducted in a research facility accredited by the Bureau of Animal Industry. Efforts were made to minimize animal suffering, especially during fin amputation.

DECLARATION OF COMPETING INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Phenotypic characterization of Paraoakan chicken (*Gallus gallus domesticus* Linnaeus, 1758) in selected municipalities of Palawan, Philippines

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ABSTRACT

This study assessed the Paraoakan chicken *Gallus gallus domesticus* (Linnaeus, 1758) characteristics such as the birds' morphology, productivity, adaptability, and raising challenges through survey interviews of 96 raisers from seven municipalities of Palawan. Primarily raised for personal consumption, these chickens exhibit normal and plain feather morphology with varied plumage colors and pea combs. Paraoakans predominantly had white skin, yellow shanks, red earlobes, and pea-sized combs. The roosters, on the average, weighed 2.3 kg, measured 40.83 cm in body length, and had a chest circumference of 35.35 cm, with shanks and wingspan measuring 9.98 cm and 43.90 cm, respectively. The hens are generally smaller averaging 1.67 kg weight, 36.90 cm body length, 31.63 cm chest circumference, 8.44 cm shank length, and 43.10 cm wingspan. Paraoakans lay small-sized, white, and brown-colored eggs. Raisers reported that these chickens had a hatchability rate of 86.57%. With a range of 20 to 50 eggs per year, egg production of Paraoakan peaks at 6-8 months. Paraoakan plays a vital role in local food security and rural livelihoods in Palawan, being considered the biggest native breed in the country. At a slaughter weight of 1.0 to 1.5 kg and a 0.5 kg average carcass yield, it is regarded as having a good dressing percentage of 50% to 66%. However, despite being regarded as disease-tolerant, common issues like coryza, fowlpox, and lice infestation are usual issues faced by raisers. This study provides baseline data for sustainable and productive breeding and conservation programs. A comprehensive breeding and training program can be started for growers to establish best practices, enhance disease management, and improve flock health and performance.

Keywords: livestock**,** native chicken, Palawan chicken, phenotypic characterization

INTRODUCTION

The Philippines' native chicken is an important livestock resource. The DOST-PCAARRD (2016) reported that it generates extra income for families and offers social, nutritional, and cultural benefits. The village poultry is an add-on to primary livelihoods, thereby providing additional income while also affording high-quality protein that is otherwise rare to poor families (FAO 2010).

According to Dolberg (2003), poor families in India and Bangladesh trade poultry products among themselves, alongside vegetables and cereals, thereby reducing the consumption of animal-origin foods like meat, fish, and milk and enhancing food security.

Prominent for its positive features like its muscular frame, overall vigor, and hardiness to pests and diseases (Baguio 2019; Slow Food Foundation for Biodiversity 2023), Paraoakan *Gallus gallus domesticus* (Linnaeus, 1758) is an indigenous Philippine chicken commonly raised in Palawan. As the largest among native poultry breeds in the Philippines, they are characterized by long legs, an elongated neck, and a more extensive body and head than other local breeds (Slow Food Foundation for Biodiversity 2023). Baguio (2019) supported this view by stating that of the several strains of native chickens in the Philippines, like the "Zampen" of Zamboanga and "Darag" of Bicol, Paraoakan is the biggest of them all. Female Paraoakan chickens are typically black, while males have reddish feathers. Roosters can reach a weight of 3-5 kg, typically attaining 1.2-1.3 kg within 6 months after hatching (Chicken Breed Guide 2021). Large bones, broad shoulders, and an upright stance characterize them. The eggs are usually tinted white to pale brown.

However, these characteristics and the overall population of local Paraoakan chickens may decline due to crossbreeding with non-native chickens primarily used for cockfighting (FAO 2012). To address this, the Department of Science and Technology (DOST), through the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD), is conducting a breeding program to establish sustainable breeding populations of Paraoakan native chickens in Palawan. This study characterizes Paraoakan chickens based on morphological traits and productivity that are important information to come up with conservation strategies.

The baseline survey of the breeding program explored Paraoakan chicken growers' views on the bird's feather morphology and plumage characteristics, morphological traits, productivity traits, adaptation, and resilience or the challenges growers face in raising Paraoakan. By keeping a record of the existing phenotypic characteristics of Paraoakan chicken in the province, this study provides a reference point to monitor future changes of these characteristics and evaluate the outcomes of the breeding program over time. This information is necessary to set breeding goals, select traits to improve the breed, ensure the breeding program aligns with local values, and increase breeder participation and support. Moreover, it documents the genotype variations necessary for maintaining genetic diversity and sustainability of the breed over time.

METHODS

Study Site and Respondents

The respondents in the study were 96 Paraoakan chicken raisers of seven municipalities of Palawan Province, the majority of whom are male aged between 31 and 40. They came from Puerto Princesa, the capital city, Roxas in the north, and Sofronio Española, Quezon, Rizal, Brooke's Point, and Bataraza in the south. Most raisers grew Paraoakan chicken for family use or consumption.

