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# ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF *Murraya Paniculata* LEAVES VIA METHANOL EXTRACTION

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*Murraya paniculata* (*M. paniculata*), a well-known medicinal plant, has widely been used in Ayurveda and other oriental healing practices for the treatment of a number of human diseases. The extract of *M. paniculata* can be prepared from different parts of the plant, especially the leaves. The main objective of this study is to evaluate the antibacterial and antioxidant activities of the methanolic extract of *M. paniculata* leaves using DPPH and disk diffusion methods. *M. paniculate* methanolic extract (MpME) was prepared from the leaves using the maceration method, which resulted in an extract yield of 13.27%. The 1,1-diphenyl-2-picrylhydrazil (DPPH) method was used to evaluate the antioxidant activity of MpME. Ascorbic acid was used as a positive control, and methanol as a negative control to ensure the validity of the assay. The highest radical scavenging activity (RSA) for MpME was 54.47%  $\pm$  0.51 at a concentration of 1000  $\mu$ g/mL (IC<sub>50</sub> value: 1862  $\mu$ g/mL). The antibacterial activity of MpME was evaluated by the agar disk diffusion method against two bacterial strains: a Gram-positive bacterium (*Staphylococcus aureus* (ATCC 12600) and a Gram-negative (*Pseudomonas aeruginosa* (ATCC 10145). Each assay was done in triplicate. In this assay, the positive controls vancomycin and gentamicin were used as indicators to prove the validity of the assay. The Gram-positive bacteria were more susceptible to the extract (average diameter of inhibition zone for *Staphylococcus aureus* = 6  $\pm$  0.06 mm) than the Gram-negative bacteria (average diameter of inhibition zone for *Pseudomonas aeruginosa* = no inhibition zone). The results of the study show that MpME has antibacterial and antioxidant properties. Thus, these findings indicated that MpME could be developed as an antioxidant and antibacterial agent in various applications.

**Keywords:** *Murraya paniculata* extract, maceration, antibiotic resistance, phytochemical, phenol, flavonoid

## INTRODUCTION

According to the World Health Organization (WHO), an infectious disease is caused by pathogenic microbes such as bacteria, viruses, multicellular parasites, or fungi (World Health Organization Infectious Diseases). This disease can be transmitted either directly or indirectly from one person to another. Antibiotics are drugs that cure infections by destroying or suppressing the development of the bacteria responsible for the infection. Antibiotics are usually classified according to their narrow or broad-spectrum properties. Narrow-spectrum antibiotics target specific types of bacteria, while broad-spectrum antibiotics target a wide range of microorganisms (CDC, 2019). According to the Centers for Disease Control and Prevention (CDC), antibiotic resistance refers to the ability of microorganisms to resist the drugs designed to eliminate them (CDC, 2019). It is currently one of the most prominent global health threats to mankind. To address the problem of antibiotic resistance, it is essential to develop new antibacterial agents from natural sources as a way to effectively improve the body's defence mechanisms.

*M. paniculate* (Figure 1a), also known as orange jasmine, kemuning, and kamini, belongs to the *Rutaceae* family in the plant kingdom (Arya *et al.*, 2017), as depicted in Table 1. This plant is native to areas in Southeast Asia (Perry & Metzger, 1980), such as Southern China, Vietnam, and the Malay Peninsula. In China, the ornamental plant *M. paniculata* is widely cultivated on the sides of the road for decorative purposes. In Southeast Asia, it has been used for topical medicinal applications as health food (Imai *et al.*, 1989). For example, a solution of the bark of *M. paniculata*, when mixed with other ingredients, is used as an antidote for snake bites. Furthermore, the leaves of *M. paniculata* are known to possess antibacterial activity against *Mycococcus pyogenes* and *Escherichia coli* (Sastri, 1956).

