Antibacterial Activity of Flower, Leave and Stem Extract of *Melastoma decemfidum*

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***Abstract*—Melastoma decemfidum (*M. decemfidum*) or locally identified as "senduduk putih" is a small shrub with white petals. Locally, different part of *M. decemfidum* has been used as traditional remedies for various illnesses. It is used to treat postpartum conditions, hepatitis, leucorrhea, swelling, mouth ulcer, toothaches, and sinusitis. The objective of this study is to determine the antibacterial activities of methanolic extract of different parts of *M. decemfidum* which includes flower, leaves and stem. These extracts of *M. decemfidum* was used to study the antibacterial activity against two Gram positive bacteria (*Bacillus cereus, Staphylococcus aureus*) and two Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) by disc diffusion method at different concentrations (100, 150, 200, 250 mg/ml). The antibacterial activity is determined from the evaluation of the zone of inhibition. Based on the results, the different parts of *M. decemfidum* exhibited diverse activity against the tested bacteria. The largest diameter of zone of inhibition was 22.6 mm which was recorded by stem extract (250 µg/mL) against *Pseudomonas aeruginosa.* The maximum diameter of zone of inhibition recorded by flower and leave extract were both 15.8 mm against *P. aeruginosa* at 250 µg/mL. The antibacterial activities shown by *M. decemfidum* could indicates its potential for development of new drugs for pharmaceutical application.**

***Index Terms*—antibacterial activity, *Melastoma decemfidum*, Melastomataceae, medicinal plant, methanolic extract**

I. Introduction

Melastoma decemfidum (*M. decemfidum*) or locally identified as "senduduk putih" is a small shrub with white petals [1]. *M. decemfidum* is from the family of Melastomataceae [2] and have wide geographic distribution that range from Madagascar to India and Australia. In Malaysia, the lowland and mountain forest are the habitat for the *M. decemfidum* where it populates the open area [3], [4].

Locally, different part of *M. decemfidum* has been used as traditional remedies for various illnesses. The flowers are used to heal wound and chronic cough, the leaves are used for hemorrhoids and diarrhea while the stem are used to treat leucorrhea. Apart from that, Khadijah and Noraini (2007) stated that the plant is used to treat postpartum conditions, hepatitis, leucorrhea, swelling, mouth ulcer, toothaches, and sinusitis [5]. Some of the illness stated is related to microbial infections. Hence, this study aims to examine the antibacterial properties of different parts of *M. decemfidum*. Besides, according to Mahesh and Satish (2008), plants that are utilized for its medicinal attributes are a source of many potent and powerful drugs [6]. Thus, since *M. decemfidum* is recognized as a medicinal plant, this indicates that the plants may have potentiality as sources for antimicrobial drugs.

The medicinal value of a medicinal plant can be associated with the presence of bioactive compounds found within the plant [7]. Sarju *et al.* (2012) stated that *M. decemfidum* contains flavonoids that may exert the antimicrobial effect due to the presence of hydroxyl group located at C-3 in the C ring that may contributes to the antibacterial action of flavonoids by reducing the membrane fluidity [2]. Previous reports described the isolation of several flavonoids from *M. decemfidum* which includes naringenin and kaempferol and two triterpens. Hence, in order to further understand the antimicrobial activities of *M. decemfidum*, the presence of flavonoids will be identified from extracts of *M. decemfidum* [8], [9], [4].

Previous phytochemical study on *M. decemfidum* revealed that this species have high amount of secondary metabolites comprising flavonoids such are polyphenols [10]. There has been reports that stated the isolation of six flavonoids which includes naringenin and kaempferol and two triterpenes discovered on flowers and leaves of *M. decemfidum* [8], [9]. The existence occurrence of bioactive flavonoids in *M. decemfidum* may influence the antimicrobial activities of this medicinal plant.

II. Materials and Methods

A. Plant Materials

Melastoma decemfidum was collected from Tanah Merah, Kelantan, Malaysia. The leaves, flowers and stem of *Melastoma decemfidum* was used in the study.

