Phytochemical Screening, Macronutrient Content, Antimicrobial and Cytotoxic Properties of Selected Edible Plants consumed by the Palaw'an tribe in Bataraza, Palawan, Philippines

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ABSTRACT

This study investigated three edible plants, namely, *Ardisia iwahigensis* Elmer, *Baccaurea gracilis* Merr., and *Manihot glaziovii* Müll.Arg consumed by the Palaw'an tribe in Bataraza, Palawan. Specifically, the phytochemical components, macronutrient contents, antimicrobial properties, and toxicity of the crude ethanol extracts of the fresh and/or air-dried leaves were determined. Qualitative phytochemical screening of fresh leaves and chemical profiling of air-dried leaves both revealed the presence of alkaloids, flavonoids, sterols, and tannins. Macronutrient analysis indicated that *B. gracilis* contained the highest crude protein (13.4% weight by weight) and crude fiber (6.65% w/w), while *M. glaziovii* contained the highest crude fat (0.807% w/w). Disc Diffusion Assay demonstrated significant ($P < 0.05$) antibacterial property against gram-positive (*Staphylococcus aureus* Ogston, 1880 and *Bacillus subtilis* Ehrenberg, 1835) and gram-negative (*Escherichia coli* Escherich, 1885 and *Pseudomonas aeruginosa* Schroeter, 1872) bacteria. The mean zones of inhibitions for *A. iwahigensis* against gram-positive (21.65–22.58 mm) and gram-negative (19.59–22.27 mm) bacteria were comparable with the positive controls (oxacillin 19.25–19.32 mm; Amikacin 16.52–27.32 mm). However, the three plants did not exhibit antifungal properties. Brine Shrimp Lethality Assay showed that *A. iwahigensis* was the most toxic with 100% mortality at 1000 ppm (LC_{50} = 4.270 ppm) after 24h exposure followed by *M. glaziovii* (97% mortality at 1000 ppm with LC_{50} = 7.918 ppm). The three edible plants are good sources of various phytochemicals that may have essential biological activities. This indicates that they can be used, not only as food ingredient, but also for therapeutic purposes and as potential sources of bioactive compounds with antibacterial and cytotoxic activities.

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Keywords: chromatography, crude fat, crude fiber, crude protein, diffusion assay, lethality assay

INTRODUCTION

Natural products have a remarkable role in drug discovery and development due to their chemical novelties and diversity (Calixto 2019). From 1981 to 2019, there were 1,881 FDA-approved drugs derived from natural products (Newman and Cragg 2020). Although most people use conventional drugs and therapies, there are still some communities in remote rural areas, particularly in the Philippines, that rely on medicinal plants in treating common illnesses due to limited access to formal health care system (Dela Cruz and Ramos 2006).

More than 1,500 medicinal plants from over 12,000 plant species found in the Philippines are used by traditional healers (Dela Cruz and Ramos 2006) coming from 109 indigenous ethnic groups (Cariño 2012). However, there are limited ethnopharmacological studies on these ethnic groups, particularly in the province of Palawan which is home to several Indigenous peoples including the Palaw'an tribe (Cariño 2012). Palawan is also home to about 1,700-3,500 flowering plant species of which 15-20% are endemic to the province (Sopsop and Buot 2009) but only a small portion of these plants has been investigated in detail (Garcellano et al. 2019a, b).

In addition to their medicinal uses, plants are also utilized as food or food ingredients. There is an increasing research interest in the health benefits and possible clinical applications of food plants since the combination of nutrition and drug therapy may provide optimum defense against diseases. Investigations are being conducted on the potential of food plants as novel remedies to various diseases because these contain pharmacologically-active compounds (Ramalingum and Mahomoodally 2014). There is also a need to conduct toxicity tests, particularly in wild edible plants, since these contain antinutritional and toxic components in addition to their nutrient content (Guil et al. 1997).

