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Morphology, Phenolic Content, Flavonoid Content, and Antioxidant Activity of *Meistera muricarpa* (Elmer) Škorničk. & M.F. Newman (Zingiberaceae)

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ABSTRACT: For centuries, plants have served as a primary source of phytonutrients, and recently, medicinal plants have gained prominence in medicine and healthcare due to their mild effects. This study aimed to investigate the gross morphological characteristics of *Meistera muricarpa* and determine its total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant activity (TAA) of the ethanolic leaf and fruit extracts. The samples were extracted using absolute ethanol, and the TPC, TFC, and TAA were determined using the Folin-Ciocalteu, Aluminum Chloride, and Phosphomolybdenum methods, respectively. Results revealed that *M. muricarpa* exhibits bright yellow flowers, orange-red tinged indehiscent echinate fruits with dense prickles. The fruits obtained higher TPC and TAA than the leaves. Conversely, the leaves obtained higher TFC than the fruits. Specifically, the fruits had 6.99 ± 0.19 mg GAE/g sample of phenolics, whereas the leaves had 2.45 ± 0.04 mg GAE/g sample. The leaves yielded 5.11 ± 0.06 mg QE/g sample of flavonoids, exceeding the fruits with 0.29 ± 0.01 mg QE/g sample. Furthermore, the fruits exhibited higher antioxidant activity with 21.13 ± 0.87 mg AAE/g sample than the leaves with 8.28 ± 0.16 mg AAE/g sample. A strong positive linear correlation (r=0.99, p<0.001) was observed among the TPC, TFC, and TAA. These findings suggest that the leaves and fruits of *M. muricarpa* could be a valuable source of natural antioxidants.

Keywords: aluminum chloride colorimetric method, CEDAR, gingers, folin-Ciocalteu method, Philippine endemic, phosphomolybdenum method.

INTRODUCTION

Phenolics are secondary metabolites in plants (Alara *et al*., 2021). These plant metabolites are essential in the physiological defense response of the body, such as anti-aging, anti-inflammatory, antioxidant, and anti-proliferative activities (Lin *et al*. 2016). One of the many observed compounds in plant phenolics is antioxidants (Sroka, 2005). Antioxidants are substances that delay or prevent oxidation, while antioxidant activity is the reaction of antioxidants and oxidants at a constant rate (Santos-Sanchez *et al*., 2019).

Zingiberaceae are flowering plants common in the tropics and subtropical parts of the mountains (Panchareon *et al*., 2000). This family consists of at least 53 genera and over 1200 species (Kress *et al*., 2002). *Meistera* is one of the genera of Zingiberaceae that formerly shared with the genus *Amomum* Roxb. After the recent morphological revision of de Boer *et al*. (2018), it was established as a distinct genus and was reinstated. *Meistera muricarpa* (Elmer) Škorničk and M.F. Newman is a Philippine endemic species and was recorded in Mt. Hamiguitan Range Wildlife Sanctuary in Davao Oriental (Acero *et al*., 2019), Cinchona Forest Reserve in Bukidnon (Jayme *et al*. 2019), and Mt. Musuan in Bukidnon (Mendez *et al*., 2023a). This species is distinct from other species of *Meistera* by having a spatulate labellum and echinate fruits (Acero *et al*., 2019).

In ethnomedicinal practices in Bukidnon, the seeds from the ripe fruits of this species are consumed by the locals as it is affirmed to cure stomach-related disorders (Acma, 2010). The qualitative phytochemical screening of leaves, rhizomes, and fruits of *M. muricarpa* revealed the presence of various phytochemical compounds, including alkaloids, carbohydrates, glycosides, fixed oils, fats, saponins, tannins, and proteins (Acma 2013). However, the quantitative phytochemical tests of this species have not been investigated, making this paper the first report on the TPC, TFC, and TAA, which can be used for future applications of this species. Thus, this study was conducted to investigate the gross morphological characteristics of *M. muricarpa* and determine its total phenolic content (TPC), total flavonoid content (TFC),

and total antioxidant activity (TAA) of the ethanolic leaf and fruit extracts.