Research Instrument

A structured interview and a questionnaire were used as the study's instruments. Using a checklist from the Food and Agriculture Organization (FAO 2012) as a guide, the questionnaire was developed, comprising three parts. One is the phenotypic characterization, which includes feather morphology, plumage color, plumage pattern, skin and shank color, ear lobe color, comb type, body measurements, adaptation, and the chicken's resilience to pests, diseases, and climatic conditions. The second part encompasses specific chicken traits, including egg production, egg size, egg color, as well as egg-quality traits, reproduction characteristics, growth performance, mortality, and carcass characteristics. The third part was on the issues or problems encountered by the raisers.

Data Gathering Procedure

Since the study considered Paraoakan chicken raisers as the actual population, purposive sampling was employed to select respondents. The selection was facilitated by barangay officials and representatives from the Department of Agriculture, particularly the heads and personnel of the Municipal Agriculture Offices. To obtain the phenotypic identification and body measurements, the study examined 96 pairs of 1-year-old male and female chickens randomly picked from each respondent's flock of Paraoakan chickens (Figure 1).

Figure 1. Male and female Paraoakan chickens.

The chicken's weight was obtained in kilograms. A tape measure was used to measure the body, which comprised the chickens' wingspan, chest circumference, body length, and shank length. To ensure accuracy and comparability across different birds, and to provide a consistent location for measurement, the chest circumference was measured around the pectus. The body measurement was taken with the neck fully extended, starting from the tip of the beak to the tip of the tail, excluding the feathers (Figure 2). The chicken's wingspan was obtained by fully stretching the wings and measuring across the back of the bird. To get the shank length, the hock joint to the spur of either leg was measured. Data related to the productivity traits, adaptation, and resilience, and the issues encountered in growing the Paraoakan chicken were collected through surveys and interviews.

Figure 2. Measuring of wing span and body length of chicken done during the baseline survey.

Data Analysis

The study employed descriptive statistics, specifically frequency counts, range, mean, and percentages, to analyze the collected data.

RESULTS

Feather Morphology and Plumage Characteristics

The feather morphology of the Paraoakan in Palawan is generally normal. However, 36.46% of raisers reported having chickens with muffs and beards, 19.79% with naked necks, 10.42% with feathered shanks and feet, and 7.29% with crested chickens (Table 1). Plumage patterns include plain (67.70%), mottled (30.21%), laced (26.04%), and barred (26.04%). Plumage colors vary (Figure 3), with black and wheaten/light brown being the most common (85%), followed by white (52%).

Table 1. Percentage distribution of feather morphology of Paraoakan native chicken Respondents have multiple answers (n=96).

Phenotypic Characteristics	Frequency	Percentage
Feather Morphology		
Normal	96	100
Frizzle	8	8.33
Silky	0	-
Feather Morphology		
Normal	96	100
Muffs & Beard	35	36.46
Naked Neck	18	19.79
Feathered Shanks & Feet	10	10.42
Crest	7	7.29
Plumage Pattern		
Plain	96	100
Mottled	65	67.7
Laced	29	30.21
Barred	25	26.04
Plumage Color		
Black	82	85.42
Wheaten/Light Brown	82	85.42
White	50	52.08
Red	38	39.58
Gray	11	11.46
Blue	4	4.17
Golden	2	2.08

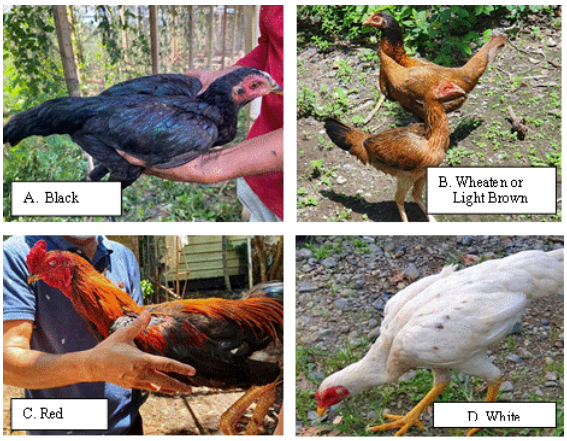

Figure 3. Various Paraoakan chicken plumage colors raised by respondents: A-Black; B- Wheaten or Light Brown; C–Red and D-White

Morphological Traits

Skin, shank, and earlobe colors. Predominantly white skin (71.88%) and yellow skin (54.17%) were reported. Yellow shanks (90.63%) were the most common, followed by black shanks (52.08%) and white shanks (41%). Red earlobes (44.79%) and white earlobes (38.54%) were most common. Some raisers also noted yellow and red/white earlobes (Table 2).

Comb type. Pea combs (66.67%) and cushion combs (33.33%) were prevalent. Small combs were also noted in 73.96% of the chickens (Table 3).

