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Phytochemical, Antioxidant and Antibacterial Evaluation of *Melastoma* malabathricum L.: an Indonesian Traditional Medicinal Plant

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ARTICLE INFO	ABSTRACT

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Antibiotic resistance has become a worldwide threat to public health and traditional medicinal plants may provide a simple solution to overcome this problem. Many plants are known to contain antioxidants such as polyphenols and flavonoids which are active agents against pathogens. To find phytochemicals to boost the immune system naturally, it is essential to investigate new plant sources of antioxidants and antimicrobials. Melastoma malabathricum L. belongs to the Melastomataceae family and is traditionally used to treat several ailments related to infectious diseases, *i.e.* wounds, dysentery, diarrhea, stomachache, and toothaches. This study aimed to evaluate the phytochemical, antibacterial and antioxidant activities of extracts and fractions of M. malabathricum leaves. Antibacterial effect was tested against selected strains from the American Type Culture Collection (ATCC) including Staphylococcus aureus, Escherichia coli, and methicillin-resistant Staphylococcus aureus (MRSA) using microdilution assay while antioxidant activity was analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. Ethyl acetate extract showed the highest antibacterial activity against S. aureus and MRSA with MIC 1 mg/mL (0.1% b/v) and the highest antioxidant activity with IC50 of 43.30 µg/mL with DPPH free radical scavenging assay. Ethyl acetate fraction exhibited the presence of the flavonoid contents by citroboric reagent. Extracts and fractions of M. malabathricum leaves revealed that the potential activity against selected bacterial and DPPH assay might be due to the presence of phenolic substances. These findings support the usage of these plants as a traditional medicine for wound healing and further investigations are needed to identify the active constituents.

Keywords: Antioxidant, Free radical scavenging, Infectious disease, *Melastoma malabathricum* L., Methicillin-resistant *Staphylococcus aureus*.

Introduction

In the late 1990s, the use of medicinal plants became increasingly popular as an alternative form of medical treatments.¹ Medicinal plants have continued to gain more interest and play a significant role in the discovery of new pharmaceuticals preparations. The importance of phytochemicals is proven by the discovery of about 20% of known plants that have been used in pharmaceutical studies, affecting the health care system in positive ways such as helping to heal cancer and prevent harmful diseases.² Plants are extremely complex and provide important sources of diverse bioactive compounds.³ Various studies have shown that many medicinal plants are rich in antioxidants.⁴

Antioxidants in many plants prevent oxidative stress-related illnesses including Parkinson's disease, Alzheimer's disease, cardiovascular disease, and cancer which are often fatal due to the imbalance between dangerous free radicals and the endogenous antioxidant system.⁵ The high content of the phenolic compounds in medicinal plants plays a key role in the antioxidant activity regarding protection against the damage from reactive oxygen species which can lead to cell damage and death.⁶

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The investigation of new active and safe antioxidant agents is crucial. The use of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) is a simple and efficient method to evaluate the scavenging power of antioxidants towards the stable radical DPPH molecules.⁷ In recent years, antibiotic resistance has become a worldwide threat to public health and the discovery of new medicinal plants can provide a simple solution to overcome the problem.^{8,9} New resistance mechanisms are occurring and spreading globally while threatening the ability to treat common infectious diseases and resulting in prolonged illness, disability and death.¹⁰ The emerging resistance of pathogens against the currently available antimicrobial agents demands the discovery of new antimicrobial agents.¹¹ Therefore, it is important to search among traditional medicinal plants for new antimicrobial and antioxidant sources with diverse mechanisms of action.

