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Phytochemical content, thin layer chromatographic profile, and pharmacologic activities of the Philippine native *Melastoma malabathricum* Linn. from Benguet

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ABSTRACT

Melastoma malabathricum Linn. is a traditional medicinal plant used to treat wounds, infections, and diabetes. We investigated the phytochemical content, thin–layer chromatography (TLC) profile, and pharmacological properties of *M. malabathricum.* An ethanolic extract of *M. malabathricum* was prepared and subjected to phytochemical screening and analysis of the total phenolic content (TPC) and total flavonoid content (TFC). TLC was performed to characterize the constituents. Antioxidant activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Antibacterial activities against antibiotic-resistant bacterial pathogens (*Staphylococcus aureus*, *Enterobacter cloacae, Enterococcus faecium, and Klebsiella pneumoniae*) were determined using a resazurin-based assay. Hypoglycemic activity was determined using the alpha-glucosidase inhibition assay. Phytochemical analysis revealed the presence of tannins, polyphenols, flavonoids, triterpenoids, and saponins. TPC was 298.68±7.79 mg GAE/g, and TFC was 37.34±1.87 mg QE/g. The ethanolic extract inhibited DPPH free radical formation by 82.59% (IC_{50}, 57.98±2.80 µg/mL) and was active against the gram-positive pathogens *E. faecium* and MRSA (MICs, 4 mg/mL and 8 mg/mL, respectively). In addition, the extract inhibited alpha-glucosidase activity more potently (99.35%) than the positive control, acarbose (93.57% inhibition). Overall, this study demonstrates *M. malabathricum* contains bioactive compounds with antioxidant, antibacterial, and potent hypoglycemic activities. These pharmacologic activities support the traditional use of this plant in the management of infections and diabetes.

Keywords: *Melastoma malabathricum*; antioxidants; antibacterial; alpha-glucosidase; hypoglycemic; phytochemicals

1. INTRODUCTION

Melastomataceae is an angiosperm family of 4200–4500 species in approximately 166 genera that exhibit a pantropical distribution (Renner, 1993). The genus *Melastoma* is widely distributed in tropical and subtropical countries, such as Southeast Asia (Diris et al., 2017), and is commonly mentioned in many traditional medicinal approaches for treating various conditions (Zheng et al., 2021). Samad et al. (2018) reviewed the ethnobotanical, phytochemical, and pharmacological properties of the genus *Melastoma. M. malabathricum*, an evergreen flowering shrub, is one of the most studied species in this genus due to its medicinal properties and therapeutic potential (Diris et al., 2017). In their review, Joffry et al. (2012) stated that *M. malabathricum* is considered as an herbal medicine in Malay culture as well as in Indian, Chinese, and Indonesian traditional medicine. Consumption of the shoots of the plant has been reported as a traditional remedy for the treatment of diabetes, high blood pressure, and puerperal infections (Joffry et al., 2012). Other traditional uses of this plant include treatments for ulcers and wounds, infections, toothache, diarrhea, dysentery, hemorrhoids, flatulence, and inflammation (Wong et al., 2012; Zheng et al., 2021).

M. malabathricum species is also native to the Philippines, occurring naturally in the Cordillera administrative region (CAR) and in the provinces of Aurora, Cagayan, Ilocos Norte, Ilocos Sur, Isabela, and Nueva Vizcaya. In Cordillera, the plant is known as *bakgi* (Elmido et al., 2019). Several review papers and researchers have delved into the biological activities of *M. malabathricum.* A review by Zheng et al. (2021) stated that *Melastoma* species possess anti-inflammatory, anticoagulative, cytotoxic, anti-bacterial, antioxidative, hepatoprotective, and gastroprotective activities. Diris et al. (2017) reported that *M. malabathricum* also exhibits antinociceptive, antipyretic, and antidiarrheal properties.

The search for new antibacterial and antidiabetic agents is always a worthwhile endeavor, especially as the emergence of antibiotic resistance and the need to manage diabetes are major concerns. The World Health Organization considers antibiotic resistance as one of the current priority public health issues (Begum et al., 2021). In a study of the polluted surface waters of Metro Manila, Philippines, Vital et al. (2018) found that more than 50% of the bacterial isolates from water, soil, and vegetables grown in this environment were resistant to at least one antibiotic. Diabetes is also a growing concern in the country. Tan (2015) estimated that the incidence of type 2 diabetes in the Philippines is around 16.3%, with an estimated 3.2 million cases and around 1.7 million people remaining undiagnosed.