MATERIAL AND METHODS

Entry Protocol. Before conducting the study, the necessary permits and approvals were secured. A formal letter was personally submitted to the office of the Municipal Mayor of Impasug-ong, while a prior informed consent (PIC) was obtained from the Barangay Captain of Barangay Impalutao, Municipality of Impasug-ong. Additionally, a Wildlife Gratuitous Permit (WGP) with permit number R10-2024-14 was obtained from the Department of Environment and Natural Resources (DENR) - Region X, located in Cagayan de Oro City, Misamis Oriental. This permit is a requirement for researching wildlife, as outlined in the DENR Administrative Order No. 2004-55, which streamlines the procedures for wildlife research and conservation

Place and Duration of the Study. The leaf and fruit samples and voucher specimens of *M. muricarpa* were collected from the Center for Ecological Development and Recreation (CEDAR), Impalutao, Impasug-ong, Bukidnon, Philippines (8.254137ºN, 125.036881ºE) (Fig. 1). The morphological dissection, measurements, and description of *M. muricarpa* were done in the field. The samples for laboratory analyses and voucher specimens were transported to Central Mindanao University for processing. The analyses of TPC, TFC, and TAA were carried out at the Natural Science Laboratory of the Natural Science Research Center (NSRC), Central Mindanao University, Musuan, Bukidnon, from December 2023 to April 2024.

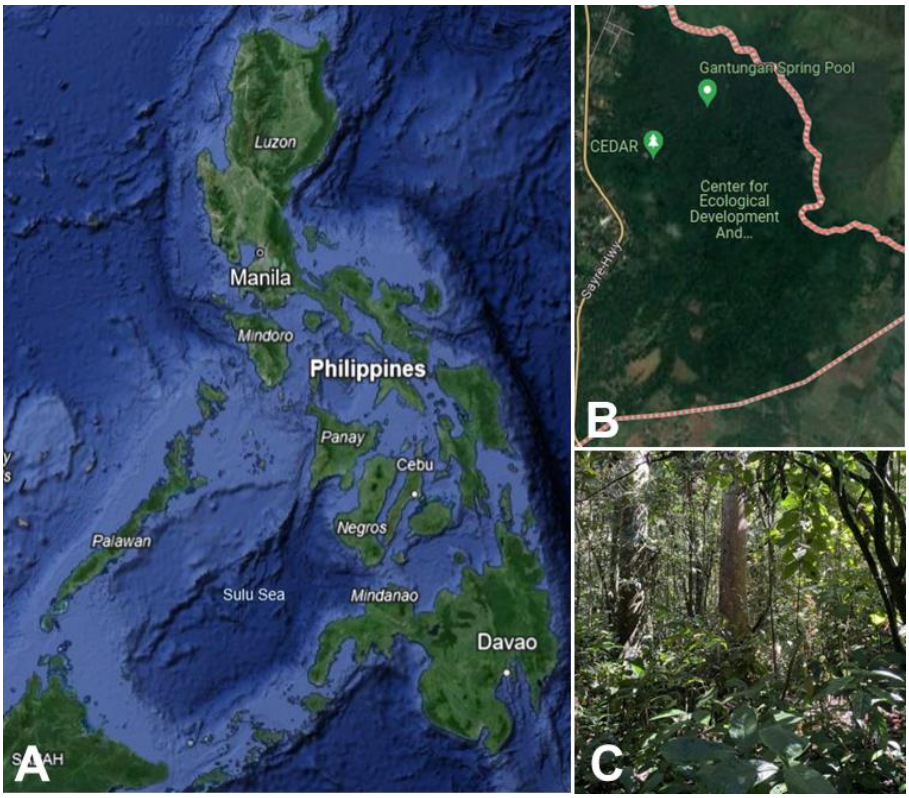

Fig. 1. Study site. A) Map of the Philippines; B) Map of CEDAR (red circle indicates the location of species; C) Habitat of *M. muricarpa* (A & B: ©2024 Google Earth).