B. Preparation of Plant Extract

The leaves, flower and stem was oven dried for 3 days at 45 ºC and then converted into a very fine powder. The methanol extract was prepared by soaking 200 g of the plant material in 1 000 mL methanol for three days then the mixture will be filtered with filter paper (Whatman No. 1) and extracted under compact pressure in a rotating

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evaporator to yield the crude extract. Prior to use, the crude extract will be dissolved in distilled water to a final concentration of 100 mg/mL, 150 mg/mL, 200 mg/mL and 250 mg/mL.

C. Test Microorganisms

A total of four common pathogenic microorganisms was used in the study. They includes two Gram-positive bacteria; (*Bacillus cereus*, and *Staphylococcus aureus*) and two Gram-negative bacteria; (*Escherichia coli*, and *Pseudomonas aeruginosa*).

D. Kirby-Bauer Disc Diffusion Technique

Disc diffusion technique was employed to measure the ability of the antimicrobial compound found in the plant extracts to inhibit bacteria growth in-vitro. The positive controls used was tetracycline. Sterile filter paper disc was impregnated with 20 µL of sample extracts with different concentration of 100 mg/mL, 150 mg/mL, 200 mg/mL and 250 mg/mL each. The impregnated discs was placed on the inoculated Mueller-Hinton agar (MHA). The tested plate will be incubated at 37ºC for 24 hours. The antibacterial activity was measured and expressed in terms of zone of inhibition of bacterial and fungal growth around each disc to the nearest millimetre (mm) by using a ruler.

E. Minimum Inhibitory Concentration(MIC)

Each extract was dissolved in distilled water and diluted in a concentration of 100 mg/ml. All wells used was filled with 200 µl of media. About 100 µl of extract was transferred to the first well and mixed. The initial content of the first well will contain 300 µl of media and extract mixture. Two-fold serial dilutions was performed by transferring 100 µl of the mixture in the first well into the next consecutive well until the end of the row while making sure that 200 µl of the mixture will remains in each well. In the last well, 100 µl of the mixture was discharged so that the total mixture in each well is 200 µl. 10µl of the bacteria was transferred into all wells. The microtiter plate was incubated at 37 ºC for 24 hours. The microbial growth was determined by observing the turbidity of the extract in the wells. The MIC was determined by analysing the lowest concentration of samples in which there is no visible growth of microorganism. All the tested samples was assayed in triplicates.

F. Minimum Bactericidal Concentration (MBC)

In order to determine MBC, all wells that showed no visible growth were subcultured on MHA (for bacteria).The agar was incubated at 37 ºC for 24 hours. The lowest concentration of extract that exhibit complete killing of the microorganism was considered as the MBC.

III. Results

The antibacterial activity was analysed from the diameter of the zone of inhibition which was measured to the nearest mm and was compared with the control drug (tetracycline). The antibacterial activities of *M. decemfidum* extract were shown in Table I. The flower extract showed antibacterial activity against all tested bacteria. As compared with the antibacterial drug used, the tested organism showed more susceptibility against flower extract except for *B. cereus* which at lower concentration of flower extract (100 and 150 µg/mL) showed lower antibacterial activity compared to control. As the concentration of flower extract increase, only *P. Aeruginosa* showed an increasing susceptibility against the flower extract. However, *B. cereus* and *S. aureus* showed an increase susceptibility with increasing concentration of flower extract only up to 200 µg/mL. On the other hand, *E. coli* showed no relationship between concentration and the antibacterial activity. The highest antibacterial activity was against *P. Aeruginosa* with 15.8mm ZOI at extract concentration of 250 µg/mL. *E. coli* demonstrated the highest resistance against flower extract as the diameter of zone of inhibition is the lowest compared to other bacteria species.