Treatment for diabetes commonly aim to reduce blood glucose levels. Established treatments include biguanides, thiazolidinediones, sulphonylureas, D-phenylalanine derivatives, and meglitinides (Jyothi et al., 2017). These anti-hyperglycemic drugs are the first-choice treatment for diabetes but are commonly described as having limited efficacy with significant adverse effects (Mehrzadi et al., 2021). In addition, existing drugs fail to maintain normal blood glucose levels, do not prevent microvascular and macrovascular complications, and are financially expensive, especially for the economically deprived (Jyothi et al., 2017). Inhibition of alpha-glucosidase and alpha-amylase, enzymes involved in carbohydrate digestion and absorption, represents a promising approach for managing hyperglycemia in patients with diabetes (Yusuf et al., 2022). Andrade et al. (2020) reported that 62 species of plants that grow in Thailand can significantly inhibit alpha-glucosidase. Based on its use in traditional medicine, *M. malabathricum* may potentially contain compounds with alpha-glucosidase inhibitory activity. To date, few studies have established the traditional use of this native species for diabetes and bacterial infections. Hence, to contribute to the current literature on this Philippine native plant, this study aimed to elucidate the phytochemical and TLC profiles of *M. malabathricum* from Benguet province, investigate its antioxidant and antibacterial activities, and assess the inhibitory potential of the ethanolic leaf extract against the enzyme alpha-glucosidase.

2. MATERIALS AND METHODS

2.1 Plant materials

M. malabathricum plants were collected from Kapangan, Benguet province, Philippines. The species of the collected samples was authenticated by the plant taxonomist of the Institute of Biology, University of the Philippines Diliman, where a voucher specimen was deposited. The leaves were cleaned, washed, oven dried, homogenized to a fine powder, and stored in tightly sealed plastic bags.

2.2 Preparation of the extract

To prepare crude leaf extracts, 50 g of the powdered leaf samples were placed in glass beakers, 500 mL of 80% ethanol was added, the samples were macerated and occasionally stirred for 48 h, and then filtered into an Erlenmeyer flask (Punzalan and Villaseñor, 2019). The ethanolic extract was concentrated under reduced pressure at 50 °C in a rotary evaporator, dried using the Biotage V10-Touch solvent evaporation system at 48 °C, and stored in a refrigerator until analysis.

2.3 Phytochemical screening

A preliminary qualitative analysis was conducted to determine the presence of phytochemicals using standard colorimetric methods.

2.3.1 Test for tannins (gelatin test)

An aqueous extract was prepared by macerating 10 g of powdered leaf sample in 100 mL of distilled water for 48 h. The sample was filtered and dried using a Biotage V10-Touch evaporation system. Four drops of gelatin-salt solution (1% gelatin solution + 10% NaCl) were added to 1 g of the plant extract dissolved in 5 mL distilled water in a test tube. The formation of a white precipitate indicates the presence of tannins (Shaikh and Patil, 2020; Ben et al., 2013).

2.3.2 Test for phenolics (ferric chloride test)

Four drops of a 10% ferric chloride solution were added to 1 mL of the ethanolic extract. The formation of a dark green or bluish-black color indicates the presence of phenolics (Shaikh and Patil, 2020; Vimalkumar et al., 2014).

2.3.3 Tests for flavonoids

Flavonoids, which include flavanones and flavonols, were detected using the following techniques:

Alkaline reagent test

One milliliter of the ethanolic extract was treated with two drops of 2% sodium hydroxide (NaOH). An intense yellow color formed, and then 1 mL of 2 M hydrochloric acid (HCl) was added dropwise until the solution became colorless; this color change indicates the presence of flavonoids (Shaikh and Patil, 2020; Gul et al., 2017; Vimalkumar et al., 2014).

Lead acetate test

One milliliter of the ethanolic extract was treated with three drops of a 10% lead acetate solution. The formation of a yellow precipitate indicates the presence of flavonoids (Shaikh and Patil, 2020; Vimalkumar et al., 2014).

Shinoda's test

Dried ethanolic extract was dissolved separately in 10 mL of distilled water and 5 mL of 95% ethanol. The extract dissolved in ethanol was thoroughly mixed, filtered, and warmed in a water bath, whereas the extract dissolved in distilled water was thoroughly mixed and filtered. Three fragments of magnesium ribbon were added to 1 mL of the plant extract in water and in ethanol, followed by a few drops of concentrated HCl. The appearance of red coloration generally indicates the presence of flavonoids (Shaikh and Patil, 2020; Gul et al., 2017; Bijekar et al., 2015).

2.3.4 Tests for saponins

Froth test

In a test tube, 10 mL of distilled water was mixed with 2 mL of ethanolic extract and shaken vigorously for 1 min. The formation of a one-centimeter-thick foam layer indicates the presence of saponins (Ben et al., 2013).

2.3.5 Test for alkaloids

The presence of alkaloids in the ethanolic extract was tested using four protocols: Mayer's test, Wagner's test, Dragendorff's test, and Hager's test (Shaikh and Patil, 2020; Vimalkumar et al., 2014; Ben et al., 2013).

2.3.6 Test for steroids and triterpenoids

Liebermann-Burchard Test

One gram of the dried ethanolic extract was dissolved in 1 mL of chloroform and 1 mL of acetic anhydride. Concentrated H_2SO_4 (1–2 drops) was added slowly along the side of the test tube. The formation of a range of colors, including blue to green, violet, and red, confirms the presence of steroids and triterpenoids (Shaikh and Patil, 2020; Jagessar, 2017).

2.3.7 Test for cardiac glycosides

To determine the presence of cardiac glycosides, the following tests were conducted, together with the Liebermann-Burchard test described above, to test for three moieties.