Sample Preparation and Extraction. To prevent dehydration, the collected leaf and fruit samples were placed in plastic cellophane bags with moist tissue paper. The samples were then washed to remove any soil or debris and air-dried at room temperature (25°C) for 14 days. The dried samples were subsequently homogenized using a household blender and stored in zip-lock cellophane bags until further analysis. Ethanol extracts were prepared using a modified version of the method described by Padda and Picha (2008). Specifically, 1 g of dried leaf or fruit powder was extracted with 25.0 mL of absolute ethanol at room temperature (25°C ± 2°C). The mixtures were shaken at 300 rpm for 1 hour using an orbital shaker, followed by centrifugation at 5,000 rpm for 5 minutes. The resulting extracts were collected in 15 mL conical tubes and stored at 2–8°C.

The stored ethanolic leaf and fruit extracts were used to determine the total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant activity (TAA) using 96-well plate format colorimetric assays.

Determination of Total Phenolic Content. The total phenolic content (TPC) of the plant extracts was determined using a modified version of the Folin-Ciocalteu assay described by Ainsworth and Gillespie (2007). The assay involved mixing 200 µL of plant extract with 200 µL of 10% Folin-Ciocalteu reagent in a 2-mL centrifuge tube. After a 5-minute incubation, 800 µL of 10% sodium carbonate was added, and the mixture was incubated for an additional 30 minutes at room temperature. The mixture was then centrifuged at 11,000 rpm for 3 minutes, and 200 µL of the resulting solution was transferred to a microplate well. The absorbance was measured at 750 nm using a microplate

reader. A standard calibration curve was prepared using gallic acid (GA) standards with concentrations ranging from 0 to 100 ppm, in increments of 10 ppm, from a 100 ppm GA stock solution in absolute alcohol. The TPC was calculated and expressed as milligram gallic acid equivalent per gram dried sample (mg GAE/g sample) by comparing the sample absorbance to the standard calibration curve using the following formula:

$$\text{Total Phenolic Content} = \left(\frac{\text{mg GAE}}{\text{g dried sample}} \right) = \frac{A}{B} \qquad (1)$$

Where A = gallic acid concentration of the sample solution determined from the calibration curve (mg GAE/L)
B = concentration of test solution (g/L, gram dried sample per L solution)

Determination of Total Flavonoid Content. The TFC was assessed using the aluminum chloride colorimetric method, as described by Nurcholis *et al.* (2021). Briefly, 50 µL of plant extract was added to a 96-well plate, followed by the addition of 10 µL of 10% aluminum chloride, 130 µL of 96% ethanol, and 10 µL of 1M sodium acetate. The mixture was incubated in the dark for 40 minutes at room temperature. The absorbance was measured at 415 nm using a microplate reader. The TFC was expressed as milligram quercetin equivalent per gram dried sample (mg QE/g dried sample) by comparing the sample absorbance to a standard calibration curve. The TFC was calculated using the following formula:

$$\text{Total Flavonoid Content} = \left(\frac{\text{mg QE}}{\text{g dried sample}} \right) = \frac{A}{B} \qquad (2)$$

where A = quercetin concentration of the sample solution determined from the calibration curve (mg QE/L)
B = concentration of test solution (g/L, gram dried sample per L solution)

Determination of Total Antioxidant Activity. The TAA was assessed using the phosphomolybdenum method described by Prieto *et al.* (1999), with minor modifications to the reagent solution volume. Briefly, 50 µL of the extracts were diluted with 200 µL of a 1:1 ethanol-water solution in centrifuge tubes. Then, 600 µL of the reagent solution, comprising equal parts of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate, was added. The mixture was incubated at 95°C for 1 hour and 30 minutes and cooled to room temperature (25°C). After centrifugation at 11,000 rpm for 3 minutes, the absorbance of the supernatant was measured at 695 nm against a blank using a microplate reader. A standard calibration curve was generated using ascorbic acid (AA) standards with concentrations ranging from 0 to 150 ppm, in increments of 15 ppm, prepared from a 300 ppm AA stock solution in absolute alcohol. The TAA was determined by comparing the sample absorbance to the standard calibration curve. The TAA was calculated using the following equation:

$$\text{Total Antioxidant Activity} = \left(\frac{\text{mg AAE}}{\text{g dried sample}} \right) = \frac{A}{B} \qquad (3)$$

where A = ascorbic acid concentration of the sample solution determined from the calibration curve (mg AAE/L)
B = concentration of test solution (g/L, gram dried sample per L solution)

Statistical Analysis. All TPC, TFC, and TAA analyses were performed in triplicate, with three trials conducted per replicate to ensure reliability and accuracy. The data obtained from the leaf and fruit samples of the species were then subjected to statistical analysis. A t-test was performed at a 0.001 level of significance to determine significant differences between the means of the two groups. Additionally, Pearson's correlation analysis was conducted at a 0.001 level of significance to examine the relationships between TPC, TFC, and TAA. This comprehensive statistical approach allowed for a thorough understanding of the differences and correlations between the phytochemical parameters of the leaf and fruit samples.