**Table 1:** Taxonomy of *Murraya paniculata* (Adapted from India Biodiversity Portal).

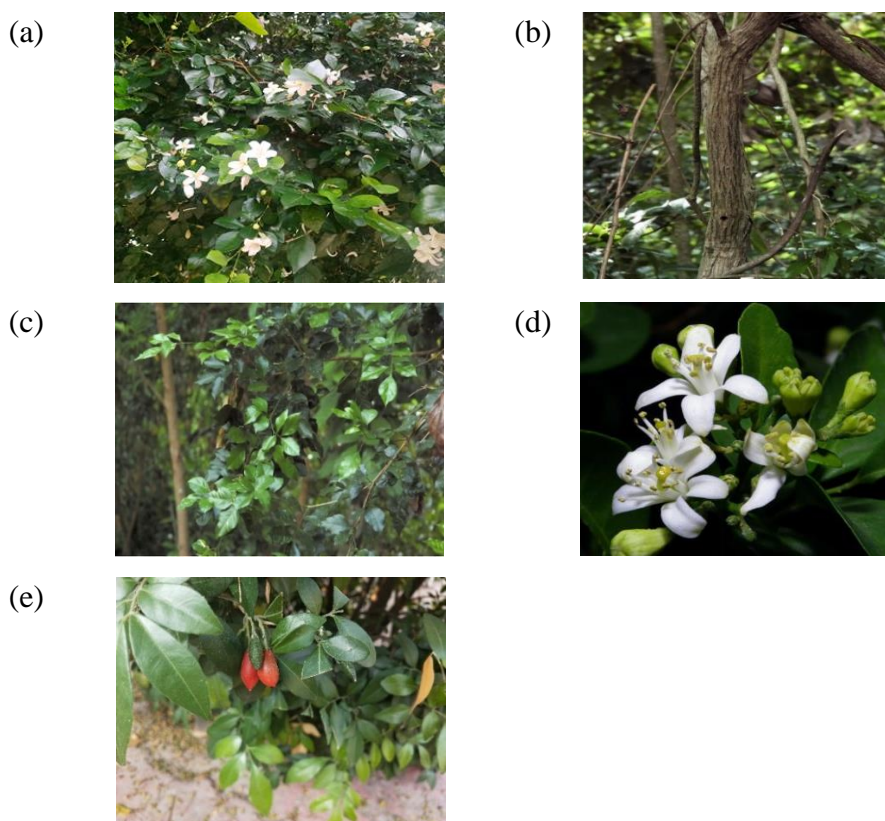
<b>Plant Profile: Orange Jasmine</b>	
<b>Family</b>	Rutaceae
<b>Kingdom</b>	Plantae
<b>Division</b>	Magnoliopsida
<b>Order</b>	Sapindales
<b>Genus</b>	Murraya
<b>Species</b>	<i>Murraya paniculata</i> (L.) Jacq.

Oxidation caused by reactive oxygen species (ROS) leads to the degeneration of cell membranes and membrane proteins, as well as DNA alterations that cause aging and initiate or promote the progression of numerous diseases (Dontha, 2016). Antioxidants are chemical substances that bind to oxygen-free radicals to prevent their damage to healthy cells. A few research have extensively investigated the antioxidant activity of *M. paniculata*. For example, Chen and co-workers have reported that *M. paniculate* extract (MPE) is a stronger antioxidant than vitamin E (Chen *et al.*, 2009). In a preclinical study, Gautam and collaborators found that MPE decreases the free radical level in diabetic rats significantly (Gautam *et al.*, 2012). Rodríguez and collaborators have also reported that the antioxidant activity of *M. paniculata* essential oil is stronger than that butylated hydroxyanisole and butylated hydroxytoluene

(Rodríguez et al., 2012). In addition, Mita and collaborators have determined that total phenolic content of MPE is high and its free radical scavenging activity is excellent (Mita et al., 2013).

Several secondary metabolites (>70 flavonoids) have been isolated from the leaves and roots of *M. paniculate*. Flavonoids, with their hydroxyl groups and unsaturation, which are present in several medical plants, are widely recognized for their natural antioxidant properties (Olawore et al., 2005; Saied, 2005; Rao et al., 2011; Kinoshita & Firman, 1996; Sukari et al., 2003; Zhang et al., 2011). Frequently, the antioxidant activity of an extract correlates linearly with the total flavonoid content of the extracts (Tchoumtchoua et al., 2013; Lotito & Frei, 2004). Furthermore, the leaves and roots of this plant are utilized in folk medicine to treat stomachache, toothache, gout, diarrhea, dysentery, rheumatism, cough, and hysteria (Rahman et al., 1997; Ghani, 1998). The use of *M. paniculata* has also been recommended for the treatment of cuts, joint pains, and body aches (Azizi et al., 2010).

*M. paniculate* plant grows into a small, spreading shrub about 2.5 meters tall. The main stem is 16 cm in diameter, dark green to brownish, and covered with many spots. Removing the bark (Figure 1b), the white wood underneath becomes visible. The leaves (Figure 1c) are exstipulate, bipinnate, 30 cm long, and have a reticulate venation. A fully bloomed flower (Figure 1d) has an average diameter of 1.12 cm. The flowers are bisexual, funnel-shaped, and sweetly scented. The ripe fruits (Figure 1e) are red with a gleaming surface and contain a single seed that is spinach green in color (Wagay et al., 2017). In China, India, and Indonesia, *M. paniculata* is traditionally used in phytotherapy (Selestino Neta et al., 2016).