Keller-Kiliani test for deoxy sugars

A solution of glacial acetic acid (1.5 mL) containing one drop of 5% ferric chloride was mixed with 1.5 mL of ethanolic extract, and then 1 mL of concentrated sulfuric acid (H_2SO_4) was added gradually along the side of the test tube. The formation of a brown ring between the layers confirms the presence of deoxy sugars (Shaikh and Patil, 2020; Gul et al., 2017).

Kedde's test for unsaturated lactone ring

Ethanolic extract (4 mL) was evaporated to dryness and then dissolved in 1–2 mL of methanol. Alcoholic KOH (1–2 mL) and 1% alcoholic 3,5-dinitrobenzene (3–4 drops) were added, then the solution was heated. The formation of a violet color indicates the presence of an unsaturated lactone ring (Shaikh and Patil, 2020; Jagessar, 2017).

In general, the presence of cardiac glycosides is confirmed when the extract is positive in the Keller-Kiliani, Liebermann-Burchard, and Kedde's tests.

2.4. Quantification of total phenolic content and total flavonoid content

The dried ethanolic extract was dissolved in distilled water at concentrations of 2 mg/mL and 1 mg/mL for estimation of the total phenolic content (TPC) and total flavonoid content (TFC), respectively.

2.4.1 Total phenolic content

Total phenolic content was quantified using the Folin-Ciocalteu method as described by Al-Dhabi et al. (2017). Briefly, three replicates of 100 µL of the diluted plant extract (2 mg/mL), 150 µL of Folin & Ciocalteu's phenol reagent (Sigma-Aldrich, USA), and 1 mL of distilled water were vortexed in a 2 mL microtube for 1 min, then 600 µL of sodium carbonate (10% *w/v*) was added, and the solution was vortexed for another 1 min. The samples were incubated in the dark for 2 h at room temperature, and the absorbance was read at 760 nm using a BMG Labtech FLUOstar Omega microplate reader. Total phenolic content was quantified using a gallic acid standard curve, and values were expressed as gallic acid equivalents (mg GAE/g).

2.4.2 Total flavonoid content

Total flavonoid content was analyzed using an aluminum chloride assay (Alnajar et al., 2012). Three replicates of 200 µL of the diluted plant extract (1 mg/mL), 600 µL of 95% ethanol, 40 µL of 1 M potassium acetate, 40 µL of aluminum chloride, and 1.12 mL of distilled water were incubated in a 2-mL microtube for 30 min at room temperature, and absorbance was determined at 415 nm using a BMG Labtech FLUOstar Omega microplate reader. Total flavonoid content was estimated using a quercetin standard curve, and values were expressed as quercetin equivalents (mg QE/g).

2.5 Antioxidant activity assay

The antioxidant activity of the plant extract was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Masuku et al., 2020). The dried ethanolic extract was dissolved in distilled water at 400 µg/mL and serially diluted to 12.5 µg/mL. Ascorbic acid standards were prepared at similar concentrations (12.5 to 400 µg/mL). In a 96-well microplate, 100 µL of 0.051 mM of DPPH in methanol was mixed with 100 µL of the extracts or standard. DPPH in methanol served as a negative control, and methanol was used as a blank. Each treatment was performed in triplicate. The microplate was incubated at ambient temperature for 30 min, then absorbance was measured at 517 nm using a BMG Labtech FLUOstar Omega microplate reader. Percentage inhibition values were determined using Equation (1):

$$\%\ Inhibition = \left[1 - \left(Abs_{sample} \div Abs_{control}\right)\right] \times 100 \quad (1)$$

where Abs_{sample} is the absorbance of the sample and $Abs_{control}$ is the absorbance of the negative control.

The IC_{50} values of the plant extract and ascorbic acid (the concentration required for 50% inhibition of DPPH free radicals) were determined by linear regression.

2.6 Thin-layer chromatography profiling

The dried extract was re-dissolved in 80% ethanol. The preparation and development of the TLC plates, detection of spots, and computation of the retention factor values followed the protocol of Punzalan and Villaseñor (2019).

Four solvent systems (25% hexane:75% ethyl acetate, 50% hexane:50% ethyl acetate, 70% hexane:30% ethyl acetate, and 75% hexane:25% ethyl acetate) were prepared by mixing n-hexane (Duksan Pure Chemicals, South Korea) and ethyl acetate (Scharlau ACS Basic, Spain/European Union); 70% hexane:30% ethyl acetate resulted in the best resolution of the components in *M. malabathricum* extract, and the data for this solvent system are presented in this paper. The glass TLC chambers were initially saturated with the developing solvent system by pouring a small amount of the solvent (around 20–21 mL) into the chamber in order to not submerge the origin line of the TLC plate, placing a small piece of filter paper, covering the chamber, and leaving the setup to stand for at least 5 min. Aluminum sheets of TLC silica gel (Merck TLC Silica gel 60 F_{254}) were cut into plates measuring approximately 9 cm by 4.5 cm. On the shorter sides, a straight line was lightly drawn 1 cm from the end of the plate, corresponding to the origin line at the bottom and the solvent front at the top of the plate. The marked TLC plates were heated at 50–60 °C in an incubator (Tryte Technologies Electrothermal Thermostatic Incubator TNP-9082-11) for 20 min and then carefully spotted with two replicates of the sample on the origin line using a capillary tube (Kimble Chase 34500-99 Borosilicate Glass Melting Point Capillary Tube).