Table 2. Percentage distribution of skin, shank, and earlobe colors of Paraoakan native chicken (Respondents have multiple answers n=96)

Phenotypic Characteristics	Frequency	Percentage
Skin Color		
White	69	71.88
Yellow	52	54.17
Blue-Black	2	2.08
Shank Color		
Yellow	87	90.63
Black	50	52.08
White	41	42.71
Green	10	10.42
Gray	8	8.33
Blue/Bluish	2	2.08
Brown	1	1.04
Earlobe Color		
Red	43	44.79
White	37	38.54
Yellow	32	33.33
White & Red	25	26.04
Black	1	1.04
Orange	1	1.04

Body measurements. Roosters had an average weight of 2.3 kg. with the heaviest recorded in Rizal, a body length of 40.83 cm with the longest observed in Quezon, chest circumference of 35.35 cm with the most significant found in Espanola, a shank length of 9.98 cm with the most extended shank length recorded in Brookes' Point, and wingspan of 43.90 cm with the broadest wingspan observed in Roxas. Hens averaged 1.67 kg in weight, 36.90 cm in body length, 31.63 cm in chest circumference, 8.44 cm in shank length, and 43.10 cm in wingspan (Table 4).

Table 3. Percentage distribution of comb characteristics of Paraoakan native chicken (Respondents have multiple answers. n=96)

Phenotypic Characteristics	Frequency	Percentage
Comb Type*		
Pea	64	66.67
Cushion	32	33.33
Strawberry	18	18.75
Rose	18	18.75
Single	13	13.54
Walnut	7	7.29
Carnation	5	5.21
V-shaped	-	
Double/ Buttercup	1	1.04
Comb Size*		
Small	71	73.96
Medium	55	57.29
Large	10	10.42

Productivity Traits

Egg production. Some of the respondents (43.75%) reported that their Paraoakan chickens laid eggs at 6 months, while 16.67% reported at 7-8 months. The highest annual egg production recorded was 20-50 pieces, 69.76% (Table 5).

Egg size and color. Most chicken raisers (43.75%) reported that their Paraoakan chickens laid small-sized eggs (45-54 g), with only 29.17% indicating medium-sized eggs, 55-59 g (Table 6). Sixty-nine percent (69%) of Paraoakans lay cream-colored, while 44% lay white-colored eggs (Figure 4).

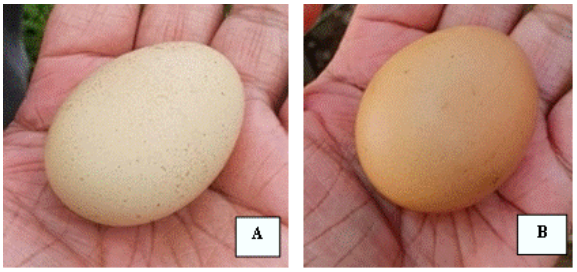

Figure 4. Chicken lay either cream-colored A) or brown-colored eggs (B).

Table 4. Body measurements (±sd) of Paraoakan roosters and hens in the respondent municipalities.

Municipality	Weight (kg)		Body Length (cm)		Chest Circumference (cm)		Shank Length (cm)		Wing Span (cm)	
	Rooster	Hen	Rooster	Hen	Rooster	Hen	Rooster	Hen	Rooster	Hen
Roxas	2.18 (±0.21)	1.52 (±0.18)	41.03 (±1.24)	38.96 (±1.23)	33.73 (±2.28)	30.1 (±1.85)	8.58 (±1.12)	7.75 (±0.6)	48 (±2.43)	48.36 (±2.53)
Espanola	2.55 (±0.21)	1.95 (±0.18)	41 (±1.24)	36.04 (±1.23)	38.38 (±2.28)	35.14 (±1.85)	10.38 (±1.12)	8.75 (±0.6)	40 (±2.43)	41.5 (±2.53)
Quezon	2.32 (±0.21)	1.88 (±0.18)	42.54 (±1.24)	37.33 (±1.23)	37.17 (±2.28)	32.17 (±1.85)	10.42 (±1.12)	8.38 (±0.6)	45 (±2.43)	42.08 (±2.53)
Rizal	2.63 (±0.21)	1.58 (±0.18)	40.88 (±1.24)	36.13 (±1.23)	37.58 (±2.28)	31.33 (±1.85)	10.13 (±1.12)	8.96 (±0.6)	44.67 (±2.43)	42.42 (±2.53)
Brookes' Point	2.17 (±0.21)	1.58 (±0.18)	41.67 (±1.24)	36.67 (±1.23)	32.96 (±2.28)	29.58 (±1.85)	11.17 (±1.12)	8.67 (±0.6)	43.25 (±2.43)	43.83 (±2.53)
Bataraza	2.15 (±0.21)	1.68 (±0.18)	38.57 (±1.24)	37.83 (±1.23)	34.36 (±2.28)	32.39 (±1.85)	10.93 (±1.12)	9.05 (±0.6)	43.57 (±2.43)	42.89 (±2.53)
PPC	2.12 (±0.21)	1.49 (±0.18)	40.14 (±1.24)	35.35 (±1.23)	33.25 (±2.28)	30.73 (±1.85)	8.28 (±1.12)	7.5 (±0.6)	42.83 (±2.43)	40.65 (±2.53)
Average	2.3	1.67	40.83	36.9	35.35	31.63	9.98	8.44	43.9	43.1

Table 5. Egg production characteristics of Paraoakan native chicken in the respondent municipalities.

