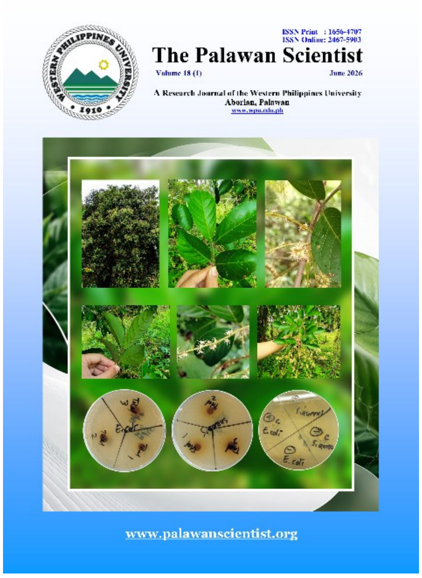

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Evaluation of the antioxidant and phytochemical analysis of Tabon-tabon (*Atuna racemosa* Raf) methanol fruit extract

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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.

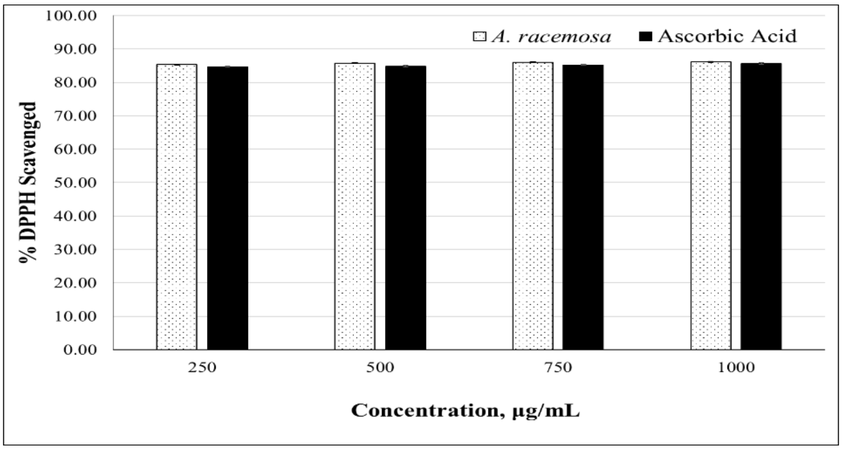

Figure 2. DPPH scavenging assay of *Atuna racemosa* Raf methanol fruit extract. Both *A. racemosa* and ascorbic acid show high percentages of DPPH scavenging across all concentrations tested (around 85-90%), indicating strong antioxidant activity. Each value represents mean ± standard deviation. Means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

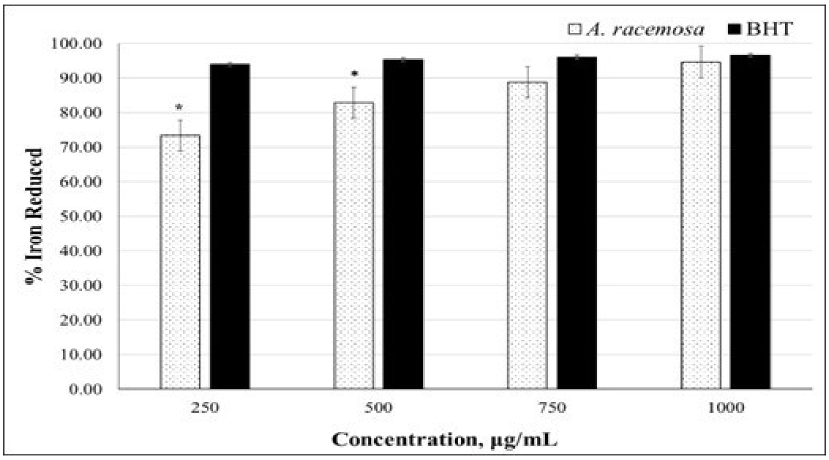

Figure 3. Reducing Power of *Atuna racemosa* Raf methanol fruit extract. *A. racemosa* methanol extract showed strong reducing power iron reduction comparable to butylated hydroxytoluene (BHT) at 1000 *µ*g/mL and maintained approximately 70% reducing activity at 250 *µ*g/mL. Each value represents mean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

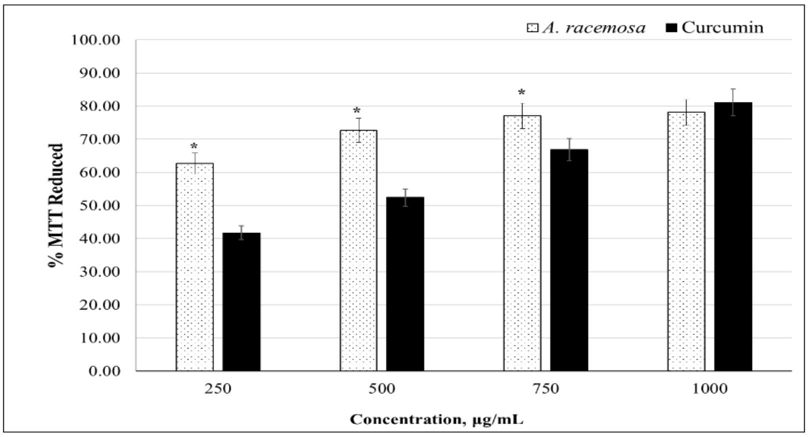

Figure 4. MTT Reducing Activity of *Atuna racemosa* Raf methanol fruit extract. *A. racemosa* methanol extract showed higher MTT-reducing activity than curcumin at 250 *µ*g/mL and maintained good reduction across concentrations. Each value represents mean ± standard deviation. Means were compared against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