After saturation of the chamber, the spotted TLC plates were placed vertically in a slightly tilted position in their respective chambers using forceps, covered, and the development solvent was allowed to rise along the adsorbent layer of the TLC plates to the solvent front, then the chromatograms were removed from the chamber using forceps and air-dried for 5 min.

The chromatograms were viewed under UV light (Analytik Jena UVP UVGL-58, Analytik Jena UVP Chromato-Vue® Cabinet C-10) at short (254 nm) and long (365 nm) wavelengths. Dark, non-fluorescent spots against a fluorescent background and bright fluorescent spots against a faintly fluorescent background were marked with a pencil. Next, the TLC plates were placed vertically against a glass spraying box inside a fume hood, and vanillin-sulfuric acid spray reagent (4 g of vanillin in 25 mL of concentrated sulfuric acid) was sprayed onto each chromatogram until it was evenly wet. Each TLC plate was then placed horizontally using forceps on a hot plate at 110 °C and heated until coloration on the chromatogram was observed. The colored spots were marked with a pencil. The distance traveled by the solvent from the origin line to the solvent front (determined as 7 cm all throughout) and from the origin line by each spot detected using UV light and vanillin spray were measured in centimeters. The retention factor (Rf) values were computed using Equation (2):

$$R_f = \frac{distance\ traveled\ by\ the\ sample\ component\ from\ the\ origin}{distance\ traveled\ by\ the\ solvent\ from\ the\ origin} \quad (2)$$

2.7 Antibacterial assay

The minimum inhibitory concentration (MIC) of the plant extract was determined by the broth microdilution method using a resazurin microtiter assay (Sarker et al., 2007; Elshikh et al., 2016). Briefly, the test pathogens *Klebsiella pneumoniae* (NCTC 13440), Methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 33592), *Enterococcus faecium* (NCTC 12204), and *Enterobacter cloacae* (ATCC BAA-2341) were separately inoculated into a cation-adjusted Mueller Hinton Broth (CAMHB) and incubated overnight prior to the bioassay. Each bacterial culture was adjusted to an optical density equivalent to 0.5 McFarland standard.

The specific antibiotic resistance mechanisms of these pathogens are presented in Table 1.

Table 1. Strain characteristics of the bacterial pathogens used

Bacteria	Catalogue number	Strain characteristics
Klebsiella pneumoniae	NCTC 13440	MecA-positive SCCmec type III-positive Methicillin- and gentamicin-resistant
Methicillin-resistant *Staphylococcus aureus* (MRSA)	ATCC 33592	Metallo-beta-lactamase-positive, VIM-1 Beta-lactam antibiotic-resistant
Enterococcus faecium	NCTC 12204	VanA-type glycopeptide-resistant Vancomycin-resistant
Enterobacter cloacae	ATCC BAA-2341	BlaKPC-positive Beta-lactam antibiotic-resistant

The dried ethanolic extract was dissolved in 2% dimethyl sulfoxide (DMSO) at a concentration of 16 mg/mL, serially diluted to 0.25 mg/mL, and 50 µL of each dilution was aliquoted to microplate wells in triplicate. Fifty microliters of the adjusted bacterial solution were added to the wells, except for the blanks and sterility controls. The final concentrations of the diluted plant extracts were 8, 4, 2, 1, 0.5, 0.25, and 0.125 mg/mL. The microplate was incubated at 37 °C for 22 h, then 20 µL of 300 ppm Alamar blue was dispensed into the wells and incubated for 15 min at room temperature. Fluorescence was measured using a BMG Labtech FLUOstar Omega microplate reader with excitation at 544 nm, emission at 590–10 nm, and gain at 700 nm. The concentration that inhibited the growth of 99% of the pathogen was considered the MIC. Percent inhibition was determined using Equation (3):

$$\%\ Inhibition = [\ 1 - \frac{Abs_{sample} - Abs_{blank}}{Abs_{negative\ ctrl} - Abs_{sterility\ ctrl}}\ ]\ x\ 100\% \quad (3)$$

where Fl_{sample} is the fluorescence of the sample wells (50 µL plant extract plus 50 µL bacterial solution), Fl_{blank} is the fluorescence of blank wells (50 µL plant extract plus 50 µL CAMHB), $Fl_{negative\ ctrl}$ is the fluorescence of negative control wells (50 µL CAMHB plus 50 µL bacterial solution), and $Fl_{sterility\ ctrl}$ is the fluorescence of the sterility control wells (100 µL MHB).

The minimum bactericidal concentration (MBC) was determined by inoculating the contents of the wells into nutrient broth and observing bacterial growth after 24 h of incubation at 37 °C. Ciprofloxacin (at 100 ppm final well concentration) was the positive control for all test pathogens. All conditions were tested in triplicate in two independent experiments.

