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SHORT COMMUNICATION

Antimicrobial activities and phytochemicals of *Murraya paniculata* L. flowers, leaves and bark crude extracts

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ABSTRACT

Aims: *Murraya paniculata* (L.) has been widely employed in medicine, has also been modified to serve as an ingredient in health foods and found application in cosmetics. This study was aimed to assess the biological activities of *M. paniculata* by analyzing the chemical compositions of its flowers, leaves and bark.
Methodology and results: Crude extracts drawn from the flowers, leaves and bark of *M. paniculata* underwent testing to determine the antibacterial properties in terms of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), as well as the overall chemical composition, total phenolic content, flavonoids and antioxidant activity**.** Crude extract of leaves exhibited the most potent antibacterial activity when tested against *Staphylococcus aureus* TISTR 1466, *Bacillus subtilis* ATCC 6633 and *Pseudomonas aeruginosa* ATCC 27853. The crude extract from bark delivered the most significant antibacterial activity when tested against *Micrococcus luteus* TISTR 9341, *Escherichia coli* ATCC 1261, *Pseudomonas* sp., *Streptococcus* sp. and Methicilin resistant *S. aureus* (MRSA). For all crude extracts, the MIC value against *M. luteus* TISTR 9341 was 12.5 mg/mL. Meanwhile, the MBC value for the crude extract of leaves against *B. subtilis* ATCC 6633 was 12.5 mg/mL, whereas, for flower and bark crude extracts, the MBC value against *S. aureus* TISTR 1466 was 25 mg/mL. Antioxidant activity was at its highest for the crude extract from bark (IC_{50} = 1.36 mg/mL). The highest phenolic content was recorded for the crude extract from bark, while the highest flavonoid content came from the crude extract of leaves (70.81 ± 0.31 mgGAE/g extract and 115.73 ± 1.18 mgQE/g extract, respectively).
Conclusion, significance and impact of study: The research findings suggest that the crude extracts of *M. paniculata* leaves and bark show greater significant levels of bioactivity than was the case for crude extracts from flowers. The research findings could help in exploring the possibilities of using *M. paniculata* for pharmaceutical purposes and in aquaculture.

Keywords: Antimicrobial, antioxidant, crude extract, *Murraya paniculata*

INTRODUCTION

Medical herbs are an important bioactive resource. For example, anthraquinone glycoside is known for its laxative property. Phenolic compounds and flavonoids are known as antioxidants or have anti-inflammation, antimicrobial and anticancer properties, including immune system promotion (Tungmunnithum *et al.*, 2018).

Andaman satinwood or Chinese box tree or Orange jessamine (*Murraya paniculata* L.) is generally found in Cambodia, South Vietnam, East Africa and also in Thailand for garden decoration. Zhu *et al*. (2015) reported that a solution of bark was an important active ingredient for snake venom antidotes, while the leaves and roots were also helpful for healing stomachache, toothache, coughing and diarrhea. Phytochemical studies of

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Andaman satinwood showed that it contained flavonoids, coumarins, alkaloids and essential oils (Ng *et al*., 2012). This tree has been used as a medicinal plant for its pharmacological efficiency (Sonter *et al*., 2021). Moreover, this plant is also used for healthy food, including as a flavoring agent for foods and cosmetics. Essential oils from leaf extracts could inhibit the fungus *Sclerotinia sclerotiorum*, which is a plant pathogen causing stem rot in many agricultural products, including cotton, vegetables, fruits and soybeans. In this study, we aim to analyze and evaluate the biological activities and chemical compositions of this plant's flower, leaf and bark crude extracts.

MATERIALS AND METHODS

Plant materials and preparation of extract

The leaf, bark and flower components of *M. paniculata* used in this research were gathered in Chanthaburi province, Thailand (Figure 1). The components were initially dried at a temperature of 40 °C for 3 days before grinding took place separately. The extracts were obtained using 70% ethanol for 72 h. In order to evaporate the solvents, the filtered contents were placed in a rotary vacuum evaporator, allowing the solid crude extracts to be removed. These dried materials were placed in a desiccator for 3-5 days and then refrigerated before experimental use.

Antibacterial activity

The microorganisms to be used underwent preparation from 18-24 h broth cultures, whereupon the suspensions were set as 0.5 McFarland standard. Each part of the plant extract was dissolved in 10% dimethyl sulfoxide (DMSO) and diluted for testing purposes to a concentration of 100 µg/ mL. Further two-fold dilutions were carried out in series to create a concentration range from 1.56-100 µg/mL with samples placed in 10 mL sterile test tubes which contained a nutrient broth. A micro-well dilution approach was then used to determine the MIC and MBC values for the *M. paniculate* extract when tested against five principal strains of pathogenic bacteria: *S. aureus* TISTR 1466, *E. coli* ATCC 1261, *P. aeruginosa* ATCC 27853, *B. subtilis* ATCC 6633 and *M. luteus* TISTR 9341, along with a pair of fish pathogenic bacteria: *Pseudomonas* sp., *Streptococcus* sp., obtained from tilapia sourced from the School of Science, King Mongkut's Institute of Technology Ladkrabang and Methicilin resistant *S. aureus* (MRSA) (Zgoda and Porter, 2001).