Egg Production Characteristics	Frequency	%
Age at First Egg (month)		
4	7	7.29
5	5	5.21
6	42	43.75
7	16	16.67
8	16	16.67
9	2	2.08
10	1	1.04
No Data	7	7.29
Annual Egg Production		
20-50	67	69.76
51-70	16	16.67
71-90	6	6.25
Above 90	-	-
No Data	7	7.29

Table 6. Egg quality traits of Paraoakan native chicken in the respondent municipalities (n=96).

Egg Quality Traits	Frequency	%
Egg Weight (g)		
Peewee <45	1	1.04
Small 45-54	42	43.75
Medium 55-59	28	29.17
Large 60-64	17	17.71
Extra-large 65-69	-	-
Jumbo >70	1	1.04
No Data	7	7.29
Shell Color		
White	42	43.75
Brown	11	11.46
Cream	66	68.75
Dotted	1	1.04

Reproduction characteristics. The majority of the raisers (85%) disclosed the Paraoakan chicken "usually" reproduces, with an estimated hatchability of 86.57% (Table 7).

Growth Performance. At eight weeks, most raisers (41.67%) claimed roosters weigh 0.6-1.0 kg; at 12 weeks, 42% claimed 0.5-1.0 kg. In adulthood, 35% weigh 2.1-3.0 kg, followed by 33% at 1.1-2.0 kg. Paraoakan hens, on the other hand, weigh 0.2-0.5 kg at eight weeks (49%), 0.35-1.0 kg at 12 weeks (64%), and 1.1-2.0 kg. at adulthood (57%) (Table 8).

Mortality. Very few raisers did not answer the question related to mortality, but 6% disclosed chicks usually die between 0-1 weeks (Table 9).

Carcass characteristics. A predominant 45.83% of the chicken raisers prefer slaughtering chickens at 4-6 months. At this stage, most roosters (42.70%) and hens (58.33%) typically have a live weight of 1.0-1.5 kg. The raisers further reported an average dressing loss of approximately 0.5 kg, representing the difference between live weight and carcass weight (Table 10; Table 11).

Adaptation and Resilience

Most raisers noted high tolerance to feed and water availability, diseases, parasites, and temperature extremes. Coryza was the most common disease reported (83%), followed by fowlpox (23%), poultry lice, and mites (14%), which were also noted as the common problems raisers raised in growing Paraoakan (Table 12).

Table 7. Reproduction characteristics of Paraoakan native chicken in the respondent municipalities.

Reproduction Characteristics	%
Broodiness Usual Sometimes Rare	 85 6
Hatchability (Average)	86.57%

Table 8. Weight and growth of Paraoakan roosters and hens in respondent municipalities (n=96).

Weight	Roosters		Hens	
	Frequency	%	Frequency	%
8 Weeks (kg)				
0.25-0.5	34	35.42	49	51.04
0.6-1	40	41.67	30	31.25
1.1-1.5	6	6.25	6	6.25
1.6-2	6	6.25	2	2.08
No data	10	10.42	9	9.38
12 Weeks (kg)				
.5-1	40	41.67	61	63.54
1.1-1.5	29	30.21	18	18.75
1.6-2	9	9.38	7	7.29
2.1-2.5	7	7.29	-	-
>2.5	1	1.04	1	1.04
No Data	10	10.42	9	9.38
Adult (kg)				
.75-1	3	3.13	6	6.25
1.1-2	32	33.33	55	57.29
2.1-3	34	35.42	24	25
3.1-4	14	14.58	2	2.08
>4	3	3.13	-	-
No Data	10	10.42	9	9.38

Table 9. The mortality rate of Paraoakan chicken in the respondent municipalities.

Age of Mortality (Weeks)	%
0-1	6
1-8	1
8-20	-
20-n	-

Table 10. Preferred slaughter age of respondents' chickens.

Characteristics	Roosters		Hens	
	Frequency	%	Frequency	%
Age (mos.)				
3-Jan	19	19.79	19	19.79
6-Apr	44	45.83	44	45.83
9-Jul	15	15.63	15	15.63
12-Oct	3	3.13	3	3.13
No data	15	15.63	15	15.63

Table 11. Live and carcass weights of Paraoakan roosters and hens in the respondent municipalities.

Live Weight (kg)	Carcass Weight (kg)	Roosters		Hens	
		Frequency	%	Frequency	%
1-1.5	.5-1	41	42.71	56	58.33
1.6-2	1.1-1.5	26	27.08	14	14.58
2.1-2.5	1.6-2	6	6.25	7	7.29
2.6-3	2.1-2.5	6	6.25	1	1.04
>3	>2.6	2	2.08	-	-
No Data		15	15.63	18	18.75

Table 12. Adaptability traits of Paraoakan native chicken in the respondent municipalities.