TABLE I. Antibacterial Activity of Methanolic Extract of *M. Decemfidum*

	Extract concentration (µg/mL)	Zone of inhibition diameter (mm)			
		E. coli	*P. Aeruginosa*	*B. cereus*	*S. aureus*
Flower extract	100	7.8 ± 0.2^{a}	12.6 ± 1.5^{ab}	7.2 ± 0.2^{a}	10.0 ± 0.3^{ab}
	150	8.4 ± 0.2^{a}	13.6 ± 1.5^{b}	7.8 ± 0.4^{a}	11.0 ± 0.3^{bc}
	200	7.2 ± 1.8^{a}	14.6 ± 1.2^{b}	12.0 ± 0.3^{c}	12.0 ± 0.3^{c}
	250	9.0 ± 0.3^{a}	15.8 ± 1.2^{b}	11.8 ± 0.2^{c}	11.8 ± 0.2^{c}
Leave extract	100	9.8 ± 0.4^{a}	12.6 ± 0.8^{ab}	7.4 ± 0.2^{a}	12.4 ± 0.4^{b}
	150	12.4 ± 0.5^{b}	13.6 ± 1.2^{b}	7.6 ± 0.2^{a}	13.2 ± 0.2^{c}
	200	13.2 ± 1.2^{b}	14.2 ± 0.7^{b}	7.6 ± 0.2^{a}	13.4 ± 0.2^{cd}
	250	13.8 ± 1.3^{b}	15.8 ± 1.7^{b}	8.0 ± 0.3^{a}	14.0 ± 0.0^{d}
Stem extract	100	10.0 ± 0.3^{ab}	16.4 ± 2.2^{b}	6.8 ± 0.2^{a}	12.4 ± 0.2^{b}
	150	11.0 ± 0.3^{cd}	14.4 ± 0.2^{b}	7.6 ± 0.2^{a}	12.6 ± 0.5^{bc}
	200	11.8 ± 0.4^{d}	20.0 ± 1.1^{c}	7.8 ± 0.6^{a}	13.4 ± 0.2^{c}
	250	10.4 ± 0.2^{bc}	22.6 ± 0.4^{c}	8.0 ± 0.3^{a}	12.4 ± 0.2^{b}
Tetracycline		9.0 ± 0.3^{a}	10.2 ± 0.4^{a}	16.4 ± 0.5^{b}	8.6 ± 0.2^{a}

Data expressed as mean of duplicate±standard deviation (SD) including the disc diameter (6 mm). All values were rounded to one decimal place.

The leaf extract of *M. decemfidum* showed antibacterial activity against all tested bacteria. *E. coli, P. Aeruginosa* and *S. aureus* were more sensitive against *M. decemfidum* leaf extract as compared to control drug. However, *B. cereus* showed more resistance against *M. decemfidum* leaf extract in comparison with the control. As the concentration of the leaf extract increase, the diameter of zone of inhibition also increased for all tested bacteria. The highest diameter of inhibition zone was recorded by *P. Aeruginosa* at the concentration of 250 µg/mL leaf extract. On the other hand, *B. cereus* exhibits the lowest susceptibility against leaf extract of *M. decemfidum* among all the tested bacteria with the range of diameter of inhibition zone between 7.4 to 8.0 mm.

All tested bacteria shows sensitivity against the stem extract. With the exception of *B. cereus*, the other bacteria displayed a higher diameter of inhibition zone as compared to control drug.

The increase in concentration used against *B. cereus* results in an increase susceptibility of the bacteria against the inhibitory effect of the stem extract. Nonetheless, the concentration of stem extract did not have effect on the diameter of the inhibition zone produced by *E. coli, P. Aeruginosa* and *S. aureus.* The highest diameter of inhibition zone of 22.6 mm was recorded against *P. Aeruginosa* at stem extract concentration of 250 µg/mL. On the other hand, *B. cereus* recorded the lowest diameter of inhibition zone at all concentration of stem extract.

The results for minimum inhibitory and bactericidal concentration were displayed in Table II. The leaves and stem extract showed the best inhibitory effects against both *E. coli* and *S. aureus* with MIC value of 12.5 µg/mL. On the other hand, the flower extract recorded a weaker inhibitory properties with MIC value of 50.0 µg/mL against *B. cereus* while no inhibition was recorded against *P. aeruginosa*. The leave and stem extract has the ability to kill *P. aeruginosa, B. cereus* and *S. aureus* at con centration of 100.0, 25.0 and 12.5 mg/mL respectively. However, *E. coli* showed resistance against the bactericidal effect of all three extract of *M. decemfidum*. In addition, flower extract also displayed a weak bactericidal effect where only *S. aureus* was killed at 25.0 mg/mL while other bacteria is resistant against the bactericidal effect of flower extract.