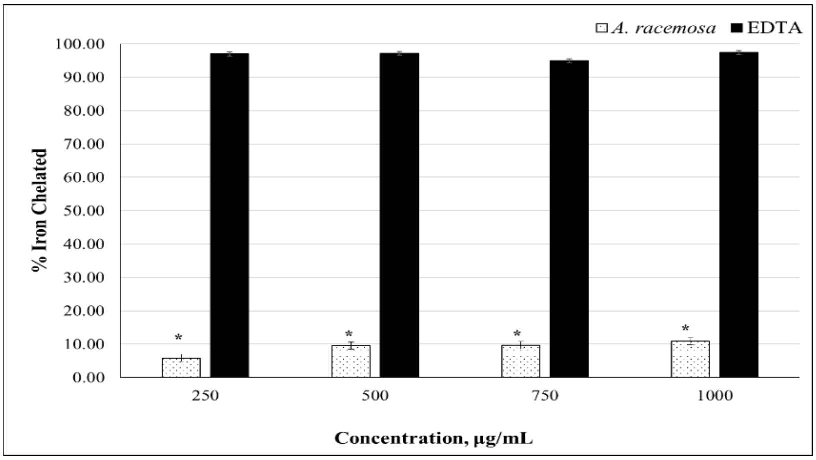

Figure 5. Metal chelating Activity of *Atuna racemosa* Raf methanol fruit extract. Significantly low iron chelating activity of less than 20% was demonstrated by *A. racemosa* compared to EDTA ($P < 0.05$). Each value represents mean ± standard deviation. Means were compared against the crude extract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

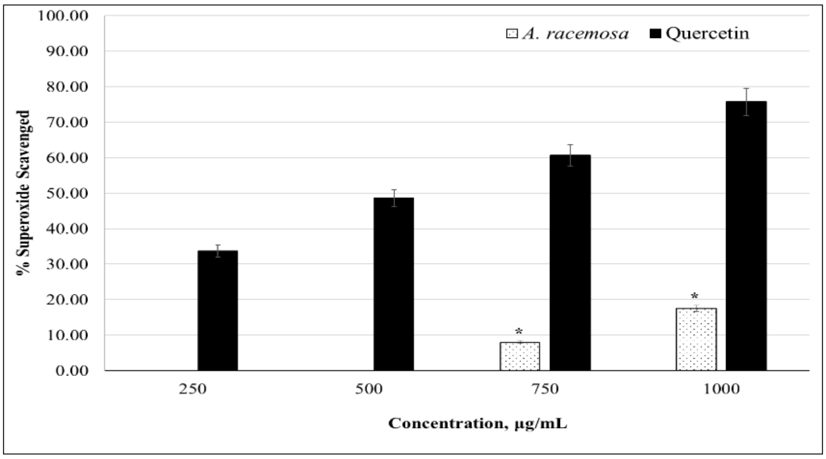

Figure 6. Superoxide radical scavenging activity of *Atuna racemosa* Raf methanol fruit extract. *A. racemosa* methanol extract displayed weak radical scavenging (<20% at 1000 *µ*g/mL) than that of quercetin which exhibited stronger activity. Each value represents mean ± standard deviation. Means were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

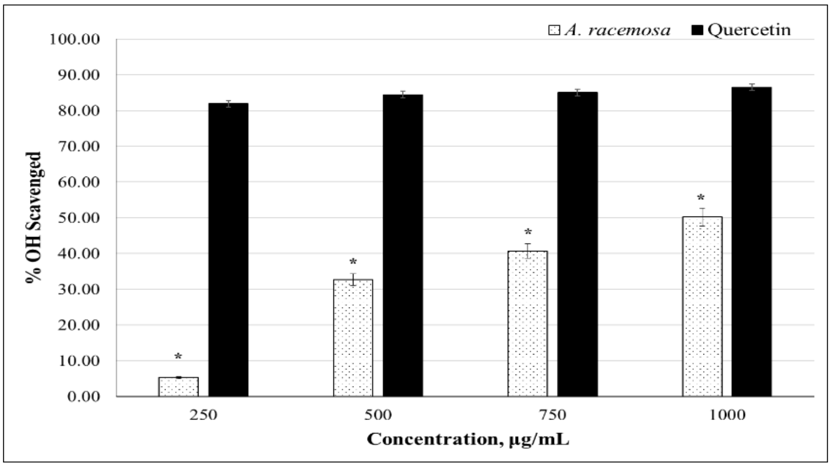

Figure 7. Hydroxyl radical scavenging assay of *Atuna racemosa* Raf methanol fruit extract. A. *racemosa* extract demonstrated increasing hydroxyl radical scavenging activity but has lower activity than quercetin across all concentrations tested. Each value represents mean ± standard deviation. Means 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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GENERATIVE AI STATEMENT

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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