Phytochemical contents

Folin-Ciocalteau reagent and gallic acid were used as the standard in assessing the total phenolic content through the use of a colorimetric assay. The crude extracts were prepared so that 10 mg was dissolved in 1 mL DMSO (10 mg/L). The solutions obtained for flower, leaf and bark crude extract underwent further dilution to the respective levels of 2.5 mg/L and 1.25 mg/L (Baba and Malik, 2015).

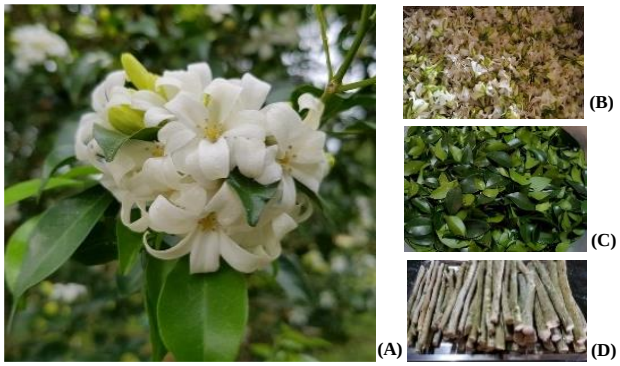

Figure 1: Plant materials in this study. (A) *M. paniculata* (L.); (B) Flower; (C) Leave; (D) Stem bark.

The approach of John *et al*. (2014) was used to assess flavonoid content, whereby a standard curve based on gallic acid was used to assess phenolic content and a standard curve based on quercetin was used to assess flavonoids. Each extract's total phenolic and flavonoid contents could be expressed in mg GAE and mg QE (gallic acid and quercetin equivalent) per gram.

Antioxidant activity

The free radical scavenging activity of the various plant extracts was examined using the DPPH assay. The 40 mg parts of the extract of *M. paniculate* were dissolved in 1 mL absolute ethanol to create a stock solution. The samples were then sequentially diluted (20.0, 10.0, 5.0, 2.5, 1.25, 0.63 and 0.31 mg/mL), while the positive control was 500 mM of alpha-tocopherol. A solution of DPPH (2,2 diphenyl-1-picrylhydrazyl) was freshly prepared using 100 µM/mL in absolute ethanol. This DPPH solution was then mixed with the plant extract, shaken strongly and incubated in darkness for 20 min at a temperature of 37 °C. Finally, the solution was placed on a microtiter plate in order to measure the absorbance at 517 nm (Singleton *et al*., 1999).

% scavenging = [(A control - A sample) × 100]/A control

A sample = absorbance of samples; A control = absorbance of control

The chemical composition

The chemical composition was analyzed using gas chromatography-mass spectrometry (GC-MS) (Agilent Model GC G1530N, MS G2573A). The starting conditions used for the GC-MS analysis were 5 min at 50 °C, 50-150 °C at 5 %/min and another increase for a further period of 5 min. The mobile phase involved He at a flow rate of 1.0 µm/min (Yang *et al.*, 2009).

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Data analysis

The data in this study are presented in the form of mean ± standard deviation (SD) and all tests were performed in triplicate. Statistical analyses involved the one-sample t-test, which employed a confidence interval of 95%.

RESULTS AND DISCUSSION

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *M. paniculata* showed the MIC value of all crude extracts against *M. luteus* TISTR 9341 was 12.5 mg/mL. The MBC value of leaf crude extracts against *B. subtilis* ATCC 6633 was 12.5 mg/ mL. Meanwhile, the MBC value of flower and bark crude extracts against *B. subtilis* ATCC 6633 and *S. aureus* TISTR 1466 was 25 mg/mL (Table 1). In addition, the MBC value of all crude extracts was more effective for Gram-positive of general pathogen bacteria (12.5, 25 mg/mL), while Gram-negative general pathogen bacteria and fish pathogen bacteria were effective on 100 mg/mL concentrations. The result of the antimicrobial activity of five general pathogenic bacteria, two fish pathogenic bacteria and one resistant bacteria showed that the crude extracts from flowers, leaves and bark could inhibit all those bacteria. Leaf and bark extracts were more efficient than flower extracts. This result is also in accordance with the quantitative findings of phenolic and flavonoid analysis. The high content of flavonoids from leaf and bark extracts was also the same as that in the study of Gautam *et al.* (2012), who also reported the efficiency of leaf extract in inhibiting both Gram-negative and Gram-positive bacteria. The inhibition involves the chemical composition of crude extract (i.e., alkaloid, flavonoid, phenolic, carbohydrate, protein and lipid). It is known that phenolic compounds offer antimicrobial properties through their capacity to cause cytoplasmic constituent leakage, whereby protein, glutamate, potassium and phosphate are drawn from bacteria. This process takes place when the cell membrane is damaged or as a consequence of the disturbance of cell peptidoglycan (Sayar *et al*., 2014).