2.8 Alpha-glucosidase inhibition assay

The alpha-glucosidase inhibition assay was performed by the Terrestrial Natural Products Laboratory, Institute of Chemistry at UP Diliman, using the protocol described by Anam et al. (2009) and Naing et al. (2019).

Stock sample solutions of the ethanol extract at 10,000 ppm and a working solution of 300 ppm in DMSO were prepared, homogenized using a vortex mixer, sonicated, and centrifuged. To each well of a 96-well quartz microplate, 190 µL of 50 mM sodium phosphate buffer (PBS) containing 100 mM NaCl at pH 6.8, 10 µL of the 300 ppm sample, and 50 µL of 120 mU/mL enzyme solution were added. Two hundred microliters of acarbose (final well concentration of 1000 ppm) and 50 µL of 120 mU/mL enzyme solution were placed in the positive control wells, and 200 µL of 5% DMSO in PBS (final well concentration of 3.33%) and 50 µL of 120 mU/mL enzyme solution were placed in the negative control wells. After incubation for 10 min at 37 °C, 50 µL of 1.86 mM ρ-nitrophenol-α-D-glucopyranoside was added to start the reaction. The absorbance of the liberated ρ-nitrophenol was measured at 405 nm every 30 s for 30 min using a Multiskan Go® UV/vis Spectrophotometer. This experiment was performed in triplicate.

The inhibitory activities of the samples and the positive control (acarbose) were determined based on the average slope of each triplicate using Equation (4):

$$\%\ Inhibitory\ Activity = \frac{Slope_{uninhibited} - Slope_{inhibited}}{Slope_{uninhibited}}\ x\ 100 \quad (4)$$

where $Slope_{uninhibited}$ is the slope of the line from the absorbance vs. time plot of the negative control group, and $Slope_{inhibited}$ is the slope of the line from the absorbance vs. time plot of the samples or positive control.

A sample was considered active if the percentage inhibition was greater than or equal to 50% and was significantly different from the negative control at $p<0.05$.

2.9 Statistical analysis

The experimental data obtained from the antioxidant assay and the alpha-glucosidase inhibition assay were presented as the mean±standard deviation (SD) of the replicates. To compare the bioactivity of the extract with that of the positive control, the Student's t-test was performed using Microsoft Excel 2019, with a significance level of $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Phytochemical analysis, TPC, and TFC

Qualitative phytochemical screening revealed that the *M. malabathricum* ethanol extract contained several phytochemicals, including tannins, polyphenols, and flavonoids, as well as saponins and triterpenoids (Table 2). Alkaloids and cardiac glycosides were not detected.

Table 2. Summary of the phytochemical screen of *M. malabathricum*

Test	Positive result	Result
Tannins		
Gelatin test	White precipitate	+
Polyphenols		
Ferric chloride test	Dark green/bluish-black color	+
Flavonoids		
Alkaline reagent test	Colorless	+
Lead acetate test	Yellow precipitate	+
Shinoda's test	Red color	+
Saponins		
Froth test	Formation of 1 cm layer of foam	+
Alkaloids		
Mayer's test	Creamy white/yellow precipitate	-
Wagner's test	Brown/reddish precipitate	-
Dragendorff's test	Red/reddish-brown precipitate	-
Hager's test	Creamy white/yellow precipitate	-
Steroids and Triterpenoids		
Liebermann-Burchard test*	Colors ranging from blue to green, violet, and red	+
Cardiac Glycosides		
Keller-Kiliani test	Brown ring between layers	+
Liebermann-Burchard test	Colors ranging from blue to green, violet, and red	+
Kedde's test	Disappearing violet color	-

Note: *The test was also conducted under tests for cardiac glycosides.

Phytochemical studies provide an overview of the chemical profile of medicinally interesting species. Zheng et al. (2021) conducted preliminary phytochemical screening of several *Melastoma* species and detected flavonoids, phenolic compounds, tannins, saponins, and triterpenoids. All of these compounds were also detected in this study. Previous studies on *M. malabathricum* reported the presence of flavonoids, triterpenoids, tannins, saponins, and steroids, with no alkaloids detected (Joffry et al. 2012). A similar study isolated specific compounds from *M. malabathricum* leaf extracts: tannins were isolated from *M. malabathricum* acetone leaf extract, and pentacyclic triterpenoids were isolated from methanolic leaf extracts (Joffry et al., 2012). In a more recent study, flavonoids, tannins, and saponins were detected in a methanol extract of *M. malabathricum*, while terpenoids were not detected (Meilawati et al., 2022); the same extract tested negative for alkaloids using Mayer's and Bouchardat's tests but was positive for alkaloids in Dragendorff's test. Similarly, Samad et al. (2018) conducted phytochemical analysis of *M. malabathricum* and detected tannins, phenols, flavonoids, and steroids; these authors found that the flowers contained the highest phenolic content and the leaves contained the highest flavonoid content.

In this study, the total phenolic and total flavonoid contents of the leaf extract were quantified using standard calibration curves of gallic acid ($y = 3.29x + 0.207$; $R^2 = 0.994$) and quercetin ($y = 3.5569x - 0.0288$; $R^2 = 0.9788$), respectively (Table 3). A relatively high TPC of 298.68 mg GAE per gram of sample was observed, and the TFC was 37.34 mg QE per gram of sample.