The discovery of antimicrobial activities from second metabolites of plants provides a promising source of antibiotic agents.¹ The usage of plants as anti-infection treatments has been implemented for many centuries.⁹ People who live in rural areas in Riau province, Sumatera Island, Indonesia have consumed "kaduduak" leaves (Melastoma malabathricum L.) to cure various ailments such as diarrhea, dysentery, wounds, toothache and diabetes. M. malabathricum L. is an important medicinal species in the Melastomataceae family which is found in tropical countries including South East Asia, India, Malaysia, Australia, and Indonesia. This plant has been shown to have several pharmacological actions such as: antibacterial¹²; antioxidant, cytotoxic¹³; wound healing and antidiarrhea¹⁴; anti-inflammatory¹⁵, and antiulcer activities.¹⁶ Several studies reported bioactive compounds from M. malabathricum L. including: flavonoids, e.g. kaempferol, quercetin, quercitrin, rutin, kaempferol-3-O-(2-6-di-O-p-trans-coumaroyl)- β -glucoside,¹⁷ tannin groups including malabathrin B, malabathrin C, malabathrin D, strictinin, casuarictin, penduculagin, nobotanin B,

nobotanin D, nobotanin G, epicatechin gallate, ellagic acid,¹⁸ phenolic (gallic acid, benzoid acid, epicatechin), and anthocyanin.

The accumulation and production of secondary metabolites from the same plant species are influenced significantly by different geographical conditions.²⁰ The factors that might affect the diversity of metabolites for individual plant species involve cultivation conditions (type of soil, H of soil, micro and macronutrients), climate (temperature, humidity, light and wind), geographical location (altitude) and harvest time.²¹ Therefore, the evaluation of phytochemical and biological activities of this plant is extremely important when it is used as a therapeutic agent. The present investigations aimed to evaluate the phytochemical, antimicrobial and antioxidant effects of various extracts and fractions of M. malabathricum L. leaves from Riau province, Sumatera Island, Indonesia. To the best of our knowledge, even though a few compounds have been isolated from extracts of M. malabathricum L., the ethnomedicinal importance, antibacterial and antioxidants properties are still poorly known empirically. As a traditional treatment for various ailments, these plants have been used by indigenous people in the Riau regions of Indonesia and other Asian countries for centuries. With the conclusive results from in-depth phytochemical testing, this research is one of the first studies to provide empirical evidence of its healing properties.

Materials and Methods

Chemical reagents

All solvents: n-hexane, ethanol, dichloromethane, ethyl acetate, formic acid, methanol, were of analytical grade supplied from Merck (Merck, Darmstadt, Germany). Citroboric reagent was purchased from Merck & Co., Germany. Ascorbic acid and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) were supplied from Sigma (Aldrich, USA). Column chromatography was performed with silica gel (0.040-0.063 mm; Silicycle, Quebec City, Canada). Extracts and fractions were monitored by thin-layer chromatography (TLC) which was done on pre-coated silica gel 60 F254 plates (Merck). All chemical solvents used were of analytical grades. In addition, Mueller Hinton Agar, Nutrient Agar, and Nutrient Broth were purchased from Oxoid, UK.

Plant material

The leaves of *M. malabathricum* were collected in June 2018 from Kuantan Singingi district of Riau province, Sumatera Island, Indonesia with coordinates: 0°33'2"S and 101°32'11"E. The plant was authenticated by a botanist (Dr. Djoko Santosa of the Department of Pharmaceutical Biology) and a voucher specimen (29560/M/03/02) was deposited at the Department of Pharmaceutical Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia. Once dried at room temperature (after a week), the leaves were powdered and stored in cool dry conditions prior to extraction.

Preparation of extract and fraction

Dried and ground leaves (500 g) were extracted sequentially by several solvents (1:10 b/v) of increasing polarity. Firstly, it was extracted with n-hexane exhaustively in a Soxhlet apparatus. The dried and defatted pulp was then soaked in ethyl acetate and finally the rest of the pulp was extracted with 5 L ethanol. Ethanol extract was separated using a liquid-liquid partition with n-hexane, dichloromethane, and ethyl acetate on separation funnel. Each extract and fraction were filtered and concentrated under vacuum on a rotary evaporator. All extract and fractions were stored at 4°C in the refrigerator for further use.