**Figure 1:** (a) *Murraya paniculate* plant; (b) Bark; (c) Leaves; (d) Flowers; (e) Fruits  
(Adapted from India Biodiversity Portal)

Extraction refers to the process of isolating the therapeutically active part of plant or animal tissue components from inactive or inert components by using selected solvents with standard extraction methods. In the current scientific literature, *M. paniculata* has been reported to have antihyperglycemic, antifungal, antibacterial, analgesic, antioxidant, antispasmodic, bronchodilator, and vasodilator activities. In addition, nephroprotective properties have been demonstrated in diabetic nephropathy (Selestino Neta *et al.*, 2016). Until recently, limited studies were found on the Malaysian native *M. paniculata* against bacteria-causing nail diseases. Previous studies have shown that the plant extract of *M. paniculata* is known to have antioxidant and antimicrobial properties (Sonter *et al.*, 2021). In this study, we evaluated the methanol extract of *M. paniculata* (MPE) for its reducing activity, lipid peroxidation inhibition, radical scavenging activity (for radicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH•), superoxide anion ( $O_2^{\cdot-}$ ), and hydroxyl ( $OH\cdot$ )), and hydrogen peroxide ( $H_2O_2$ ) scavenging activity. This study highlights the utilization of leaves from *M. paniculata*, which are derived from Malaysia, to investigate the available phytoconstituents and determine whether they exhibit antibacterial and antioxidant effects against bacteria-causing nail diseases.

## EXPERIMENTAL

### Chemicals and Materials

*M. paniculate* leaves, methanol, hydrochloric acid, Dragendorff's reagent, sodium hydroxide, 5% ferric chloride, sulfuric acid, Folin-Ciocalteu reagent, gallic acid, sodium carbonate, Mueller Hinton (MH) powder, sodium chloride, gentamicin disks, vancomycin disks, 1,1-diphenyl-2-picrylhydrazyl DPPH powder and ascorbic acid powder.

### Plant Material and Extraction

The leaves of *M. paniculata* were purchased from a local nursery in Pahang. The leaves were rinsed thoroughly with tap water and then cut into small pieces. The sample was oven-dried at 50°C for three days to remove any moisture. After drying, the leaves were ground into fine powder using a blender, and the dry powder of the leaves was stored at room temperature (RT) for subsequent use.

*M. paniculate* methanolic extract (MpME) was prepared from the leaves using the maceration method. The powder from *M. paniculate* leaves (100 g) was soaked in 80% methanol at a ratio of 1:10 and placed in a Schott bottle (Wagay *et al.*, 2017). A sufficient amount of methanol was used (i.e. enough to submerge the potency completely), and the samples were extracted three times with fresh methanol each time over a period of 3 days. The MpME was filtered and concentrated to dryness in a rotary evaporator (BUCHI, Switzerland) under vacuum at 40°C and then kept in a desiccator. The percentage yield of MpME obtained (Figure 2) resulted in an extract yield of 13.27%. The percent yield was higher than that reported by Sonter *et al.* (2021), who obtained 4.8% of the methanol extract.



**Figure 2:** Sample of *M. paniculata* extract.

## Phytochemical Screening

### Qualitative Analysis

The *M. paniculata* methanolic extract (MpME) was subjected to qualitative phytochemical analysis to determine the presence of alkaloids, flavonoids, terpenoids, and phenol by following standard procedures.

#### A. Alkaloid Test

In a small test tube, 1 mL of MpME was mixed with 2 drops of Dragendorff's reagent. The appearance of a brown-orange precipitate confirmed the presence of an alkaloid (Wardan et al., 2019)

#### B. Flavonoid Test

In a small test tube, 1 mL of MpME was added slowly with 1 mL of concentrated NaOH. The appearance of yellow color developed, confirming the presence of flavonoids (Shaikh *et al.*, 2020).

#### C. Phenol Test

The test was performed by adding drops of 5% FeCl<sub>3</sub> in a small test tube containing 0.5 mL of distilled water. Then, 1 mL of MpME was mixed into the solution. The formation of the dark green color showed the presence of phenolic compounds (Shaikh *et al.*, 2020).