The TPC of the ethanolic extract of *M. malabathricum* leaves determined in this study was within the range of TPC values found in the literature. Sharma and Kumar (2011) reported a lower TPC of 210 mg GAE/g for *M. malabathricum* leaf methanol extract, while Danladi et al. (2015) reported values ranging from 216.97 to 671.51 mg GAE/g for methanol extracts of *M. malabathricum* gathered from different locations. Alnajar et al. (2012) reported a higher TPC value of 384.33 mg GAE/g for an ethanolic extract of *M. malabathricum*, which they stated was a high TPC. The TFC value obtained in the current study was within the lower range of values in previous reports. Methanol extracts of *M. malabathricum* were previously reported to have TFC values ranging from 25.27 to 102.92 mg QE/g (Sharma and Kumar, 2011; Danladi et al., 2015), whereas aqueous and ethanol extracts of *M. malabathricum* were reported to have TFC values of 14.7 mg QE/g and 85.8 mg QE/g, respectively (Alnajar et al., 2012).

Table 3. Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of *M. malabathricum* leaf ethanolic extract

	TPC (mg GAE/g)±SD	**TFC (mg QE/g)±SD**	**DPPH % Inhibition**	**IC_{50} (µg/mL)±SD**
M. malabathricum	298.68±7.79	37.34±1.87	82.59%*	57.98±2.83*
Ascorbic acid	-	-	93.87%	23.31±0.90

Note: *Considered significant at $p<0.05$.

3.2 Antioxidant activity

Antioxidants, including phenolics and flavonoids, play a crucial role in the maintenance of health by mitigating the damaging effects of free radicals. Research has linked free radicals to a variety of chronic diseases, including diabetes, atherosclerosis, and hypertension (Singh et al., 2015).

The DPPH scavenging assay is commonly used to measure the antioxidant properties of extracts because it is both rapid and cost-effective. The test offers a basic understanding of an extract's capacity to donate hydrogen atoms to counteract the damaging effects of oxidative molecules (Kedare and Singh, 2011). The inhibitory activity of *M. malabathricum* leaf ethanolic extract was 82.59% at 200 µg/mL (Table 3). Moreover, the scavenging activity of the extract in this study (IC_{50} 57.98 µg/mL) was stronger than the *M. malabathricum* extract prepared by Danladi et al. (2015) (IC_{50} ranging from 102 to >1000 µg/mL) but weaker than that prepared by Sharma and Kumar (2011) (IC_{50} of 21.86 µg/mL). Although our extract exerted lower inhibitory activity compared to the positive control, ascorbic acid, our findings are consistent with the inhibitory and IC_{50} values obtained in recent studies on *Melastoma* species (Sari et al., 2018; Zheng et al., 2021).

Previous studies have reported the antioxidant properties of tannins, flavonoids, and triterpenoids in *M. malabathricum* (Sirat et al., 2010; Sari et al., 2018). In particular, the flavonoids quercitrin, quercetin, and kaempferol-3-*O*-(2″,6″-di-*O*-*p*-*trans*-coumaroyl)-β glucoside isolated from the leaves of *M. malabathricum* were found to exhibit high DPPH radical scavenging activities (Sirat et al., 2010). The antioxidant activity of ursolic acid, a pentacyclic triterpenoid carboxylic acid, was also previously observed (Checker et al., 2012). Thus, the presence of flavonoids and triterpenoids reports may explain the antioxidant activity of the *M. malabathricum* extract.

3.3 TLC profile

TLC profiling is a useful tool for identifying and characterizing phytochemicals in plant extracts in order to understand their pharmacological properties. The chromatograms and TLC profiles generated by this technique offer a means of evaluating the chemical composition and integrity of a given plant extract (Talukdar et al., 2010). Moreover, TLC profiling is an important technique for quality control and for the determination of adulterants in herbal medicines and other plant products (Braz et al., 2012).

Of the four developing solvent systems tested in this study, 70% hexane and 30% ethyl acetate resulted in the best resolution of the components of *M. malabathricum* extract; hence, the results obtained for this solvent system were presented in this paper. Photodocumentation and the summary of the TLC profile of the extract are shown in Figure 1 and Table 4. The largest numbers of spots were visualized using UV_{365} and after post-derivatization with vanillin-sulfuric acid. Eight red fluorescent spots were observed using UV_{365}; nine spots of various colors, including violet/purple, grey, and blue, were detected after spraying vanillin-sulfuric acid, and only four quenching spots were visualized under UV_{254}.