TABLE II. MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATION OF METHANOLIC EXTRACT OF FLOWER, LEAF AND STEM OF *M. DECEMFIDUM*

		Concentration (mg/mL)			
		E. coli	*P. aeruginosa*	*B. cereus*	*S. aureus*
FLOWER	MIC	25.0	-	50.0	25.0
	MBC	-	-	-	25.0
LEAVES	MIC	12.5	25.0	25.0	12.5
	MBC	-	100.0	25.0	12.5
STEM	MIC	12.5	25.0	25.0	12.5
	MBC	-	100.0	25.0	12.5

IV. DISCUSSIONS

The extracts from various parts of *Melastoma decemfidum* exhibited different range of reaction when tested against the selected microorganism which include gram positive (*E. coli, P. Aeruginosa*) and gram negative bacteria (*B. cereus, S. aureus*). These findings from *M. decemfidum* is in agreement with antibacterial studies on other species from the same family of melastomacae, namely *Melastoma malabathricum* which had shown antibacterial properties. The *M. malabathricum* showed antibacterial activity against *E. coli, Streptococcus sp., Staphylococcus aureus, S. agalactiae* and *P. aeruginosa* [11]-[13].

These antibacterial activities demonstrated by *M. decemfidum* can be attributed to the presence of active compound present in the extract of *M. decemfidum*. Previous study had found two flavonoids which are naringenin and kaempferol from the leaf extract of *M. decemfidum* [2]. The same compound had also been identified by Susanti *et al.* in the flower extract of *M. decemfidum*[8]. Both of these compound were classified as flavonoid [14]-[16] and Sarju *et al.* had suggested that the presence of hydroxyl group located at C-3 in the C ring structure of the flavonoid may contribute to the antimicrobial effect by reducing the membrane fluidity of cell [2]. There were various studies that had indicated that kaempferol possess numerous pharmacological activities which include antibacterial activity [15], [17].

However, the variation in the size of inhibition zone produce by extracts from different parts of *M. decemfidum* show that there are variation in the distribution of active chemical compound in the plant. The variation could be attributed to the function or location of the parts. This is parallel to several studies which had identified different chemical composition on different parts of the plant. Sunday *et al.* expressed that leaf of *Abrus precatorius* had the highest amount of tannin and phenol while the root showed a lower quantity of other compound [18]. The study also suggest that the higher phytochemical concentration in the aqueous extract of leaf and root of *Abrus precatorius* may attributes to their higher antimicrobial activity as compared to the seed extract [18]. Apart from that, a study on chemical diversity of essential oil from different part of *Rhanterium epapposum* by Awad and Abdelwahab found that different chemical diversities

were identified from its flower, leaves and stem in which the oils from flower bearing the highest chemical diversity as compared to oils from other parts [19]. Moreover, Euch *et al.* reported that *Cistus salviifolius* showed significant difference in the plant chemical composition in which phenol and flavonoids were highest in flower buds while the leaves has a higher amount of tannin and anthocyanins [20]. Besides, a comparative study on elemental composition between leaves and flowers of *Catharanthus roseus* conducted by Aziz *et al*. found that the leaves contain a higher concentration of elemental composition compared to the flower part [21]. These studies showed that the quantitative and qualitative aspect of chemical composition in different parts of plants used were varied and also depends on the species used in those studies.

V. Conclusion

Based on the results, it can be concluded that *M. decemfidum* possess antibacterial properties which may contributes to its healing qualities as claimed by locals. This can justify the use of *M. decemfidum* by locals to treat various kind of illness. However, more research need to be developed to investigate the medicinal potential of *M. decemfidum.* Its antibacterial properties could utilized for the developments of new antibacterial drug.

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