#### D. Saponin Test

In a small test tube, 1 mL of MpME was mixed with 2 mL of distilled water. The was added to the same test tube, and the mixture was shaken for at least 15 minutes. The formation of foam at the 1 cm layer indicated the existence of saponins (Shaikh *et al.*, 2020)

### Quantitative Analysis

#### Total Phenolic Content

The extract of *M. paniculata* leaves was performed by quantitative phytochemical analysis based on total phenolic content (TPC). The phenolic content of the defatted MpME was determined by the spectrophotometric method (Singh et al., 2012) using the Folin-Ciocalteu (FC) method. Different concentrations of standard gallic acid (GA) and distilled water were

prepared in test tubes. This is followed by the addition of 1.5 mL of the test solution FC to each test tube containing GA solution. The mixture was vortexed and kept in a dark cabinet for 15 minutes. Then, 1.5 mL of Na<sub>2</sub>CO<sub>3</sub> solution was added to each test tube and left in the darkroom for 90 minutes. These steps were also repeated with the *M. paniculata* extract. Absorbance was measured at 725 nm against the blank sample, and gallic acid was used as a standard. All determinations were performed in triplicate. TPC was expressed as gallic acid equivalent per gram dry weight of extract (mg GAE /g extract) (Sonter et al., 2021).

## **Determination of Antioxidant Activity**

### ***DPPH radical scavenging activity assay***

DPPH (1,1-diphenyl-2-picrylhydrazil) free radical scavenging was carried out for the determination of antioxidant activity (Samritsakulchai, 2016). In 100 mL of methanol, 2.4 mg of DPPH powder was dissolved to prepare a DPPH solution. As a positive control, 20 mg of ascorbic acid was dissolved in 20 mL of distilled water. Then, 1 mL of the ascorbic acid solution was added to a test tube, and a serial dilution was performed to obtain concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL. This step was performed in triplicate and was also performed with the *M. paniculata* extract. Then, 2.7 mL of DPPH solution was added to 300 µL of the different concentrations of the sample. Then, the mixtures were stored in a dark cabinet for 15 min. For the negative control, 300 µL of methanol was mixed with 2.7 mL of DPPH solution, and the mixture was stored in a dark cabinet for 15 minutes. The absorbance was measured after 15 minutes at 517 nm using methanol as a blank on a UV-vis spectrometer. The following formula was used to calculate the radical scavenging activity:

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100 \%$$

## **Determination of Antibacterial Activity**

### ***Bacteria***

The bacterial organisms used in this study consisted of two bacterial strains, which were purchased from American Type Culture Collection (ATCC). The bacterial strains were composed of Gram-positive bacteria (*Staphylococcus aureus* (ATCC 12600)) and Gram-negative bacteria (*Pseudomonas aeruginosa* (ATCC 10145)). These bacteria were obtained from the laboratory of UniKL-RCMP. The bacteria were cultivated in a solid medium of Nutrient Agar (NA) plates by streaking method and stored at 4°C.

### ***Preparation of Culture Media (Agar)***

Mueller Hinton (MH) agar was used for the antibacterial test, whereby 13.3 g of MH powder was dissolved in 350 ml of distilled water and autoclaved at 121°C for 20 minutes. Once the agar cooled to 45°C, the medium was poured into a sterile disposable petri dish. The pouring plate process was operated in the laminar airflow and left at room temperature until solidified. The prepared plates were put in upside-down positions and kept in the refrigerator.

### **Preparation of Bacterial Suspension**

A single colony of each bacteria culture in the plate for Gram-positive and Gram-negative were transferred in 10 ml of MH using a sterile loop. The prepared bacterial suspension was incubated for 3 hours at 37°C to achieve the exponential phase of 0.5 McFarland Standard (Abdallah, 2016). Each bacterial suspension was subjected to turbidity check using a single-beam spectrophotometer at 600 nm. A 0.5 McFarland Standard has an absorbance reading of 0.08 to 0.1. All bacterial suspensions were directly used in the antibacterial activity assay.

### **Disc Diffusion Method**

The antibacterial activity assay uses the disk diffusion method described by Sonter et al. (2021). This method is based on the clear zone formed around the disk. Partial inhibition was indicated by a semi-clear zone and complete inhibition by a clear zone. The sterilized media was poured onto sterilized Petri dishes (20 ml per Petri dish) and allowed to stand until solidified. Wells of 6 mm diameter were made in the solidified media with the help of a sterile borer. A sterile swab was used to spread the bacterial suspension evenly on the surface of the solidified media, and extracts of *M. paniculata* at various concentrations of 1, 2, 2.5, and 5 mg/mL were added to each well using a micropipette. The plates were incubated at 37°C for 24 hours. Ampicillin and gentamycin were used as antibacterial standards. This experiment was carried out in triplicate, and zones of inhibition were measured at the mm scale.