Phytochemical screening of plants used by Indigenous people is considered an effective approach in discovering bioactive components with potential therapeutic applications. In this study, the authors investigated the edible plants used by the Palaw'an tribe. This group, in earlier times, were hunters and lived in upland areas near the forest where they get their food and other daily needs (Ethnic Group Philippines 2017). This study examined and documented the bioactive profile (phytochemical components, macronutrient contents, antimicrobial properties and toxicity) of three plants (*Ardisia iwahigensis* Elmer, *Baccaurea gracilis* Merr., and *Manihot glaziovii* Müll.Arg) used as food ingredient by the Palaw'an tribe in Bataraza, Palawan.

METHODS

Plant Materials

The plant samples for this study, *Ardisia iwahigensis* (fam. Primulaceae), *Baccaurea gracilis* (fam. Phyllanthaceae), and *Manihot glaziovii* (fam. Euphorbiaceae) (Figure 1) are known to the Palaw'an tribe as "Tambilikan", "Duro-manok", and "Sapikol", respectively. *Ardisia iwahigensis* is endemic to Palawan while both *B. gracilis* and *M. glaziovii* have a more widespread distribution (Pelser et al. 2011). The fresh mature leaves of *A. iwahigensis* and young leaves of *B. gracilis* and *M. glaziovii* are used as food ingredients by the Palaw'an tribe in Sitio Budis-Budis, Bataraza, Palawan. In this study, about three kilograms (3 kg) of fresh leaves from each plant were collected in Sitio Budis-Budis.

Figure 1. The three edible plants consumed by the Palaw'an tribe in Sitio Budis-Budis, Brgy. Tarusan, Bataraza, Palawan. A,B: *Ardisia iwahigensis* (Tambilikan); C,D: *Baccaurea gracilis* (Duro-manok); E,F: *Manihot glaziovii* (Sapikol).

Phytochemical Screening, Macronutrient Content Determination, and Disc Diffusion Assay of Fresh Leaves of the Three Edible Plants

Three hundred grams of fresh leaves of each plant were submitted to Department of Science in Technology – Mindoro Marinduque Romblon Palawan Regional Standards and Testing laboratory (DOST-MIMAROPA RSTL) in Puerto Princesa City. The samples were subjected to ethanol extraction and the crude extracts were then sent to the Department of Science and Technology – Industrial Technology Development Institute–Standards and Testing Division (DOST-ITDI-STD) in Bicutan, Taguig City, Philippines for phytochemical screening, macronutrient content determination, and disc diffusion assay (Figure 2).