RESULTS AND DISCUSSION

Plant Description. Terrestrial perennial herb, arching, reaching as high as 2–2.5 m tall. *Stilt roots* absent. *Rhizomes* below ground, pinkish, sometimes deep red, 2.4–2.8 × 3.6–3.9 cm diam., brownish. Leaves subsessile, distichous, oblong to lanceolate, 24–41 × 5.5–5.9 cm, adaxially dark green and glabrous, abaxially pale green and glabrous; *midrib* adaxially greenish to yellowish, continuous, abaxially greenish to yellowish, glabrous; *margin* entire, wavy, brownish; *base* acute; *apex* acuminate, slightly recurved. *Leaf sheath* green with prominent hairs, grooved, green. Leaf *internodes* 5 cm distance from each other, glabrous, green. *Petiole* short, green, 0.9–1.2 cm, pubescent. *Ligule* greenish, pubescent, 0.6–0.8 × 0.7 cm, yellowish to green. *Swollen base* brownish, glabrous, 7–7.2 cm. *Inflorescence* short, lateral, bearing 1–3 flowers, 7.5 × 1.5–2 cm. *Bracts* delicate, ovate with acute apex, reddish, margin entire, apex acute, 0.8 × 0.3 cm, glabrous. *Bracteoles* tubular, pale red, acute apex, 2 × 0.3 cm, glabrous. *Calyx* tubular, fused, pinkish in color, 2–2.5 × 0.3–0.5 cm wide, glabrous. *Flower* 6 × 2.5 cm. *Lateral corolla lobes* oblong to lanceolate, glabrous, trilobed, pinkish to red, with visible veins, entire margin, rounded apex, rounded apex, dorsal lobe pale red, 1.5 cm × 0.2–0.3 cm. *Dorsal corolla lobe* reddish, rounded apex, 2.0–2.5 × 0.3–0.4 cm. *Labellum* spatulate, frilly, lower lobes tucked, stamen, glabrous, bright yellow, orange-red tinge, entire margin, acute apex, 3.5–4.0 × 0.2–0.3 cm. *Style* white, 1.7 × 1–2 cm; *anther sac* 0.5–0.7 cm long × 1–2 cm wide. *Fruit* capsule, indehiscent with dense prickles, echinate. *Seeds* globose, creamy white when young, black when mature, and white, transparent aril, 0.1–0.2 cm (Fig. 2).

Local name and Uses: Locally called “tugis” (Govaerts, 2005). The seeds are edible and used as a relief for stomach illnesses (Acma, 2010).

Distribution: LUZON: Bataan, Mariveles. Isabela and Quezon. MINDANAO: Bukidnon, Cotabato, Davao Oriental, Davao del Sur, Misamis Oriental, and Zamboanga. Endemic to the Philippines (Pelser *et al.*, 2011 onwards).

Habitat and Ecology: *Meistera muricarpa* is found in the Mindanao mountains, including Mt. Musuan

(Mendez *et al.* 2023a), Cinchona Forest Reserve (Jayme *et al*., 2020), and Mt. Hamiguitan Expansion Site (Acero *et al*., 2019). This species is mostly found in wet tropics (Govaerts, 2005), lowland, and montane evergreen forests at elevations of 216–1112 masl (Laxmay & Newman 2012).