### **Statistical Analysis**

All data were analyzed by ANOVA and expressed as mean  $\pm$  SD. Analysis of variance and the difference between the samples were determined by Duncan's multiple range test, and  $p < 0.05$  was used to determine statistical significance.

## **RESULTS AND DISCUSSION**

### **Phytochemical Analysis**

Qualitative and quantitative analyses are essential to identify phytochemical constituents present in the extract of *M. paniculata* leaves. Therefore, phytochemical screening was conducted to evaluate the active phytoconstituents in MpME.

### **Qualitative Test**

Phytochemicals are biologically active, where the secondary metabolites may exert antimicrobial properties (Aziman et al., 2012). In this study, the presence or absence of phytochemicals was confirmed by observing colour intensities upon reaction with different reagents. The overall results of the phytochemical analysis are shown in Table 3. It could be derived from the results that all samples of MpME had shown positive indications of alkaloid, flavonoid, phenol and saponin. This is partly supported by Barile et al. (2007), who reported that saponins are active constituents which are known for their antimicrobial activity, a trait which is desirable for disease resistance in plants.



**Table 3:** Phytochemical analysis of *M. paniculata* methanolic extract.

No.	Test	Extracted Sample		
		1	2	3
1	Alkaloid	+	+	+
2	Flavonoid	+	+	+
3	Phenol	+	+	+
4	Saponin	+	+	+

(+) indicates positive results.

### Total Phenolic Content (TPC)

TPC was quantified for MpME extraction by Folin-Ciocalteu (FC) method using Gallic acid as standard. TPC for MpME was equivalent to  $16.88 \pm 4.98$  GAE /mg. The reaction between the FC reagent and the MpME resulted in the blue colour formation, which absorbed the radiation and allowed quantification.

A phenol loses an H<sup>+</sup> ion and forms a phenolate ion, which reduces the folic acid-Ciocalteu reagent under basic reaction conditions (Sontner et al., 2021). The results were carried out in triplicates, and the values were expressed as mean. It has been documented that the secondary metabolites isolated from medicinal plants are highly reactive in neutralizing free radicals by donating the odd electron or hydrogen atom due to the presence of hydroxyl groups in their chemical structures (Sayed et al., 2015).

### Antioxidant Properties by DPPH Scavenging Activity

The DPPH stable radical scavenging model is a widely used method to evaluate antioxidant activities in a relatively short time compared to other methods (Birasuren et al., 2012). The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable molecule. The reducing ability of DPPH radicals was determined by the decrease in absorbance at 517 nm caused by the antioxidants. It can be visually noticeable as discoloration from the purple color to the yellow color. The overall results of the percentage radical scavenging activity of ascorbic acid and plant extract are shown in Tables 5-6 and Figures 8-9.

Table 5 shows an increasing RSA of ascorbic acid with increasing concentrations from 31.25-1000  $\mu\text{g/mL}$ . Similar pattern could be observed for RSA of *M. paniculata* extract in Table 6, whereby an increasing RSA is reported from as low as 9.83% at the lowest concentration of 31.25  $\mu\text{g/mL}$  to the highest RSA at 54.47% for 1000  $\mu\text{g/mL}$ . This shows an increasing antioxidant potential of *M. paniculata* extract.

**Table 5:** Percentage of radical scavenging activity (RSA) of ascorbic acid.

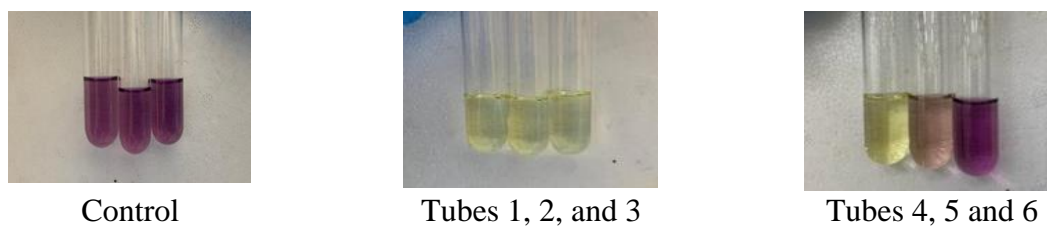
Concentration ( $\mu\text{g/mL}$ )	Absorbance			% RSA			
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Mean
31.25	0.57	0.55	0.53	11.1	13.63	16.72	13.82 $\pm$ 2.81
62.5	0.23	0.16	0.15	63.90	76.25	77.11	72.42 $\pm$ 7.39
125	0.05	0.03	0.03	92.60	96.11	95.79	94.84 $\pm$ 1.94
250	0.05	0.03	0.03	93.23	96.08	95.97	95.09 $\pm$ 1.61
500	0.05	0.03	0.03	92.84	96.14	95.45	94.81 $\pm$ 1.75
1000	0.03	0.03	0.03	95.75	96.14	96.08	95.99 $\pm$ 0.21