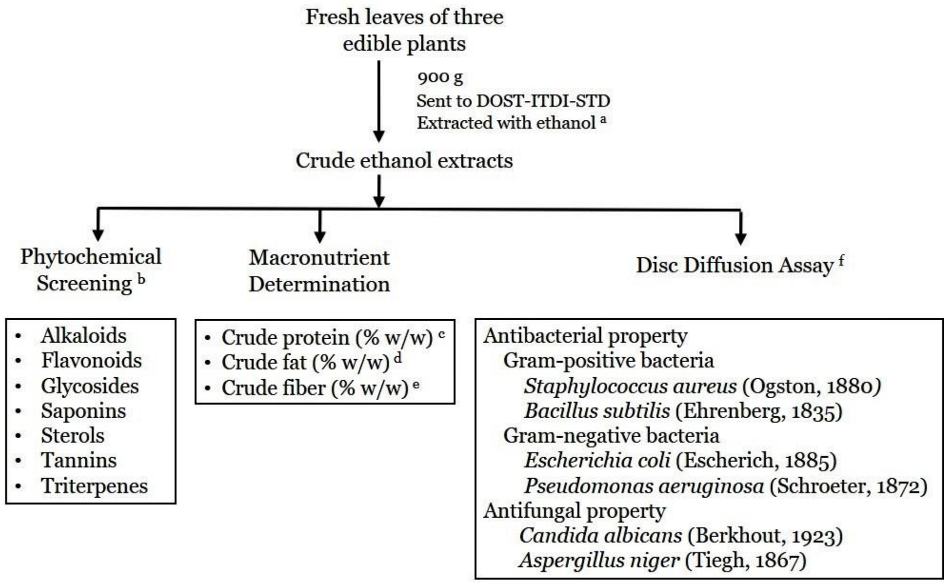

Figure 2. Schematic diagram for the tests conducted on the fresh leaves of *Ardisia iwahigensis*, *Baccaurea gracilis*, and *Manihot glaziovii* at DOST-ITDI-STD. Reference methods: [a] BTD Manual Qualitative; [b] Evans 2002; [c] Block digestion-Kjeldahl (*A. iwahigensis* and *M. glaziovii*) and Combustion (*B. gracilis*) methods; [d] Direct ether extraction; e962.09 AOAC official method; [f] USP30-NF25 2007.

At DOST-MIMAROPA RSTL, 200 g of finely cut fresh plant materials were soaked in 300 ml of 95% ethyl alcohol for 24 h and then filtered using filter paper. The flask and plant material were rinsed with fresh alcohol and then combined with the first filtrate. The residue was discarded. The filtrate was concentrated over a water bath at 40-60°C to about 20 ml and then stored in a desiccator.

At DOST-ITDI-STD, the ethanol leaf extract of the three edible plants were screened qualitatively to determine the presence of phytochemicals while various methods were utilized to determine the macronutrients present (Figure 2).

Disc Diffusion Assay was also conducted to assess the antibacterial and antifungal properties (Figure 2) in terms of reactivity based on the mean zones of inhibition (MZI) of bacterial growth (Table 1). The data were subjected to one-way analysis of variance (ANOVA) and Kruskal-Wallis Test (using SPSS and MegaStat software) to determine the level of significant difference ($P < 0.05$) among the three edible plants against each bacterial strain.

Table 1. Reactivity grades based on mean zone of inhibition (MZI) (USP30-NF25 2007).

Grade	Reactivity	MZI
0	none	No detectable zone around or under specimen
1	slight	Malformed or degenerated cells under the specimen
2	mild	Zone limited under the specimen
3	moderate	Zone extends 5-10 mm beyond specimen
4	severe	Zone extends greater than 10 mm beyond specimen

Chemical Profiling and Brine Shrimp Lethality Assay (BSLA) of Air-dried Leaves of the Three Edible Plants

Plant extraction. Two kilograms each of fresh leaves were air-dried for a month then ground to a fine powder (20 g) and soaked in a 95% ethanol solution (250 ml) for 72 h. The mixture was filtered and simple distillation was conducted to recover the solvent and obtain the crude extracts which were then subjected to chemical profiling and Brine shrimp lethality assay (BSLA) (Figure 3).

Chemical profiling. The crude ethanol extracts were separated on silica gel thin layer aluminum plates. Solvent systems of different polarities were prepared to best separate the components of the ethanol extract of air-dried leaves. Extracts were spotted manually using capillary tubes on pre-coated plates and developed in a glass chamber using different solvent systems namely, petroleum ether:methanol, petroleum ether:ethanol, petroleum ether:acetone, and petroleum ether:chloroform. The developed chromatograms were observed under ultraviolet light (long wavelength) and visible light, then dipped in various visualizing agents such as Dragendorff, Bornträger, potassium ferricyanide-ferric chloride, and acetic anhydride-sulfuric acid. Suitable color of the spots and zones were noted as an observable result for a positive test. The retention factor (Rf) value of the spots was calculated by dividing the distance traveled by the solute (mm) over the distance traveled by the solvent (mm).