Phytochemical screening

Qualitative phytochemical screening (saponin, alkaloid, phenols, tannin, steroid, terpenoid and flavonoids) of three extracts was done according to the standard qualitative analysis of phytogenic compounds described by Harborne et al.²²

Flavonoid screening

Flavonoid screening was done by TLC using silica F₂₅₄ as stationary phase and EtOAC: MeOH: water: formic acid (100: 13: 10: 2) as mobile phase. The flavonoid test involved the test of citroboric reagent and ammonia on n-hexane, dichloromethane, and ethyl acetate fractions. The chromatogram was then visually observed under visible ray and ultraviolet (UV) ray 254 nm and 366 nm. Then, its R_f values were determined.

Column chromatography

The fractions with the highest antioxidant activity were subjected to column chromatography for further separation and purification using column chromatography silica gel. Ethyl acetate fraction showed the highest activity on DPPH assay and was then subjected on column chromatography. Silica gel (230-400 Mesh) was used as the stationary phase (absorbent) while the mobile phase was step gradient mixture of dichloromethane: ethyl acetate: formic acid in the volume ratio used started from 50: 45: 5 to 0: 95: 5. The eluted fractions (each 15 mL) were collected in bottles and resulted in 105 fractions. All fractions were TLC and the same R_f of TLC were joined together, which yielded a total of 9 fractions.

Derivatization by DPPH

After development, TLC plates of column chromatography fractions were dried at room temperature (28°C) 15 min and derivatized with 0.2% freshly prepared methanol solution of DPPH. Plates were stored in dark at room temperature. The yellow spots on purple background represent the substance with antioxidant activity.

Antibacterial activity

The examined bacteria were obtained from American Type Culture Collection (ATCC) including Gram-positive strains: Staphylococcus aureus (ATCC 25923) and methicillin-resistant Staphylococcus aureus (ATCC 33591) with Gram-negative strain Eschericia coli (ATCC 25922). Microbroth dilution method was used to determine antibacterial activity of extracts with slight modification.²³ Into each well of the microtitration plate, 80 µL of Broth Heart Infusion was placed. A volume of 20 µL each plant extract with various concentrations: 1%, 0.5%, 1.25%, and 0.125% was pipetted onto the plate and mixed using micropipette. Each examined bacterial strain suspension (e.g. Staphylococcus aureus, Escherichia coli, and MRSA) was inoculated with 10 μ L of a bacterial suspension (from 5x10⁶ CFU/mL bacterial suspension) into all wells except medium control. Chloramphenicol was used as positive control. Negative control was only the bacterial inoculums; the inoculated bacteria must grow because it is only a broth and if the bacteria did not grow, then this means there was a problem in preparation of the media or bacterial inoculums. The inoculated plate was incubated at 37°C for 18-24 h. The lowest concentration of plant extracts that inhibited visible bacterial growth was considered the minimum inhibitory concentration (MIC) according to Clinical and Laboratory Standards Institute (CLSI).

Antioxidant activity

Antioxidant activity of the extracts and fractions was determined by free radical scavenging DPPH assay with slight modification.²⁴ The testing samples were prepared using various concentrations starting from 10 µg/mL to 1000 µg/mL and dilution in methanol. Blank solution and ascorbic acid were used as a negative and positive control, respectively. One milliliter of DPPH 0.4 mM, as a control solution, was prepared with 4 mL methanol into the tube. Variation concentrations of samples were added into 1 mL DPPH and 3.5 mL of methanol as a solvent. The mixtures were shaken and left to stand in the darkroom for 30 min. The solutions were measured at 517 nm with methanol as a blank using a UV-Visible spectrometer (Shimadzu 1800). The assay was performed in triplicates. The percentage of

DPPH free radical scavenging activity was calculated as: DPPH scavenging activity (%) = $\left[\frac{(A0-A1)}{(A0)} \times 100\%\right]$ (1) where A₀ = absorbance of blank; A₁ = absorbance of sample.