**Table 6:** Percentage of radical scavenging activity (RSA) of *M. paniculata* extract.

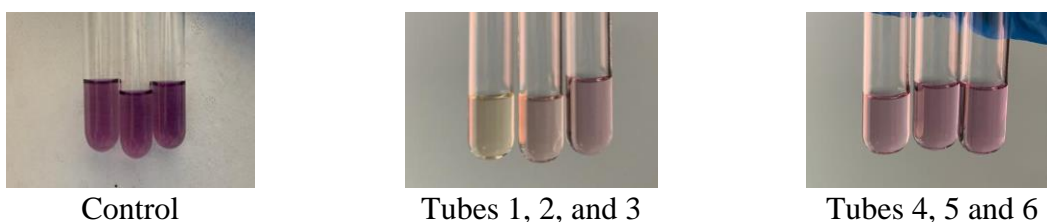
Concentration ( $\mu\text{g/mL}$ )	Absorbance			% RSA			Mean
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	
31.25	0.58	0.58	0.58	10.05	9.62	9.83	9.83 $\pm$ 0.22
62.5	0.57	0.57	0.57	11.37	11.00	11.60	11.32 $\pm$ 0.31
125	0.56	0.53	0.56	12.73	17.81	12.15	14.23 $\pm$ 3.12
250	0.50	0.52	0.50	22.38	19.08	21.93	21.13 $\pm$ 1.79
500	0.47	0.46	0.47	26.26	28.90	26.56	27.24 $\pm$ 1.45
1000	0.29	0.30	0.30	55.05	54.15	54.20	54.47 $\pm$ 0.51

The plant methanol extract was significantly different to the standard of ascorbic acid where  $p < 0.005$

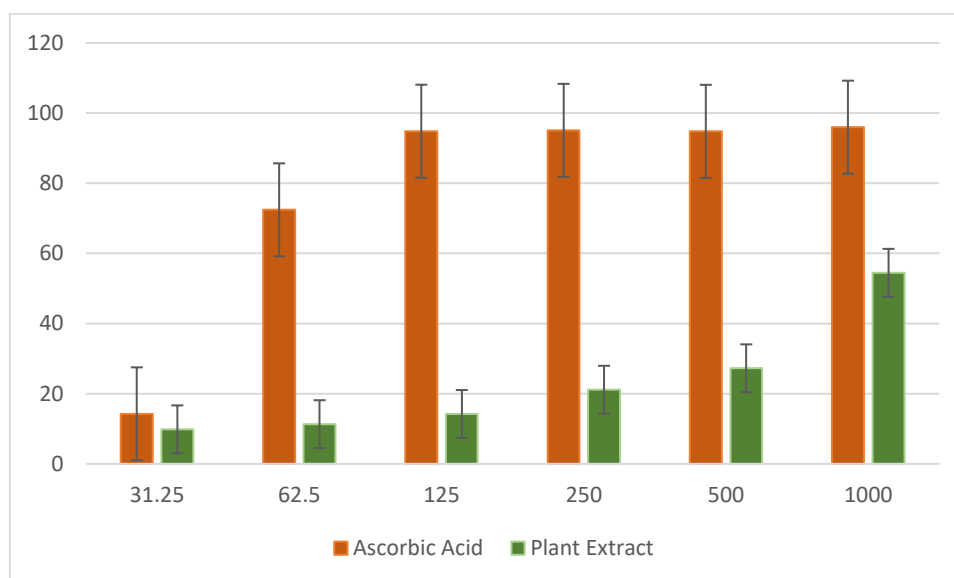
Based on Figure 8, tubes 1, 2, 3, and 4, with different concentrations of 1000, 500, 250, and 125  $\mu\text{g/mL}$  of ascorbic acid, respectively, showed yellow-colored solutions as a result of DPPH decolorization. With decreasing concentrations of ascorbic acid in tubes 5 and 6 (62.5 and 31.25  $\mu\text{g/mL}$ , respectively), purple-colored solutions were observed. For *M. paniculata* extract, yellowish solution was only observed at the highest concentration of 1000  $\mu\text{g/mL}$ . Subsequently, light purple-colored solutions corresponding to the concentrations of 500, 250, 125, 62.5, and 31.25  $\mu\text{g/mL}$ , respectively, were observed in tubes 2 to 6 ( Figure 9), which indicated moderate antioxidant activities.