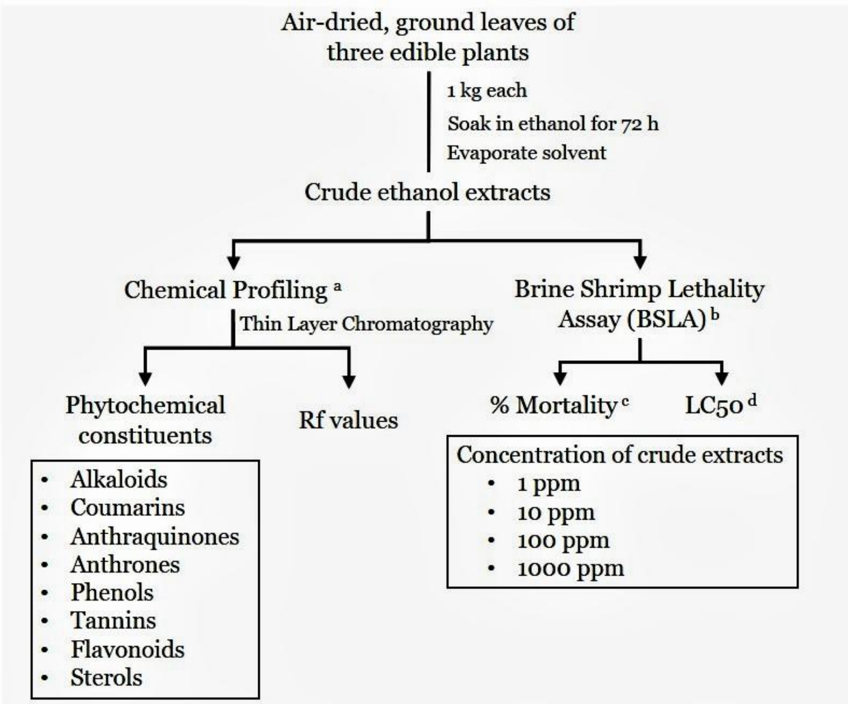

Figure 3. Schematic diagram for the tests conducted on air-dried leaves of *Ardisia iwahigensis, Baccaurea gracilis*, and *Manihot glaziovii*. Reference methods: [a] Guevara et al. 2005; [b] Sarah et al. 2017; [c] Meyer et al. 1982; [d] Mekapogu 2016.

Brine shrimp lethality assay (BSLA). This assay has been widely used to screen the toxicity of plant extracts and as a bioassay guide for cytotoxic and antitumor agents (Sarah et al. 2017). A small tank was filled with filtered and sterilized seawater, fully aerated, and divided into two compartments interconnected with holes: darkened and illuminated area. Brine shrimp eggs (*Artemia salina* L.) were placed on the darkened compartment. The hatched brine shrimp nauplii then migrated into the illuminated compartment after 48 h of incubation to reach their mature state (Figure 4A). The illumination is important to simulate the optimum temperature (30°C) of their natural habitat.

Twenty (20) mg each of crude extracts was dissolved in 3 ml of 95% ethanol to prepare 10,000 parts per million (ppm) stock solution. This was then used to prepare four concentrations - 1000 ppm, 100 ppm, 10 ppm, 1 ppm - through serial dilution (Figure 3). Triplicates were prepared for each concentration and placed in separate Eppendorf tubes (Figure 4B-D). The volume of each sample was adjusted to 5 ml by adding artificial seawater. Negative controls were used containing artificial seawater and 0.5 ml of 95% ethanol. Ten brine shrimp nauplii were collected with a pipette, added to each vial, and left uncovered under the lamp. The number of brine shrimp survivors was observed, counted, and recorded after 24 h.