Statistical analysis

Results were expressed as mean \pm standard deviation (SD) of three parallel reactions. The data were subjected to analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS version 16.0, IBM Corp., USA).

Results and Discussion

Traditional use of M. malabathricum L.

Due to its geographical location, Indonesia is a tropical archipelago country with an abundance of indigenous medicinal plants. The indigenous plants species are found to be highly diverse in each island. Sumatera island is one of the five biggest island in Indonesia that exhibits a rich and large range of vegetation types.²⁵ Traditional medicine is a heritage that has been developed since ancient times. In spite of the progress of pharmacological methods, the traditional medicines from plants are mainly used for discovering novel compounds for particular ailments. Several modern drugs have been discovered derived from medicinal plants, *i.e.* aspirin, morphine, and pilocais rapine.²⁶

Melastoma malabathricum L. is native to tropical and subtropical regions, in temperate Asia and Pacific island.²⁷ *M. malabathricum* L. has been reported to treat various types of ailments, for instance: dysentery, diarrhea, hemorrhoids, cuts and wounds, infection during toothache, stomachache, and thrush.^{19,28,29} In traditional uses, the leaves of *M. malabathricum* L. are chewed up, pounded and applied as paste on cuts or wounds or squeezed to apply the juice onto the wound to stop bleeding and accelerate the dryness of wounds. For healing diarrhea and dysentery, 3-4 pieces of young leaves are boiled and consumed orally twice a day.

Extraction and fractionation

Extraction process was done by sequential extraction from nonpolar solvent (n-hexane), semipolar (ethyl acetate) and polar solvent (ethanol) to exclude all the non-polar substances. Therefore, the chemical compounds of the crude extract could be separated based on their polarity. Ethanol extracts were continued to the liquid-liquid partition method and this step resulted in three fractions: n-hexane, dichloromethane and ethyl acetate fraction. The yield of extracts and fractions are presented in Table 1. The highest extraction yield was obtained using ethanol (12.82%), followed by ethyl acetate and n-hexane. For liquid-liquid partition fractions, ethyl acetate (12.06%) showed the highest yield compared to dichloromethane and n-hexane fractions.

Phytochemical analysis

The relationship between the antioxidant activity and secondary metabolite was investigated in the phytochemical analysis. Phytochemical analysis was detected in each collected extract as shown in Table 2. The most effective fraction was ethanol extract since it is rich in various bioactive phytochemical substances, and contains saponin, phenols, tannin, steroid, terpenoid and flavonoid. Qualitative analysis revealed that flavonoids, phenols, and tannin were found in ethanol and ethyl acetate extract; steroids and terpenoid were identified in ethanol and n-hexane, while saponin was only found in the ethanol extract and alkaloid was found in the n-hexane extract. Liquid-liquid fractions such as n-hexane, dichloromethane and ethyl

Liquid-liquid fractions such as n-hexane, dichloromethane and ethyl acetate fractions were analyzed using a citroboric reagent to detect flavonoid compounds as shown in Figure 1. Based on the chromatogram, the fractions exhibited a clear separation when developed using a mobile phase of EtOAC: MeOH: water: formic acid (100: 13: 10: 2 v/v). Citroboric reagent is one of the specific identifications for detection of flavonoid groups. The compounds containing the flavonoid groups revealed yellow spots in the TLC plate.³⁰ The TLC profile of ethyl acetate fraction exhibited some spots with R_f value : 0.30, 0.50, and 0.70 with yellowish fluorescense under UV 254 nm, 366 nm and visible ray after sprayed by citroboric reagent. Dichloromethane fraction showed a few yellow spots on the plate. It indicated that in this fraction, only a few flavonoid substances were still present while the n-hexane fraction did not exhibit yellow

fluorencens spot on the chromatogram contain indicating the absence of flavonoid compounds.