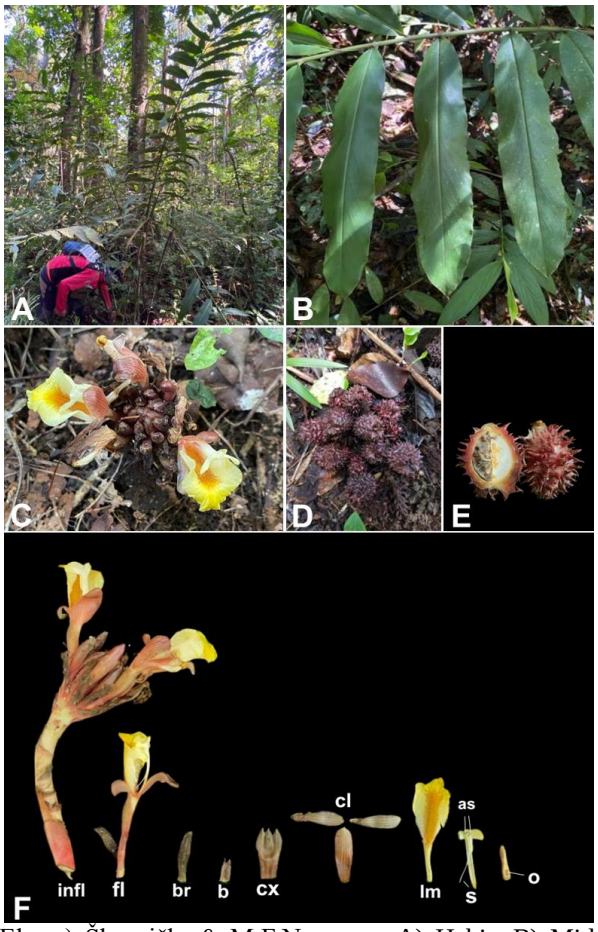

Fig. 2. *Meistera muricarpa* (Elmer) Škorničk. & M.F.Newman. A) Habit, B) Mid foliage, C) Inflorescence, D) Infructescence, E) Longitudinal section of the fruit, F) Floral dissection: infl–inflorescence; fl–flower; br–bracts; b–bracteoles; cx–calyx; cl–corolla lobes; lm–labellum; s–stamen; as–anther sacs; o–ovary (Scale bar: 1 cm).

Total Phenolic Content. The TPC of the ethanolic extracts of leaves and fruits of *M. muricarpa* is presented in Table 1. The results were derived from a calibration curve (equation: $y = 0.0671x + 0.0267$; $R^2 = 0.9951$) of gallic acid (0–200 mg/mL). The TPC of the leaves of *M. muricarpa* in this is study with 2.45 ± 0.04 mg GAE/g sample is lower compared to *Etlingera fimbriobracteata* (K.Schum) R.M.Sm. with 13.20 ± 0.35 mg GAE/g sample and *E. philippinensis* (Ridl.) R.M.Sm. with 7.21 ± 0.33 mg GAE/g sample (Mendez *et al*., 2023b), and *E. dostseiana* Naive, Demayo & Alejandro with 15.44 ± 0.80 mg GAE/g sample (Mendez *et al*., 2023c).

Table 1: Mean TPC of the ethanolic extract of the leaves and fruits of *M. muricarpa.*

Species	Plant parts (mg GAE/g sample)	
	Leaves	Fruits
Meistera muricarpa	2.45 ± 0.04	6.99 ± 0.19

In plant parts, fruits exhibit a good source of compounds with phenolic relevance, such as phenols, lignins, lignans, coumarins, tannins, phenolic acids, and flavonoids (Xu *et al*. 2017). These phenolics are also called secondary plant metabolites. The group of compounds with single or more aromatic rings that are attached to hydroxyl groups with more than 8000 known structures (Balasundram *et al*., 2006). These compounds hold a significant role in protecting plants from ultraviolet (UV) rays, and diseases, as well as deterring parasites, insects, and predators (Caleja *et al*. 2017; Durazzo *et al*. 2019).

The fruit of *M. muricarpa* has been traditionally used to alleviate stomachaches, and locals often consume the ripe seeds from its fruits (Acma 2010). Previous studies have reported that *M. muricarpa* exhibits high radical scavenging activity in its rhizomes (Barbosa *et al*., 2016). The rhizomes of ginger species are known to accumulate high levels of antioxidants and secondary metabolites compared to other plant parts (Jitoe *et al*., 1992).