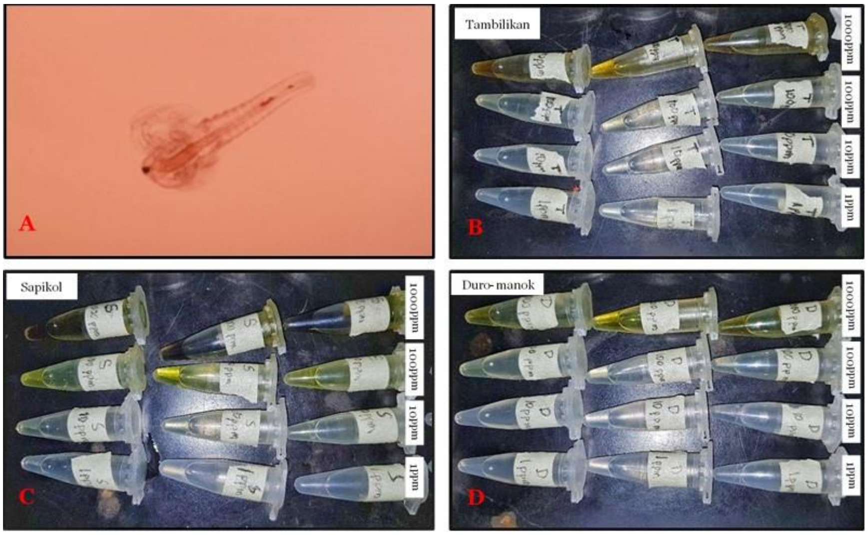

Figure 4. A: Brine shrimp under the dissecting microscope. Eppendorf tubes for Brine Shrimp Lethality Assay containing the edible plants' crude extracts in four concentrations (1 ppm, 10 ppm, 100 ppm, 1000 ppm). B: Tambilikan (*Ardisia iwahigensis*); C: Sapikol (*Baccaurea gracilis*); D: Duro-manok (*Manihot glaziovii*).

Toxicity Analysis and Testing Criteria

A calculator developed by Mekapogu (2016) based on Finney's (1952) probit analysis method was used to compute the Lethal Concentration on 50% of the population (LC_{50}). The relative toxicity of the extracts to the living organisms were then determined (Table 2). The percentage mortality was calculated by dividing the number of dead nauplii by the total number, and then multiplying by 100.

Table 2. Stephan's (1977) toxicity assessment of plant extracts.

LC_{50} (ppm or μgml^{-1}) concentration	Toxicity profile
<100 ppm	potent (active)
<1000 ppm	toxic
>1000 ppm	non-toxic

RESULTS

Phytochemical Screening

The fresh leaves of the three edible plants contained six to seven kinds of phytochemicals. Flavonoids and tannins were abundant in the fresh leaves *M. glaziovii* while *B. gracilis* was rich in saponins (Table 3).

Table 3. Phytochemical constituents present in the fresh leaves of three edible plants. Note: (+) Traces, (++) Moderate, (+++) Abundant, and (-) Absence

Constituents	*Ardisia iwahigensis*	*Baccaurea gracilis*	*Manihot glaziovii*
alkaloids	(+)	(+)	(+)
flavonoids	(+)	(+)	(+++)
glycosides	(+)	(+)	(+)
saponins	(-)	(+++)	(+)
sterols	(+)	(++)	(++)
tannins	(++)	(+)	(+++)
triterpenes	(+)	(-)	(+)

Macronutrient Content

All macronutrients tested were present in fresh leaves of the three plants with concentrations ranging from 0.125 to 7.34%. *Baccaurea gracilis* had the highest crude protein and crude fiber contents while *M. glaziovii* showed the highest crude fat content (Table 4).

Table 4. Macronutrient content in fresh leaves of the three edible plants in % weight by weight.

Macronutrient (% weight by weight)	*Ardisia iwahigensis (%)*	*Baccaurea gracilis (%)*	*Manihot glaziovii* (%)
crude protein	2.75	13.40	7.34
crude fat	0.13	0.37	0.81
crude fiber	2.79	6.65	3.27

Antibacterial Property

Among the three plant species, *A. iwagensis* had the highest MZI for all bacterial strains tested and were significantly different when compared to the positive controls. Both *B. gracilis* and *M. gracilis* had similar MZI for the bacterial strains tested (Table 5).

Antifungal Property

The three edible plants did not show antifungal property against *C. albicans* and *A. niger* (Table 6).