Column chromatography

Column chromatography was used to isolate the bioactive compounds. Ethyl acetate fraction was eluted on column chromatography with composition of solvents: dichloromethane: ethyl acetate: formic acid (50: 45: 5) and silica gel packed with dichloromethane as the solid phase. All fractions were tested using qualitative analysis of DPPH assay to evaluate the potential fractions for antioxidant properties (Figure 2). Several fractions showed the yellow band in violet background of DPPH with the following retardation factor (R_f): fraction 1 (0); 2 (0.75); 3 (0.50); 4 (0); 5 (0.10); 6 (0.05, 0.10, 0.30, 0.80); 7 (0.05); 8 (0.05, 0.10); 9 (0); Quercetin (Q1-Q3) was used as a positive control (0.80).

Column chromatography fractions showed the potential antioxidant activity based on qualitative DPPH test. Fraction 8 exhibited various spots with yellow bands on the TLC plates. This might be due to the several compounds that have been found in the previous studies such as kaempferol, quercetin, quercitrin, tannin, malabathrin, and other phenolics.^{13,17,18}

Antimicrobial properties

The extracts of n-hexane, ethyl acetate, and ethanol exhibited antimicrobial activity against growth of selected microorganisms in this study (Table 3). Ethyl acetate and ethanol extracts revealed the potential activity against *S. aureus* and MRSA while n-hexane, ethyl acetate and ethanol extracts showed the similar activity against *E. coli*. Data results were significant (p < 0.05) based on ANOVA analysis.

Ethyl acetate and ethanol extracts showed potential against the selected microorganisms with the lowest MIC value (0.5 mg/mL, 0.1 mg/mL, and 6.25 mg/mL) for *S. aureus*, MRSA, and *E. coli*, respectively.

 Table 1: The yield of extracts and fractions of M.

 malabathricum L. leaves

Extracts	Amount of extracts (g)	Yield (%)
n-hexane	10.80	2.16
ethyl acetate	44.70	9.41
ethanol	62.86	12.82
Liquid-liquid partition fractions		
n-hexane	0.69	4.6
dichloromethane	1.55	10.33
ethyl acetate	1.81	12.06

Table 2:	Phytochemical	ethanol,	ethyl	acetate,	and n-hexane
extracts					

Phytochemical compounds	Extracts			
	Ethanol	Ethyl acetate	n-hexane	
Saponin	+	-	-	
Alkaloid	-	-	+	
Phenols	+	+	-	
Tannin	+	+	-	
Steroids	+	-	+	
Terpenoids	+	-	+	
Flavonoids	+	+	-	

Antibacterial activities of ethyl acetate and ethanol were found to be comparatively higher than that of n-hexane extract. The inhibition of antibacterial activity of the three extracts is higher against S. aureus and MRSA (Gram-negative bacteria) than E. coli (Gram-negative bacteria). Overall, antibacterial activity of the extract of M. malabathricum leaves was noticeably more effective against the Gram-positive bacteria compared to Gram-negative bacteria strains. This is a similar finding with the previous study conducted by Osonga et al.³¹ that reported the certain groups of flavonoid compounds revealed greater inhibition effect on Gram-positive bacteria compared to Gram-negative bacteria. In fact, Gram-negative bacteria are particularly more resistant to antimicrobial agents than Gram-positive bacteria because of the presence of the hydrophilic surface of their outer membrane, composed of lipopolysaccharides compounds that provide a barrier to penetration as well as with enzymes in the periplasmic space that are capable of breaking down any unknown molecule from outside. These could be explained by the presence of outer membrane permeability barrier of Gram-negative bacteria which limits the access of the antimicrobial agents to the target in the bacterial cell³² and provides an empirical explanation why Gram positive bacteria are more sensitive than Gram-negative bacteria.