Adaptability Traits	Frequency	%
Has tolerance or resistance to diseases and parasites		
Yes	78	81
No	18	19
Diseases or parasites incurred n=18		
Coryza	15	83.33
Not specified	3	16.66
Has tolerance to extremes of temperature		
Yes	75	78
No	21	22
Has tolerance or susceptibility to relevant diseases, ectoparasites, and endoparasites		
Yes	74	77
No	22	23
Relevant diseases, ectoparasites, and endoparasites n=22		
Fowl Pox	5	23
Poultry Lice and Mites	3	14
Pests (immunization)	3	14
Round Worms	1	5
Not Specified	10	45

Adaptability Traits	Frequency	%
Has tolerance relevant to feed and water availability		
Yes	84	88
No	12	13
Adaptability Traits	Frequency	%
Has tolerance or resistance to diseases and parasites		
Yes	78	81
No	18	19
Diseases or parasites incurred n=18		
Coryza	15	83.33
Not specified	3	16.66
Has tolerance to extremes of temperature		
Yes	75	78
No	21	22
Has tolerance or susceptibility to relevant diseases, ectoparasites, and endoparasites		
Yes	74	77
No	22	23
Relevant diseases, ectoparasites, and endoparasites n=22		
Fowl Pox	5	23
Poultry Lice and Mites	3	14
Pests (immunization)	3	14
Round Worms	1	5
Not Specified	10	45
Has tolerance relevant to feed and water availability		
Yes	84	88
No	12	13

DISCUSSION

Feather Morphology and Plumage Characteristics

Feather morphology among Paraoakan chickens was generally described by raisers as phenotypically normal, displaying both plain and diverse plumage colors, which are comparable to those observed in Aseel chicken populations in Bangladesh. Consistent with this observation is Sarker et al.'s (2018) report about considerable variation in plumage colors among indigenous chickens with black and wheaten as most prevalent phenotypes. Santiago et al. (2022), who documented predominantly black plumage in Paraoakan roosters and predominantly white plumage in hens, supported these findings. However, the pattern contrasts with the report of Lopez et al. (2013), which identified plain red as the most common feather color among native chicken populations in Palawan. Given the variability in phenotypic traits reported across studies, further molecular and genetic analyses are recommended to validate breed identity and establish a more definitive morphological profile of the Paraoakan chicken population.

Morphological Traits

Skin, shank and earlobe colors. Like Bangladesh's Aseel chicken, Paraoakans predominantly have white skins, red earlobes, yellow shanks, and pea-type combs. Similarly, South-western Ethiopian native male chickens have mainly white and yellow skins, shanks, and red earlobes (Tadele 2018). Indonesian native chickens also have predominantly yellow shanks (Maharani et al. 2019). Paraoakan's predominant white skin color is also shared with the other Philippine native chicken counterparts, which Santiago et al. (2022) also reported as white. Paraoakan's predominant red earlobe color in this study coincides with Santiago et al.'s (2022) report that, along with Boholano and Zampen, Paraoakan roosters exhibited red earlobes.

Lopez et al. (2013), identified yellow and white as predominant skin colors in Palawan native chickens. However, black shanks were the second-highest in this study, deviating from Lopez et al.'s identification of green shanks as the second-highest color. This supports Santiago et al. (2022), who reported that among the Philippine native chicken groups studied, Paraoakan and Darag mostly had black shanks, while samples from other native groups had green, white, and yellow. Additionally, this study identified seven shank colors, compared to Lopez's five in 2013. Except for the inclusion of yellow earlobes in this study, the same red earlobe color was also reported, as evident in the study by Lopez et al. (2013) on the characterization of Palawan native chicken.

Comb type. Paraoakan chicken's predominant pea and cushion combs are similar to those of Aseel chicken, which Sarker (2018) reported to also have pea combs. This, however, differs from the chickens in South-western Ethiopia, which commonly have single and rose combs (Tadele 2018). Moreover, Santiago et. al (2021) noted single combs among other Philippine native chickens like Paraoakan, Darag, Boholano, Camarines and Zampen. Lopez et al. (2013) also noted single comb as the most common among Palawan native chickens. This study's results also did not support the findings of Lopez et al. (2013), who identified single comb as the most common among Palawan native chickens. However, although past studies' findings on comb types differ, native chickens like Paraoakan, Camarines and Bolinao, consistently have small-sized combs (Santiago 2021).

Body measurements. Among the municipalities studied, Rizal and Española appear to offer environments that favor heavier roosters and hens, respectively. In terms of muscular build, Espanola offered the largest chest circumferences for both roosters and hens, suggesting that the chickens in these areas are more broad-breasted and have more vigorous body types. The longest body lengths of Quezon and Roxas chickens suggest better growth conditions or height-increasing genetic traits. Wing span and shank length also vary across municipalities, with the longest shanks observed in Brookes' Point and the widest wingspan in Roxas, physical characteristics that may make the chicken stand out in appearance and give them a more advantageous position in foraging and escaping from predators.

Generally, the findings provide valuable insights into the body measurements and other physical characteristics of Paraoakan chickens in Palawan, which breeders and growers can potentially use as desirable traits to improve chicken characteristics either for aesthetic or economic purposes.