Chemical Profile

The air-dried leaves of the three plants showed eight phytochemicals with Rf values ranging from 0.02 to 0.96 (Table 7). Four to five phytochemicals were observed in petroleum ether:methanol solvent systems while only one phytochemical was detected in both petroleum ether:acetone and petroleum ether:chloroform (7:3) solvent systems

Level of Toxicity in Terms of LC_{50} and Percent Mortality

The LC_{50} for *A. iwahigensis* was the highest at 4.270 ppm. At this concentration, the extract caused the death of half of the nauplii population. Maximum mortality (100%) was observed in *A. iwahigensis* at 1000 ppm whereas *B. gracilis* and *M. glaziovii*, in any concentration, did not give 100% mortality. Moreover, in the negative controls used, all the nauplii survived after 24 h indicating that the mortalities were entirely caused by the crude extracts of the three edible plants (Table 8).

DISCUSSION

Phytochemical Screening and Chemical Profiling

Qualitative phytochemical screening and chemical profiling of the fresh and air-dried leaves of *A. iwahigensis, B. gracilis*, and *M. glaziovii* showed the presence of phytochemicals like alkaloids, flavonoids, sterols, and tannins. Phytochemicals determine the biological activities of plants and its significance in traditional medicine, and their presence can be associated with the potential of plant as a source of phytomedicines (Lawal et al. 2019).

Table 5. Antibacterial activity of the three edible plants against ***Staphylococcus aureus*** (SA), ***Bacillus subtilis*** (BS), ***Escherichia coli (EC)***, ***Pseudomonas aeruginosa*** (PA); MZI, mean zone of inhibition; *Oxacillin; **Amikacin. Note: When ***P*** < 0.05 – statistically significant difference (Kruskal-Wallis Test).

Bacterial strain	*Ardisia iwahigensis*		*Baccaurea gracillis*		*Manihot glaziovii*		Positive controls		***P*-value**
	MZI (mm)	Reactivity	MZI (mm)	Reactivity	MZI (mm)	Reactivity	MZI (mm)	Reactivity	
SA	21.65	4	10.00	2	10.00	2	*19.25	*4	0.0138
BS	22.58	4	13.55	2	10.00	2	*19.32	*4	0.0145
EC	19.59	3	10.00	2	10.00	2	**16.52	**4	0.0138
PA	22.27	4	10.00	2	10.00	2	**27.32	**4	0.0135

Table 6. Antifungal activity of the three edible plants against ***Candida albicans*** and ***Aspergillus niger*** . MZI, mean zone of inhibition; Reactivity rating: 3 – Moderate.

Fungal strain	*Ardisia iwahigensis*		*Baccaurea gracilis*		*Manihot glaziovii*		Positive control Oxacillin (1 µg)	
	MZI (mm)	Reactivity	MZI (mm)	Reactivity	MZI (mm)	Reactivity	MZI (mm)	Reactivity
Candida albicans	0.00	0	0.00	0	0.00	0	16.31	3
Aspergillus niger	0.00	0	0.00	0	0.00	0	19.91	3

Table 7. Chemical profile of air-dried leaves of the three edible plants. Note: PE, petroleum ether; MeOH, methanol; EtOH, ethanol; Result: (+) present; (-) absent; Rf, retention factor.

Solvent System	Reagent	Constituents	*Aridisia iwahigensis*		*Baccaurea gracilis*		*Manihot glaziovii*	
			Result	Rf values	Result	Rf values	Result	Rf values
PE:MeOH 9:1	Dragendorff	Alkaloids	(+)	0.38	(+)	0.56	(+)	0.40
	Bornträger	Coumarins	(+)	0.67	(+)	0.6	(+)	0.10
		Anthraquinones	(+)	0.57	(+)	0.46	(+)	0.54
		Anthrones	(+)	0.07	(+)	0.54	(+)	0.02
PE:MeOH 7:3	Dragendorff	Alkaloids	(+)	0.52	(+)	0.96	(+)	0.60
	Bornträger	Coumarins	(+)	0.50	(+)	0.02	(+)	0.30
		Anthraquinones	(+)	0.60	(+)	0.56	(+)	0.60
		Anthrones	(+)	0.40	(+)	0.02	(+)	0.40
PE:EtOH 7:3	Potassium ferricyanide-ferric chloride	Phenols Tannins Flavonoids	(+)	0.19	(+)	0.07	(+)	0.17
PE: Acetone 3:2	Acetic anhydride-sulfuric acid	Sterols	(+)	0.04	(+)	0.04	(+)	0.08
PE: $CHCl_3$ 9:1	Potassium ferricyanide-ferric chloride	Phenols Tannins Flavonoids	(+)	0.64	(+)	0.52	(+)	0.46
PE: $CHCl_3$ 7:3	Dragendorff	Alkaloids	(+)	0.10	(+)	0.02	(+)	0.02