The MIC values of extracts are below 8 mg/mL and considered to possess some microbial activity³³ while natural products with MIC values below 1 mg/mL are considered noteworthy.³⁴ According to these finding, ethyl acetate and ethanol extract possess the greatest antibacterial activity with MIC between 0.1 - 0.5 mg/mL for MRSA and *S. aureus* and 6.25 mg/mL for *E. coli*. Extract of n-hexane also showed antibacterial activity with the range of MIC values of 1 - 6.25 mg/mL against *S. aureus*, MRSA and *E. coli*. All extracts can be concluded as having antibacterial activity against the selected microorganisms. Accordingly, this plant can be a potential source of phytochemical compounds for future investigation towards developing new anti-infective agents.

Antioxidant activity

DPPH free radical scavenging assay was adopted for evaluation of antioxidant power for each prepared n-hexane, ethyl acetate, ethanol extracts and n-hexane, dichloromethane, and ethyl acetate fraction. Ascorbic acid was used as the reference drug in various concentrations which ranged from 1 to 100 µg/mL. The blank solution was considered as the DPPH and methanol without any plant extract. The inhibition of *M. malabathricum* leaves extracts and fractions of DPPH is presented in Table 4. The results revealed that the extract and fraction of ethyl acetate showed significant antioxidant activities with IC_{50} 43.34 µg/mL and 40.34 µg/mL, respectively. Contrary to the extract and fraction of ethyl acetate, the extract and fractions of n-hexane showed low antioxidant activity with IC_{50} 2382.36 µg/mL and 1723.07 µg/mL, respectively. Ascorbic acid, as a reference drug,

exhibited excellent antioxidant properties as a free radical DPPH scavenger with IC_{50} 25.88 µg/mL.

The DPPH free radical scavenging assay was adopted for evaluation of antioxidant properties. The DPPH assay is largely used for the assessment of drugs with antioxidant potential due to its fast and simple methods to investigate antioxidant capacity. The radical scavenging activity was determined from a reduction in the absorbance at 517 nm and changing violet color to yellow color. Free radical scavenging effect of various extracts and fractions of *M. malabathricum* L. leaves was presented by their IC₅₀ values. Ethyl acetate fraction showed an excellent free radical scavenging activity that was indicated by its inhibition reaching almost as high as ascorbic acid as a positive control with IC₅₀ values 23.49 µg/mL.

The parameter used to measure the antioxidant activity is the values of inhibition concentration (IC₅₀) that is concentration of samples needed to reduce 50% of free radicals. According to Jun et al.³⁵, antioxidant activity is classified into five groups based on their IC₅₀ values: highly active (< 50 µg/mL), active (50-100 µg/mL), moderate (101-250 μ g/mL), weak (250-500 μ g/mL), and inactive >500 μ g/mL). Meanwhile, according to Molyneux³⁶, IC₅₀ value of 200-1000 μ g/mL was declared less active but still potential to be an antioxidant agent. Based on the results, ethyl acetate extract and fraction with IC₅₀ 40.34 µg/mL and 43.34 µg/mL, respectively, exhibit the highly active antioxidant activity. The extract and fraction of n-hexane were indicated as inactive antioxidants with IC₅₀ value > 1000 μ g/mL. The dichloromethane fraction belongs to the weak antioxidant category with IC₅₀ 373.30 v µg/mL, while ethanol extract belongs to the less active category with IC₅₀ value 557.30. According to the IC values obtained, it is known that ethyl acetate extract and fraction have the greatest antioxidant activity. This might be due to the hydroxyl group in the phenolic compounds in ethyl acetate extract and fraction which can be a donor of its electrons to the DPPH radicals. The radical scavenging activities of the extracts and fractions increased with the increasing of the concentrations. The extracts with high radical scavenging activity showed high phenolic content as well.