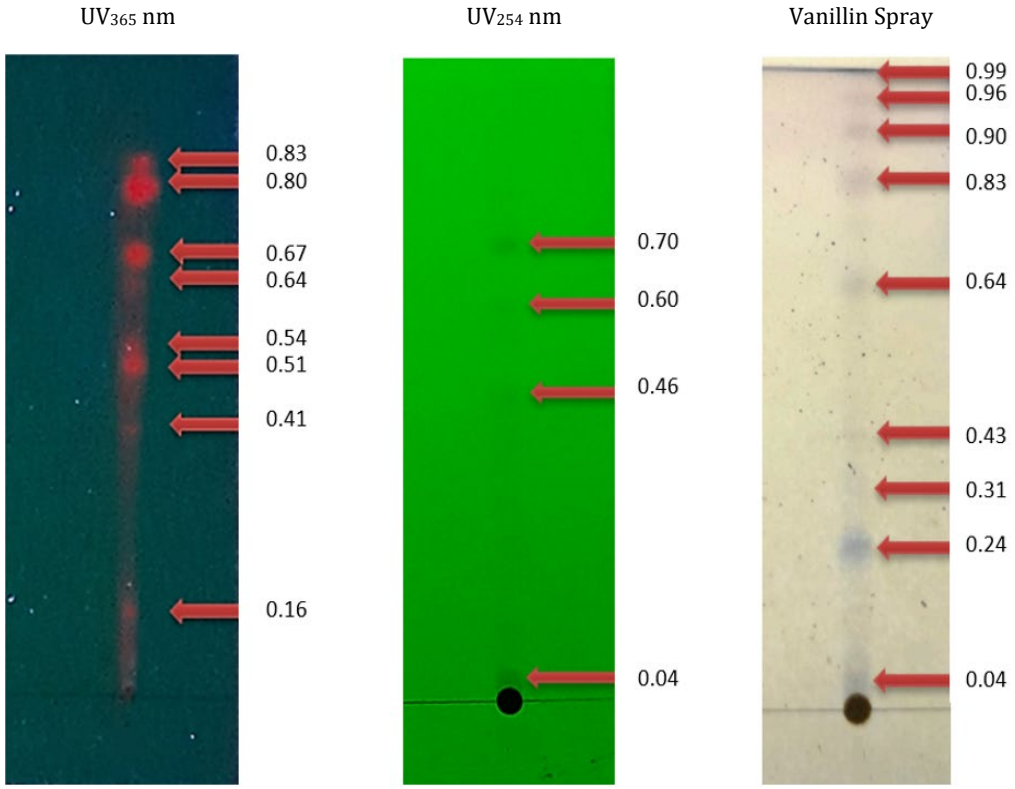

Figure 1. TLC chromatograms of *M. malabathricum* extract visualized under irradiation with UV at 365 nm and 254 nm and after spraying with vanillin-sulfuric acid
Note: Mobile phase: hexane:ethyl acetate (7:3 *v/v*)

Table 4. TLC profile of the ethanolic extract from *M. malabathricum*

R_f value	UV_{365} nm	UV_{254} nm	Vanillin spray
0.04	-	Quenching	Grey
0.16	Red	-	-
0.24	-	-	Blue
0.31	-	-	Light blue
0.41	Red	-	-
0.43	-	-	Light grey
0.46	-	Quenching	-
0.51	Red	-	-
0.54	Red	-	-
0.60	-	Quenching	-
0.64	Red	-	Purple
0.67	Red	-	-
0.70	-	Quenching	-
0.80	Red	-	-
0.83	Red	-	Purple
0.90	-	-	Grey
0.96	-	-	Light purple
0.99	-	-	Dark violet/purple

The distinct spots observed on the silica gel plate represent specific compounds with unique Rf values. These Rf values could be compared to those of known or reference compounds to determine the possible identities of the constituents of *M. malabathricum* extract (Kumar et al. 2013). Mostly red fluorescent spots were observed under UV_{365}, and spots of similar colors were detected after spraying with vanillin-sulfuric acid. Spots with the same colors may belong to the same class of compounds (Martin-Puzon et al., 2015). Hence, further analysis using other more precise separation and profiling methods, such as High Performance Thin Layer Chromatography (HPTLC), is recommended to identify the compounds present in *M. malabathricum* leaf extract.

Due to their aromatic structure, phenolic compounds exhibit strong UV absorption, resulting in the appearance of

spots in a range of colors in TLC chromatograms, including yellow, green, white to pale yellow, pink, purple, red, blue, brown, grey, or black (Martin-Puzon et al., 2015). Many of these colors were observed in the TLC spots of *M. malabathricum* leaf extract, which may mean that these phenolic compounds may contribute to the pharmacologic activities observed in this study.

3.4 Antibacterial activity

The anti-bacterial activity of the ethanolic extract was tested at concentrations up to 8 mg/mL against four bacterial pathogens with antibiotic resistance genes. As shown in Table 5, the extract was most effective against vancomycin-resistant *E. faecium* with a MIC of 4 mg/mL and was also effective against methicillin-resistant *S. aureus* with a MIC of 8 mg/mL. *E. cloacae* and *K. pneumoniae* still grew when exposed to the extract at 8 mg/mL; thus, the MIC of the extract was deemed to be >8 mg/mL for these pathogens. The positive control, ciprofloxacin (100 ppm), resulted in 99% inhibition of all four test pathogens. When re-inoculated into nutrient broth and incubated for 24 h, growth was observed for all of the pathogens exposed to the extract, which indicates that the extract was bacteriostatic and that the concentrations used were not bactericidal.