Table 8. Results of brine shrimp lethality assay for the three edible plants.

Plant sample	Concentration (ppm)	Number of surviving nauplii after 24 h			Total number of survivors	Total number of dead nauplii	% Mortality	LC_{50} (ppm)
		T1	T2	T3				
Ardisia iwahigensis	1	7	6	6	19	11	37	4.270
	10	4	4	3	11	19	63	
	100	3	4	3	10	20	67	
	1000	0	0	0	0	30	100	
Baccaurea gracilis	1	8	8	9	25	5	17	48.606
	10	6	6	6	18	12	40	
	100	6	5	6	17	13	43	
	1000	1	3	1	5	25	83	
Manihot glaziovii	1	7	6	7	20	10	33	7.918
	10	5	4	4	13	17	57	
	100	6	4	3	13	17	57	
	1000	0	0	1	1	29	97	
Negative Control								
Artificial Sea Water		10	10	10	30	0	0	
0.5 mL 95% Ethanol		10	10	10	30	0	0	

Ardisia plants are used as traditional medicine in the treatment of various ailments including cancer, heart diseases, liver poisoning, diarrhea, dysmenorrhea, gout, and mental disorder (Pournami and Pratap Chandran 2021) and have been reported to exhibit antituberculosis, antioxidant, cytotoxic, and thrombolytic activities (Khatun et al. 2013). These medicinal uses and activities may be attributed to the presence of alkaloids, steroids, flavonoids, phenols, tannins, and triterpenes reported in *Ardisia* species (Khatun et al. 2013; Amin et al. 2015). These constituents were also observed in *A. iwahigensis* used in this study.

Baccaurea species are reported to display various pharmacological activities such as analgesic, anticancer, antidiarrheal, antimicrobial, and antioxidant activities which may be ascribed to the phytochemicals abundant in this genus like alkaloids, flavonoids, saponins, sterols, and tannins (Charu et al. 2021). These phytochemicals were also present in *B. gracilis* used in this study.

An earlier study on *M. glaziovii* reported that the leaves of this plant is rich in alkaloids, saponins, and tannins (Nduche et al. 2018). These phytochemicals were also observed in the present study, in addition to flavonoids, glycosides, saponins, sterols, and anthraquinones which in turn were reported in its congener, *Manihot esculenta* (Crantz) (Ebuehi et al. 2005).

Moreover, chemical profiling showed that the phytochemicals present in the three plants have wide range of polarities as indicated by their Rf values. A high Rf value indicates that the component is less polar, while a lower Rf value means the component is more polar (Bele and Khale 2011). Sterols are very polar, anthraquinones are semi-polar, while the other phytochemicals present ranges from very polar to non-polar.

Macronutrient Content

This study provides the first report on the macronutrient content of the three edible plants. The leaves of *Ardisia* species, *Ardisia solanacea* (Poir.) Roxb., was reported for its nutrient content which includes protein (31.25%), fat (0.00067%) and fiber (6.6%) and was recommended as food additive for livestock (Pratap Chandran 2015). This species has higher protein and fiber contents than *A. iwahigensis*. Conversely, there was no study found on the nutrient content of the leaves of *Baccaurea* species but the fruit of one congener, *Baccaurea sapida* (Roxb.) Müll.Arg., was found to contain protein (5.43%), fat (1.24%) and fiber (3.60%) (Pandey et al. 2018). This species has lower protein and fiber contents than *B. gracilis*. The most studied *Manihot* species in terms of nutrient content was *M. esculenta* (cassava), a staple food in many countries. Its leaves contain much higher protein (28.02%), fat (5.63%), and fiber (21.41%) (Idris et al. 2020) than *M. glaziovii*.