Phytochemical analysis of *M. muricarpa* has revealed the presence of various bioactive compounds, including phenolic compounds such as alkaloids, flavonoids, saponins, and tannins, as well as non-phenolic

compounds like steroids, in its leaves and rhizomes (Barbosa *et al*. 2016). Fruits are known to be rich in phenolic compounds, particularly flavonoids, which are responsible for their yellow, red, and blue colors (Belitz *et al.*, 2009; Lampila *et al*., 2009). To date, over 5,000 flavonoids have been identified, and these compounds play essential roles in protecting plants against pathogens and excessive UV light (Winkel-Shirley, 2002).

Hence, the high phenolic content of the fruits of *M. muricarpa* might be due to flavonoids as well as flavonoid derivatives, anthocyanins, which are mainly responsible for the pigment of fruits, pollination, and absorption of light as well as guard plants from the damage of UV rays (Castañeda-Ovando *et al*., 2009; Ahmed *et al*., 2016). Other factors that might contribute to the high phenolic content of *M. muricarpa* are the degree of ripeness, variety, climate, soil composition, geographic location, and storage conditions (Belitz *et al*., 2009).

Total Flavonoid Content. The TFC of the ethanolic extracts of leaves and fruits of *M. muricarpa* are presented in Table 2. The results were derived from the calibration curve (equation: y=0.0394–0.003; R^2 0.9996) of quercetin (0–300 mg/mL).

Table 2: Mean total flavonoid content (TFC) of the ethanolic extract of the leaves and fruits of *M. muricarpa.*

Species	Plant parts (mg QE/g sample)	
	Leaves	Fruits
Meistera muricarpa	5.11 ± 0.05	0.29 ± 0.01

Flavonoids are a diverse group of secondary metabolites that are ubiquitous in plants, fruits, and seeds. They are responsible for the biological colors, fragrances, and flavors of plants, and play crucial physiological roles in regulating cell growth, attracting pollinators, and protecting plants from biotic and abiotic stresses (De Luna *et al*., 2020). Additionally, flavonoids function as signal molecules, ultraviolet filters, and scavengers of reactive oxygen species (Di Ferdinando *et al*., 2011; Panche *et al*., 2016; Dias *et al*., 2021).

Flavonoids are distributed throughout plant tissues, both within cells and on the surfaces of various plant organs (Ferreira *et al*., 2006). The concentration of bioactive compounds, including flavonoids, is typically higher in sunlight-exposed plant parts, such as leaves, due to photosynthesis (Kumar *et al*., 2019). This may explain why the leaves of *M. muricarpa* contain higher.

The amount of bioactive compounds in fruits can be influenced by various factors, including the degree of ripeness, variety, climate, soil composition, geographic location, and storage conditions (Belitz *et al*., 2009). These factors can contribute to variations in the phytochemical composition of fruits, highlighting the importance of considering these factors when evaluating the nutritional and medicinal properties of plant-based materials.

Total Antioxidant Activity. The total antioxidant activity of the ethanolic extracts of leaves and fruits of *M. muricarpa* is presented in Table 3. The results were derived from the calibration curve (equation: y=0.0164–0.0092; R^2 = 0.9996) of ascorbic acid (0–150 mg/mL). The TAA of the leaves of *M. muricarpa* in this is study with 8.28 ± 0.15 mg AAE/g sample is lower compared to *E. fimbriobracteata* with 12.69 ± 0.36 mg AAE/g sample and *E. philippinensis* with 7.22 ± 0.26 mg AAE/g sample (Mendez *et al*., 2023b) and *E. dostseiana* with 14.24 ± 0.25 mg AAE/g sample (Mendez *et al*., 2023c).

Table 3: Mean TAA of the ethanolic extract of the leaves and fruits of *M. muricarpa.*

Species	Plant parts (mg AAE/g sample)	
	Leaves	Fruits
Meistera muricarpa	8.28 ± 0.15	21.13 ± 0.87

Antioxidants, as molecules, possess the ability to neutralize the activity of free radicals. Their action involves the inactivation of these harmful molecules (Halliwell, 1996; Devasagayam *et al*., 2004). There are a variety of secondary metabolites produced by plants through the normal metabolic pathways. These secondary plant metabolites include flavonoids, essential oils, alkaloids, lignans, terpenes, terpenoids, tocopherols, phenolic acids, peptides, and polyfunctional organic acids (Shahidi and Ambigaipalan 2015). Among these various compounds, phenolic acids are considered the primary natural sources of antioxidants (Chanwitheesuk *et al*., 2005; Maisuthisakul *et al*., 2007).