Compared with other Philippine native breeds, Paraoakan roosters in this study appear heavier than Patani, the black native chicken of CARAGA, Philippines. Austral et al. (2022) considered Patani the heaviest among the five genetic groups of the county's native chickens they studied, namely, Patani, Zampen, Boholanon, Camarines, and Darag. The same authors reported that Patani weighed 1.9 kg, had a body length of 24 cm, and a chest circumference of 33.5 cm. However, in this study on the phenotypic characterization of Paraoakan chicken, actual measurements showed that, on average, roosters and hens in Palawan weighed 2.3 kg and 1.67 kg, respectively, with a body length of 40.83 and 36.90 cm and chest circumference of 35.35 and 31.63 cm.

These measurements were, however, lower than those of the Aseel chicken of Bangladesh, where adult males' average live weight is 3.7 kg and adult female birds' live weight is 2.06 kg. Aseel and Paraoakan chicken have comparable shank lengths, ranging from 10-12 cm among adult Paraoakans, while adult male Aseels had 12.79 cm (Sarker et al. 2018).

Study results support the other studies' findings that comparing male and female, hens' body measurements were generally smaller across all body measurements (Lopez et al. 2013; Tadele et al. 2018; Austral et al. 2022). The same authors suggested that this difference could be attributed to the general body structure of males, which is adapted for muscle development. In contrast, the body structure of hens is designed for reproduction.

Productivity Traits

Egg production. The study findings revealed that Paraoakans typically start laying eggs approximately 1.5 months later than the estimated onset of egg production of Darag chicken which usually begins at 18 weeks or 4.5 months (Manila Bulletin Agriculture 2022), and other native hens whose start of laying period is 18 to 20 weeks (Ebarle and Arevalo 2023). This delay in egg production could be attributed to an adaptive adjustment that enhances survival in scavenging environments, a situation similar to that of Paraoakan chickens, as raisers commonly keep them under free-range conditions and feed them a mix of commercial feed and natural foraging. Osei-Amponsah et al. (2015) explained that egg production requires a substantial amount of nutrients and can be affected by factors such as quantity and quality of feed, water, poor housing conditions, and parasite infestation. It is likely that the bird must first build its reserves adequately before laying eggs under a free-range setup, hence it tends to start later rather than earlier. The reproductive efficiency of chickens, including late maturity and egg production, was mainly affected by breed and dietary management (Jacob et al. 2014).

The recorded highest annual egg production of 20-50 pieces per year in this study supports Sarker et al. (2018) who reported that annual egg production was between 24 and 48 eggs per year. The finding that production peaks when hens are between 6 and 8 months old is also in conformity with Osei-Amponsah et al. (2015) who reported that the egg production curves of the experimental Ghanian chickens followed the typical egg production curve for a flock which increases during the first 8 or 9 weeks of production and decreases towards the end of the production period. A similar observation was noted in the indigenous chickens in Ethiopia, where egg production also peaked between the ages of 26 and 29 weeks (Osei-Amponsah et al. 2015).

Tadele (2018) noted that the productive performance of indigenous chickens may vary due to several factors, like disease, predation, nutrition, feed, and overall management. The elements in animal diet, like energy, protein, and calcium, are necessary for consistent egg production because hens' daily nutritional requirements also increase once they start laying eggs (Jacob et al. 2014).

Egg size and color. Tadele (2003) observed that the small eggs of native chickens usually demonstrate low productivity. Sazzad (1992) reported that exotic chicken breeds have an average egg weight of 72% higher than that of local breeds. Similarly, in addition to low egg production, local chickens exhibited low output characterized by small egg size, slow growth rate, and poor survival of chicks (Osei-Amponsah et al. 2015). He added that under village conditions, the yearly egg production per bird ranges from 20 to 100 eggs, with an average weight ranging from about 30–50 g. Bondoc et al.'s (2021) view that eggs from native Philippine chicken breeds such as Banabang Kalabaw, Joloano, Paraoakan, and Palawan Lasak were remarkably small support these viewpoints. Bondoc et al. (2021) also noted that compared to indigenous chicken breeds from other nations, these small-sized Philippine eggs closely resembled those reported for nine local breeds across Asia. One remarkable finding, however, was that despite its relatively small size, Paraoakan eggs weighed the highest among the country's native chicken breeds (Bondoc et al. 2021). Although white eggs could also be found, the Paraoakan egg color was similar to that of Aseel chicken, which was primarily light brown (Sarker et al. 2018).

Reproduction characteristics. The Paraoakan raisers' estimated chicken hatchability of 86.57% was higher than that of Lopez et al. (2014), which mean number of eggs hatched per clutch was recorded at 8.50 ± 2.12 pieces, giving an average hatchability rate of 79.38 ± 13.48%. These studies on Palawan native chickens reported a higher estimated hatchability than Aseel chickens, which stands at 71.68% (Sarker et al. 2018). This metric was influenced by various factors, including egg quality, incubation process, and the overall health of the breeding stock (Serkalem et al. 2023). Based from a study on the reproductive performance of the Philippines' Caraga black native chicken, there was a potential relationship between fertility and the age of the chicken, because it is crucial that the chicken's reproductive ova are mature and ready (Austral et al. 2022).