In addition to their macronutrient nutrient, the phytochemicals present in these plants can provide both pharmacological and health benefits when consumed as food (Demir and Akpinar 2020). For instance, the phytochemicals present in *M. esculenta* are attributed to the use of this plant in the treatment of various ailments like allergies, bone problems, celiac disease and diabetes (Zekarias et al. 2019). *Baccaurea angulata* (Merr.) was also reported as a potential functional food and exhibits effective anti-inflammatory, antioxidant, and cholesterol-lowering activities (Erwin et al. 2018).

Antibacterial and Antifungal Properties

This study provides the first report on antimicrobial test conducted on the three edible plants. *Ardisia iwahigensis* exhibited significant antibacterial property with higher MZI than the standards used, more so than the other two plants, against *S. aureus, B. subtilis* and *E. coli*. This result is of great value particularly in the case of *S. aureus*, which is known for its resistance to several antibiotics and for its ability to cause septicemia by secreting various types of enterotoxins (Al-abd et al. 2017). This antibacterial activity shown by *A. iwahigensis* infers that it could be explored as source of bioactive compounds that can be utilized against bacterial resistance. Another congener, *Ardisia elliptica* Thunb., also showed significant antibacterial activity against *S. aureus* and *E. coli*, among others. The antibacterial property could be attributed to the flavonoids and phenolics present in the leaf extracts (Al-abd et al. 2017).

The three edible plants did not exhibit antifungal property. This observed inactivity may be due to ochratoxin, a mycotoxin produced by *Aspergillus* spp. and other fungi which confers resistance to antifungal drugs. In addition, *C. albicans* is known to exhibit resistance against most antimicrobial drugs (Adeonipekun et al. 2014). However, one species of *Ardisia*, *A. solanacea*, was reported to be effective against *C. albicans* and *A. niger* (Amin et al. 2015). One species of *Manihot*, *Manihot multifida* (L.) Crantz, was also reported effective against *C. albicans* (Fabri et al. 2014). However, no report was found on the leaves *Baccaurea* species being effective against the two fungal strains used.

Toxicity

A previous study on the leaves and twigs of *A. iwahigensis* reported that it exhibited ≥50% immobilization of brine shrimp at 100 ppm and displayed an IC_{50} ≤ 20 ppm against various cancer cell lines (Horgen et al. 2001). This toxicity of *A. iwahigensis* against brine shrimp is in agreement with the results obtained from the present study. The leaves of another *Ardisia* species, *Ardisia humilis* Vahl., also displayed toxicity (LC_{50} at 2.26 ppm) against brine shrimp (Khatun 2013). These toxicity results support the traditional use of *Ardisia* plants in the treatment of cancer (Horgen et al. 2001).

As per the findings of this study, the leaves of *A. iwahigensis, B. gracilis, and M. glaziovii* are strong sources of different phytochemicals that may have essential biological activities, in addition to their use as food or food ingredient. Moreover, *A. iwahigensis* exhibited significant inhibitory property against gram-positive (*S. aureus* and *B. subtilis*) and gram-negative (*E. coli* and *P. aeruginosa*) bacteria comparable with commercial antibiotics and it also displayed high mortality against brine shrimp nauplii which may imply that it contains bioactive components that may be utilized as antibacterial and cytotoxic agents.

It is recommended that future studies be conducted to isolate the components responsible for the observed antibacterial and toxic properties in *A. iwahigensis*. Further toxicity tests should also be conducted on the three plants to ascertain their safety when taken orally, either as food or as medicine.

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