The high antioxidant activity of the fruits of *M. muricarpa* may also be due to its contained flavonoids (Barbosa *et al*., 2016) that are recognized as natural antioxidants and other bioactive compounds. These exhibited the high antioxidant activity of fruits agree with the antioxidant activity of other *Meistera* species. *M. chinensis* ethanolic fruit extracts in the DPPH assay recorded 47.62 ± 2.93 mg/L in IC50, which suggests a high potency of antioxidants in fruits (Musdalipah *et al*., 2021). Hence, *M. muricarpa* is also a great source of natural antioxidants, and this study provides additional new scientific knowledge about the plant's potential.

Correlation of Total Phenolic Content, Total Flavonoid Content, and Total Antioxidant Activity. The relationship between TPC, TFC, and TAA of *M. muricarpa* leaves and fruits was assessed using Pearson's Correlation analysis. This statistical method measures the strength and direction of linear correlations, with correlation coefficients (r) ranging

from -1 to 1. A correlation coefficient close to 1 or -1 indicates a strong positive or negative linear relationship, respectively, while a value near zero suggests no significant linear correlation (Table 4).

Table 4: Results of the correlation analysis between the TPC, TFC, and TAA of the ethanolic leaf and fruit extracts of *M. muricarpa*.

ASSAY	Total Phenolic Content	Total Flavonoid Content	Total Antioxidant Activity
Total Phenolic Content	1		
Total Flavonoid Content	0.9996**	1	
Total Antioxidant Activity	0.9982**	0.9996**	1

A strong positive linear correlation was observed between total phenolic content (TPC) and total flavonoid content (TFC) ($r=0.99$, $p<0.001$), TPC and total antioxidant activity (TAA) ($r=0.99$, $p<0.001$), and TAA and TFC ($r=0.99$, $p<0.001$). These findings suggest that plant parts with higher phenolic content exhibit significantly greater flavonoid and antioxidant activities. This implies that the phenolic compounds in *M. muricarpa* fruits are primarily responsible for their high antioxidant activity.

The presence of polyphenols in plant extracts is likely a major contributor to their antioxidant properties. The structure of phenolic compounds plays a crucial role in determining their antioxidant activity, particularly the electron delocalization over an aromatic nucleus (Jing *et al*. 2010). When phenolic compounds react with free radicals, the gained electron is delocalized over the phenolic antioxidant, stabilized by the resonance effect of the aromatic nucleus, thereby blocking the continuation of the free radical chain reaction (Tsao and Deng 2004).

CONCLUSIONS

This study revealed that M. muricarpa is characterized by its bright yellow flowers, orange-red tinged indehiscent echinate capsule fruits, and dense prickles. Phytochemical analyses showed that the fruits exhibited higher total phenolic content (TPC) and total antioxidant activity (TAA) compared to the leaves. Conversely, the leaves had higher total flavonoid content (TFC) than the fruits. Specifically, the fruits contained 6.99 ± 0.19 mg GAE/g dry weight sample of phenolics, surpassing the leaves with 2.45 ± 0.04 mg GAE/g dry weight sample. The leaves yielded 5.11 ± 0.06 mg QE/g dry weight sample of flavonoids, exceeding the fruits with 0.29 ± 0.01 mg QE/g dry weight sample. Furthermore, the fruits demonstrated higher antioxidant activity with 21.13 ± 0.87 mg AAE/g dry weight sample, compared to the leaves with 8.28 ± 0.16 mg AAE/g dry weight sample.

FUTURE SCOPE

This study suggests that the leaves and fruits of *M. muricarpa* could serve as a valuable source of natural antioxidants, offering potential applications in the development of novel antioxidant agents. There is also a need to explore quantitative phytochemical screening for other Zingiberaceae species in the Philippines that have been reported and used for ethnomedicine.

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