Among all secondary metabolites, phenolic compounds have been reported to be the main contributing compounds to antioxidant activity of plants since they have demonstrated promising antioxidant activity in many previous studies.^{24,38,39} Phenolic compounds reveal a considerable free radical scavenging agency (antioxidant activity) which is determined by their reactivity as hydrogen or electron-donating agents, the stability of the resulting antioxidant-derived radical, their reactivity with other antioxidants and metal chelating properties.⁴⁰⁻⁴² Similarly, polyphenols derived from plants are of great importance due to their significant antioxidant and antibacterial properties.⁴³ Many studies have explored traditional medicinal plants that possess great potential for wound healing because they are versatile as antioxidant and antimicrobial sources.⁴⁴

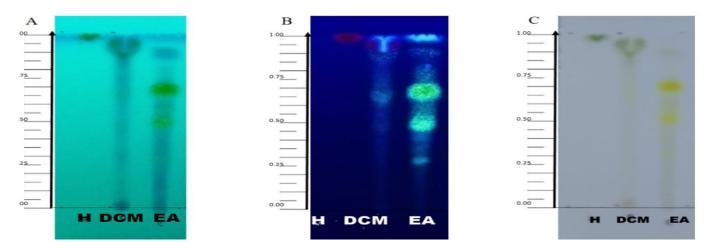


Figure 1: The chromatogram profiles of n-hexane (H), dichloromethane (DCM) and ethyl acetate (EA) fractions after sprayed by citroboric reagent under UV 254 nm ray (A), UV 366 nm ray (B) and visible ray (C).

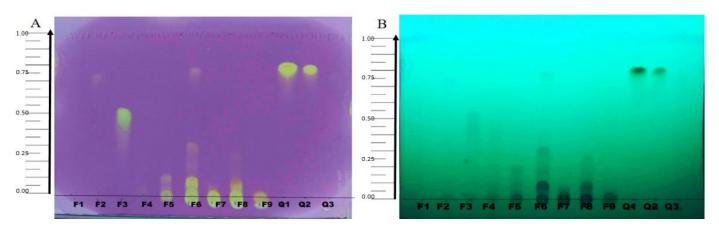


Figure 2: Chromatogram profiles of column chromatography fractions. (A) TLC plate after spraying with DPPH (B) TLC plate under UV 254 nm. F1-F9; fraction 1 – fraction 9, Q; Quercetin as standard

 Table 3: Antimicrobial activities (MICs) of n-hexane, ethyl acetate and ethanol extracts

Microorganisms	MIC, mg/mL		
	n-hexane	Ethyl acetate	Ethanol
	extract	extract	extract
Staphylococcus aureus	1 ± 0.05	0.5 ± 0.08	0.5 ± 0.08
MRSA	1.25 ± 0.01	0.1 ± 0.04	0.1 ± 0.05
Escherichia coli	6.25 ± 0.05	6.25 ± 0.09	6.25 ± 0.19

Note: MIC, minimum inhibitory concentration; MRSA, *Methicillin Resistant Staphylococcus aureus*

Table 4: DPPH free radical scavenging activity of extracts and fractions

Tested samples	IC ₅₀ (µg /mL)
n-hexane	$>1000\pm0.01$
Ethyl acetate	43.34 ± 1.43
ethanol	557.30 ± 0.04
n-hexane	$>1000\pm0.01$
dichloromethane	373.30 ± 0.01
Ethyl acetate	40.34 ± 1.43
Ascorbic acid	25.88 ± 0.05

Note: DPPH, 2,2-diphenyl-1-picrylhydrazyl

Conclusion

The extracts and fractions of *M. malabathricum* L. leaf revealed a potential source of antioxidant and antibacterial agents. The phytochemical evaluation and flavonoid test exhibited the presence of phenolic and flavonoids substances that were demonstrated to contribute to its biological activities. The results provide an explanation of the use of these plants in traditional medicine for healing of ailments related to infectious diseases. However, further phytochemical investigations are needed to determine the bioactive compound compositions, pharmacological and toxicity of these extracts and fractions. Extracts of *M. malabathricum* can become a potential source of natural antioxidants and antibacterial agents as a traditional medicinal plant from Riau, Sumatera Island, Indonesia.

Conflict of Interest

The authors declare no conflict of interest.

Authors' declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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