Growth performance. Notably, there was no consistent weight increase with age among roosters, suggesting that, other than age, there could be other predictors for chicken growth. On the other hand, Paraoakan hens, exhibited more consistent and evident weight increases. Sarker et al. (2018) noted that the growth performance of Aseel chickens was up to 3.91 months weeks or 4 months, and their seemingly higher observed growth performance was related to the breed (Chatterjee 2007; Sarker 2018). This, could be attributed to different genotypes and management practices (Sarker et al. 2018). Meanwhile, Desha et al. (2016) identified the non-genetic factors affecting the growth performance of indigenous chickens in rural villages in Bangladesh such as sex, batch, farm, brooding system, and feeding system.

Consistent with the reports of Lopez et al. (2013), Deshna et al. (2016), Tadele et al. (2018), and Austral et al. (2022) reported that the sex of birds affected growth performance at different ages, and that weight is higher in male than in female. Regarding batch effects on the growth performance of chickens, Gambo et al. (2023) observed that hatch batch had a significant effect on weekly body weight, body weight gain, and growth rate at day-old and at subsequent weeks until maturity of Nigerian indigenous Tiv chickens. Wolanski et. al (2006) noted a strong correlation between egg weight and one-day chick weight, which, in turn, affects chick size and development. On the other hand, farms or households differed in management practices, like housing and hygiene, brooding systems, and supplemental feeding, which are known to influence productivity and performance in village chicken systems. Abdelqader et. al (2007) reported that the main features of the production systems were improper housing and inadequate hygiene, and that there was a significant positive correlation between management level and chickens' performance. Hatchability, survivability, flock size, number of clutches, egg weight, and egg mass were the major parameters that improved significantly with improvement in management practices. Padhi (2016) cited that the performance of native chickens can be improved through changes in husbandry, feeding, and better health cover. Rashid et al. (2020) reported better growth performance and profitability with electricity and diesel-burner brooding for early chicks, which depend heavily on temperature stability and heat availability. On the

other hand, Roxas and Escarlos (2000) noted that different temperatures and relative humidity combinations did not affect the average final weights of native chickens.

Mortality. A low mortality rate, indicative of a healthy flock, efficient management practices, and an efficient feed conversion rate were essential characteristics for the prosperity of a poultry business (Grobbelaar et al. 2010); Osei-Amponsah et al. 2015). This is because a high mortality rate can result in financial loss due to the cost of replacing birds and the loss of potential egg production.

Paraoakan raisers reported that chicks usually survived to maturity once they are more than one week old. Tadele et al. (2003) noted an elevated mortality rate among chicks, reaching up to 49% before they reached eight weeks old. The same study showed an increase in survival rate with a larger number of chicks hatched per incubation. According to Lopez et al. (2014), native chickens in Palawan had a percent livability of chicks from hatching to weaning of 66.89 ± 20.20, indicating the proportion of chicks that survived from hatching until weaning. Lopez et al. (2014) also noted that the most common causes of chick mortality were disease and predatory attack.

Carcass characteristics. The respondents' observation of a 0.5 kg difference between the live and carcass weights points to a good dressing percentage in Paraoakan chicken for meat production, suggesting an efficient conversion of live weight into edible meat where there is less loss due to feathers, viscera, and edible parts, and therefore greater economic value per chicken. In terms of the respondents' preference for slaughtering chickens at four to six months, this is possibly associated with the ideal weight range of 1.0-1.5 kg, considering the trade-offs between feed utilization and meat standards.

Haunshi et al. (2022) noted that the carcass yield of Kadaknath, a native chicken in India, was significantly lower than that of broilers. Moreover, the authors noted that Kadaknath chicken also had significantly lower absolute weights and yields of organs such as the liver, heart, and gizzard (giblets) compared to broilers at 27 weeks of age. However, while the weight of the breast was significantly higher in the broiler, the Kadaknath chicken's weights in cut-up parts like legs, back, and neck was significantly higher.

The efficient conversion of feed into usable meat of Paraoakan, combined with the raisers' preference to slaughter the chicken at lighter weights, makes Paraoakan chickens a practical and economically viable choice for poultry meat production.

Adaptation and Resilience

The majority of the raiser respondents consider Paraoakan chickens to be resilient to feed and water limitations, as well as temperature changes. This aligns with the information shared by San Agustin in a DA-BAR online seminar, where it was noted that native chickens, like other indigenous animals, are renowned for their robustness and adaptability to local agro-climatic conditions, requiring minimal care (DA-BAR 2022). Gheyas et al.'s study in 2021 also noted that African indigenous chickens have genetically been climate-torerant, having adapted to harsh environments. Another study by Kpomasse et al. (2023) referenced Ajayi (2010), who reported that indigenous chickens possess genetic traits from seven to nine significant adaptive genes for tropical environments, thus shielding them from the adverse effects of heat stress.