Table 5. MIC values of the ethanolic extract of *M. malabathricum* against four pathogens

	MIC (mg/mL)	MBC (mg/mL)
E. faecium	4	ND
MRSA	8	ND
E. cloacae	>8	ND
K. pneumoniae	>8	ND

Note: ND - not detected.

M. malabathricum has been traditionally used to treat wounds (Diris et al., 2017), indicating that people discovered its efficacy as an antibacterial agent long ago. In this research, the antibacterial activity of the leaf extract was tested against four bacterial strains: *E. faecium*, MRSA, *E. cloacae*, and *K. pneumoniae*, all of which possess antibiotic resistance genes. At 1 mg/mL, significant percentages of inhibition greater than 50% were observed against *K. pneumoniae* (57.75%), *E. cloacae* (53.56%), and *E. faecium* (78.43%) (data not shown). In addition, the computed percentage inhibition against MRSA was 66.23% at 4 mg/mL.

In a review by Zheng et al. (2021), the antibacterial property of *M. malabathricum* was attributed to the flavonoid kaempferol-3-O-[2′′,6′′-di-O-(E)-coumaroyl]-β-D-glucoside. Wong et al. (2012) also discovered nine novel compounds in the same species that contributed to antibacterial activity, including triterpenoids and flavonoids. However, none of the pathogens tested in those studies were antibiotic-resistant. Similarly, Diris et al. (2017) assessed the antibacterial activity of *M. malabathricum* and *M. beccarianum* leaf crude extracts against bacteria lacking antibiotic resistance genes. However, the antibacterial activity of *M. malabathricum* leaf extract against bacteria with antibiotic resistance genes in this study supported the potential usefulness of this plant against pathogens that evade current methodologies for their control. The MIC against gentamicin- and methicillin-resistant *S. aureus* (ATCC 33529) and vancomycin-resistant *E. faecium* (NCTC 12204) suggests that compound purification and isolation studies on *M. malabathricum* may provide a very promising route to expand our arsenal against antibiotic-resistant bacterial pathogens.

3.5 Alpha-glucosidase inhibition activity

The effects of the *M. malabathricum* ethanolic extract in the alpha-glucosidase inhibition assay are summarized in Table 6. The extract led to significantly higher inhibition of the enzyme than the positive control, acarbose, which is the most widely prescribed alpha-glucosidase inhibitor.

Table 6. Alpha-glucosidase inhibitory activity of *M. malabathricum*

	Percentage alpha-glucosidase inhibition			
	Trial 1	Trial 2	Trial 3	Average inhibition activity
M. malabathricum	99.22	99.29	99.53	99.35%±0.16*
Acarbose (positive control)	93.80	92.77	94.13	93.57%±0.71

Note: *p<0.05 was considered significant.

In addition to its antibacterial properties, *M. malabathricum* is one of several traditional herbal medicines used for diabetes. Balamurugan et al. (2014) and Idris et al. (2022) showed that *M. malabathricum* extracts could decrease blood glucose levels *in vivo* in rats with alloxan-induced diabetes. To date, there have been no mechanistic studies of the effects of *M. malabathricum* on diabetes.

Alpha-glucosidase is an essential enzyme in the regulation of blood glucose levels. When this enzyme is inhibited, the process of breaking down glucose from oligosaccharides and disaccharides is slowed down, leading to a delay in carbohydrate digestion. Consequently, the overall carbohydrate digestion time is prolonged, resulting in a reduced rate of glucose absorption (Naing et al., 2019). The present study found that the *M. malabathricum* ethanolic extract had a very high alpha-glucosidase inhibitory activity of 99.35% at 10 ppm. This result corroborates the findings of Idris et al. (2022), who found that both alpha-glucosidase and alpha-amylase were inhibited by *M. malabathricum*. Phenolic acids (caffeic, ferulic, sinapic, syringic, and gallic acids) and the prominent flavonoids quercetin and myricetin have been strongly correlated with alpha-glucosidase inhibitory potential (Prajudtasri et al., 2019). Specifically, flavonoids were described as promising alpha-glucosidase inhibitors by Proenca et al. (2017) and in a review by Şöhretoğlu and Sari (2020).

The inhibitory effects of flavonoids on alpha-glucosidase are generally enhanced by hydroxylation, which may possibly create electrostatic interactions with the enzyme. Our phytochemical analysis and the literature indicate that *Melastoma* spp. are rich in flavonoid compounds as well as phenolic acids (Zheng et al., 2021); such components are likely to contribute to the high alpha-glucosidase inhibitory activity of the *M. malabathricum* ethanolic extract.

4. CONCLUSION

The results of this study demonstrated that *M. malabathricum* ethanol extract contains bioactive compounds with antioxidant, antibacterial, and alpha-glucosidase inhibitory properties. The ability of the crude ethanolic extract to inhibit the enzyme alpha-glucosidase is particularly interesting, especially as the extract was more potent than the positive control, acarbose. The potent alpha-glucosidase inhibition activity of the extract may be attributed to the high total phenolic content and total flavonoid content. Overall, these findings supported the traditional use of the plant species for wound healing and diabetes management. Further research on *M. malabathricum* may help to identify novel compounds with activity against antibiotic-resistant bacteria or for the treatment of diabetes.

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