**Figure 8:** DPPH of standard ascorbic acid.



**Figure 9:** DPPH of *M. paniculata* extract.



**Figure 10:** Radical scavenging activity of ascorbic acid vs. *M. paniculata* extract at different concentrations.

Figure 10 shows that the highest concentration of ascorbic acid, 1000 µg/mL, reached an RSA of more than 50% up to a certain concentration of 62.5 µg/mL. At the maximum concentration of 1000 µg/mL, the RSA value of the plant extract was  $54.47 \pm 0.51\%$ . The RSA value of the plant extract indicates that it has a moderate effect against free radicals. However, an error was found in ascorbic acid, as the RSA value decreased significantly at a concentration of 31.25 µg/mL. Ascorbic acid, or vitamin C, supports collagen synthesis in humans, strengthens the immune system by scavenging free radicals in the body, activates enzymes, and reduces oxidative stress (Yin et al., 2022). Ascorbic acid can be oxidized after being exposed to light in an unsuitable environment, which could be one of the factors contributing to this deficiency. It can also degrade after prolonged storage at room temperature (Yin et al., 2022).

The IC<sub>50</sub> value was also determined to evaluate the ability of the sample to inhibit 50% of free radicals. The IC<sub>50</sub> value of the plant extract was 1862 µg/mL, which was higher than a study (206.05 µg/mL) by Ahmed et al. (2019). There is a positive relationship between antioxidant ability and the phenolic content of the plant extract (Ahmed et al., 2019). Since the total phenolic content of the *M. paniculata* extract was lower than in a previous study by Ahmed et al. (2019), then this could provide an explanation to the reduced antioxidant activity of the plant.

### Antibacterial Activity Test by Disc Diffusion Method

Antibacterial activity of MpME against the bacterial strains, clinically isolated Gram-positive and Gram-negative bacteria are shown in Table 4 and Figure 3. The results demonstrated various antibacterial activities of MpME against the two selected bacterial strains.