In terms of disease infection, study results indicate that coryza was the most common disease among Paraoakan chickens. Coryza has respiratory symptoms that often affect semi-mature or adult birds (Serbessa et al. 2023). A reservoir hosts, birds transmit the disease through various means, reducing egg production in layer flocks. The results of the study show that most respondents were unable to specifically identify a disease other than coryza, nor an ectoparasite, nor an endoparasite affecting their chickens. This highlights a significant challenge that may hinder local chicken production, potentially leading to increased mortality rates. Improved information on poultry rearing is therefore crucial for improving production management practices.

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GENERATIVE AI STATEMENT

The author utilized OpenAI's GPT-4 to assist in surveying existing literature on the phenotypic characteristics of native chickens. The same tool was consulted when certain terms or statements from cited authors appeared unclear, for elaboration and clarification. The author acknowledges the tool's potential limitations and biases, and she takes full responsibility for the interpretation and presentation of the findings.

ETHICAL CONSIDERATIONS

This study did not involve minors or individuals below 18 as respondents. All Paraoakan raisers who participated in the study were fully informed about the purpose of the interviews, and only those who voluntarily consented were included. The respondents were not subjected to any financial burden, and the study did not involve any procedures that could harm or endanger the health of the chickens. No personally identifiable information is disclosed in this study; only aggregated results from the data analysis are presented in this paper.

DECLARATION OF COMPETING INTEREST

The author declares no competing interests in this work.

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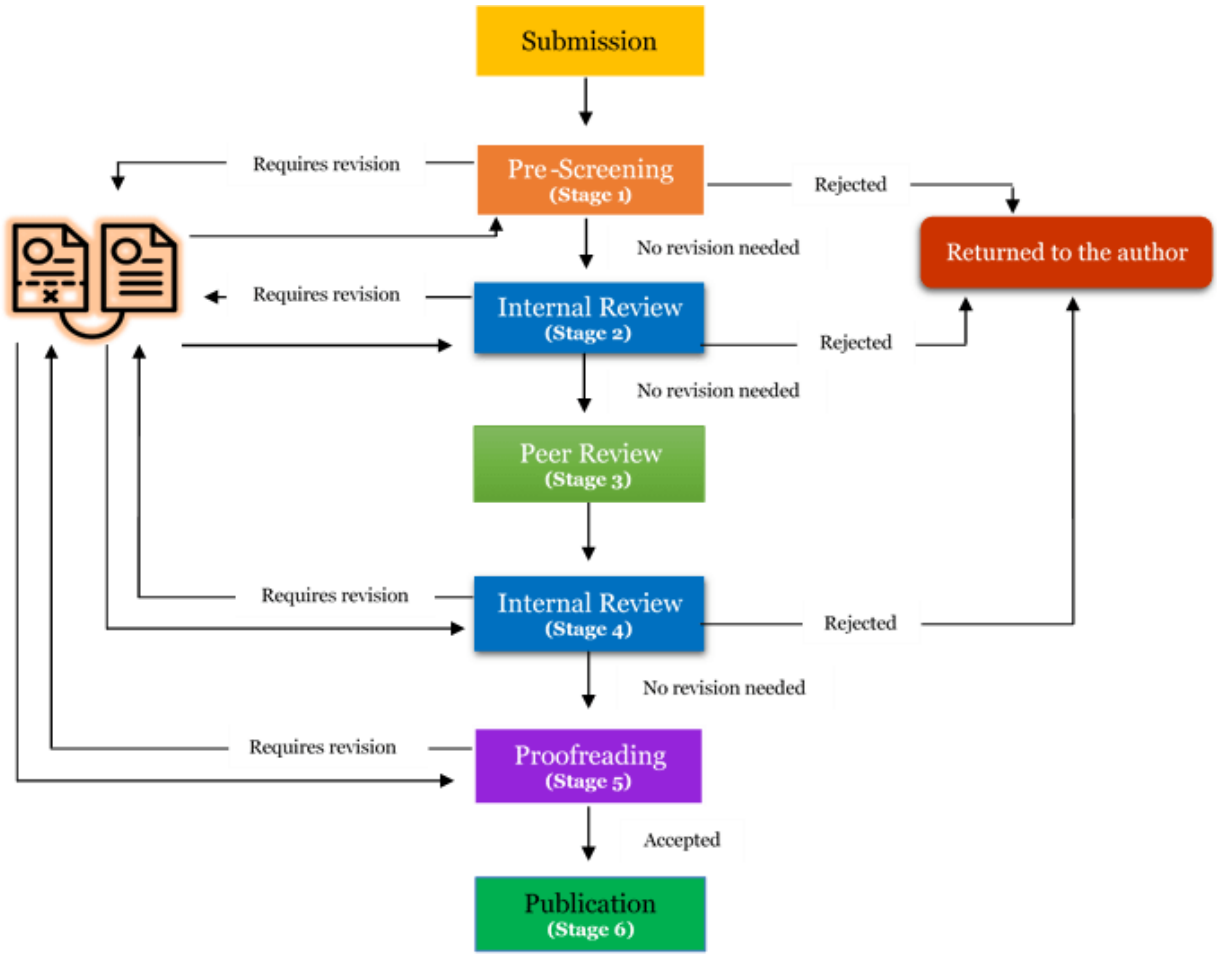

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