Ng *et al.* (2012) reported that flower, leaf, root and stem extracts of *M. paniculata* provided enriched antioxidant, antimicrobial, anticancer and anti-diabetes properties. The result of antimicrobial activity in this study was similar to that of hexane, acetone, methanol, chloroform and solutions of leaf extract that could inhibit *P. aeruginosa* (Sonter *et al.*, 2021).

Biological activities from crude extracts of the three plant parts demonstrated that the highest content level of phenolic was found in barks crude extract (70.81 ± 0.31 mgGAE/g extract). The highest content of flavonoid was in leaf crude extract (115.73 ± 1.18 mgQE/g extract) (Table 2). The flavonoid analysis from the crude extract revealed that the highest flavonoid level was found in the leaf extract. This finding was in accordance with Sayar *et al*. (2014), who reported that flavonoid and phenolic compounds account for the antioxidant and antimicrobial properties of leaf extracts. However, the quantitative performance of flavonoids depended on the species, the type and the quality of solvent in the extraction procedure. (Adaramola and Onigbinde, 2016).

The highest antioxidant activity was found in bark crude extract (IC_{50} = 1.36 mg/mL). Subsequently, the half-maximal inhibitory concentrations (IC_{50}) from leaf and flower extracts were 3.15 and 7.37 mg/L, respectively. The results of DPPH showed the lowest IC_{50} from a bark extract. This represented the highest level of antioxidant activity. Basically, antioxidant activities on the hydrogen

Table 1: Antibacterial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *M. paniculata.*

Bacteria	MIC (mg/mL) of crude extract			MBC (mg/mL) of crude extract		
	Flowers	Leaves	Stem barks	Flowers	Leaves	Stem barks
S. aureus TISTR 1466	25	12.5	25	25	25	25
M. luteus TISTR 9341	12.5	12.5	12.5	100	50	50
B. subtilis ATCC 6633	25	12.5	25	25	12.5	25
E. coli ATCC 1261	25	25	25	>100	>100	>100
P. aeruginosa ATCC 27853	50	25	25	>100	>100	>100
Pseudomonas sp.	25	25	25	>100	>100	>100
Streptococcus sp.	50	50	50	>100	>100	>100
Methicilin resistant *S. aureus* (MRSA)	100	100	100	>100	>100	>100

Table 2: Biological activities from crude extracts, biological activities on antioxidant, total phenolic content and total flavonoid from *M. paniculata*.

Crude extract	IC_{50} (mg/mL)	Total phenolic content (mgGAE/g extract)	Total flavonoid content (mgQE/g extract)
Flowers	7.37	50.78 ± 0.27^{a}	38.28 ± 0.13^{a}
Leaves	3.15	61.03 ± 0.47^{b}	115.73 ± 1.18^{c}
Stem barks	1.36	70.81 ± 0.31^{c}	101.94 ± 0.73^{b}

Each value is the mean ± S.D. of three replicates. Different letters in the same column are significantly different (p<0.05).

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transfer capability of electrons that results from plant metabolites, such as the phenolic compounds and flavonoids in the crude extracts (Nisa *et al*., 2013).

The chemical composition of flowers, leaves and stem bark crude extracts were analyzed by the use of GC-MS. It was found that 2-methoxy-4-vinylphenol, 2H-1-benzopyran-2-one, hexadecanoic acid, hexadecanamide, murrialongin and 9-Octadecenamide were the major compounds from all parts of the crude extracts**.** Those chemicals have been reported for their bioactivities, including anti-inflammatory, antioxidant, antimicrobial and antiviral properties, as well as immune stimulation (Aziz *et al.*, 2010; Ravikumar *et al*., 2012; Menezes *et al.*, 2015; Dosoky *et al.*, 2016).

CONCLUSION

The findings show that the crude extracts from leaves and bark offer a greater significant level of bioactivity than the crude extract from flowers. In addition, pathogenic bacteria can be inhibited. The results of this study may find applications in the pharmaceutical and aquaculture fields, as well as in the development of medicines. For instance, in aquaculture, crude extracts have been used to restrict and manage fish pathogenic bacteria by mixing the extracts with feed in a ratio of 0.1 kg crude extract: 1 kg feed.

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