**Table 4:** Diameter of the inhibition zones of bacterial strains growth of *Murraya paniculata* extracts

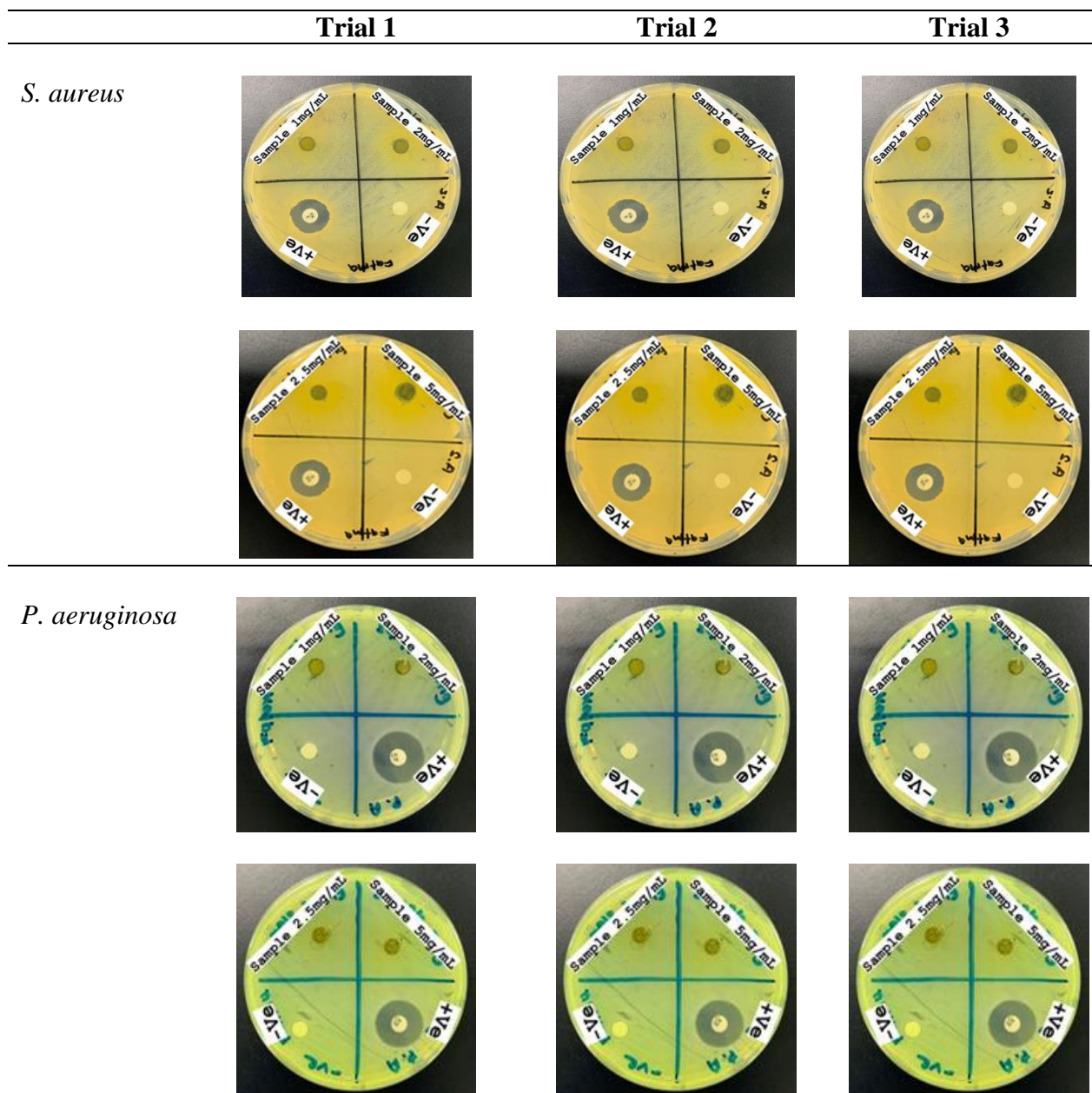
MpME extract	Inhibition Zone Diameter (mm)	
	<i>S. aureus</i>	<i>P. aeruginosa</i>
1 mg/mL	ND	ND
2 mg/mL	ND	ND
2.5 mg/mL	ND	ND
5 mg/mL	6 ± 0.06	ND
Vancomycin	16 ± 0.12	-
Gentamicin	-	20 ± 0.1

Values are expressed as mean±SD of triplicate experiments.

\*(ND) indicates not determined.

The plant extract was significantly different from the standard of vancomycin, where  $p < 0.005$ .

Based on Table 4, the MpME exhibits a zone of inhibition against *S. aureus* only at a dose of 5 mg/mL. From the obtained result, the antibacterial activity of *M. paniculata* extracts required high concentration due to its low phenolic content. In contrast, no inhibition against *P. aeruginosa* was observed at any concentration of the plant extracts. The cell walls of Gram-positive bacteria contain peptidoglycan, whereas Gram-negative bacteria lack peptidoglycan but have a high concentration of lipopolysaccharide (Berman, 2019). While peptidoglycan layers can absorb antibiotics easily, thus making Gram-positive bacteria, the presence of lipopolysaccharide, on the other hand, can potentially inhibit the permeability of the extract into the bacterial cell, making Gram-negative bacteria more resistant to kill. In a study by Sonter et al. (2021), 1 mg/mL of methanol extract was found to have an inhibition zone of 3.6 mm in *S. aureus*. At a dose of 0.25 mg/mL, the inhibition for *P. aeruginosa* was 4.3 mm. *P. aeruginosa* is an opportunistic pathogen that causes illness and death in immunocompromised individuals and exhibits remarkable resistance to antibiotics.



**Figure 3:** Antibacterial activity of *M. paniculata* extract at different concentrations.

## CONCLUSION

In conclusion, the leaves of *M. paniculata* were extracted using the maceration method, and the total yield was 13.27%. Phytochemical analysis included both qualitative and quantitative tests. The qualitative tests confirmed the presence of alkaloids, flavonoids, phenol and saponin, while the quantitative analysis showed a total phenolic content of  $16.88 \pm 4.98$  GAE /mg. In DPPH assay, the antioxidant activity was observed by decolorization of purple-colored solution to yellow. The  $IC_{50}$  value of the plant extract was  $1863 \mu\text{g/mL}$ , which was lower than the standard ascorbic acid. Lastly, the plant extract showed antibacterial activity with an inhibition zone diameter of  $6 \pm 0.06$  mm against *S. aureus*.

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