Antimicrobial Activity of *Melastoma decemfidum* Leave Extract against Human Pathogen

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***Abstract*—*Melastoma decemfidum* is a shrub that belongs to the family Melastomaceae and traditionally used by local people to treat diseases. This study was carried out with the aim to evaluate the antimicrobial activities of different concentrations of wild and *in vitro M. decemfidum* leaves extracts against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Candida albicans* and *Aspergillus brasiliensis.* The inhibitory effects of both extracts were tested using the disc diffusion method. Results showed that *Staphylococcus aureus* was the most sensitive towards both extracts, while *A. brasiliensis* resistance to both extracts. Therefore, *M. decemfidum* indicate its potential for the development of new drugs for pharmaceutical application.**

***Index Terms*—*Melastoma decemfidum*, antimicrobial activity, human pathogen**

I. Introduction

Melastoma decemfidum is a plant species from the Melastomataceae family. This white flowering plant is a small shrub with local name as "senduduk putih" [1]. They can only be found at certain area such as in open sites in mixed dipterocarp (large gaps), coastal, and sub-montane, forests up to 2700 m altitude, but most common in heavily degraded forests. They are distributed in Tropical Asia and the Pacific. In Borneo they can be collected in Sarawak, Brunei, Sabah, South- and East-Kalimantan. Sarju [2] reported the detection and quantification of important flavonoids from the plants extracts of *M. decemfidum*. These flavonoids can inhibit or kill many bacterial strains, inhibit important viral enzymes such as reverse transcriptase and protease and destroy some pathogenic protozoans yet; they have low toxicity effect on animal cells [3].

According to Mahesh and Satish [4], plants that are used medicinally are a source of many potent and powerful drugs. Thus, since *M. decemfidum* is a medicinal plant, so the plants may have potentiality as sources for antimicrobial drugs with reference to antibacterial and antifungal agents. However, the study on *M. decemfidum* is still limited compared to *M. malabathricum* that has been established studied [5] especially on their antimicrobial properties which has not been carried-out yet.

Melastoma decemfidum (synonym *M. sanguineum*) is an indigenous shrub plant in Malaysia. The plants especially their leaves can be used as alternative ways traditionally to cure toothache, mouth ulcer, to treat diarrhea, epilepsy, rheumatism and dysentery, to prevent scarring from smallpox and also to treat piles [6], [7], [2]. Unfortunately, in natural habitat, *M. decemfidum* is very difficult to be found compared to other family of Melastomaceae such as *M. malabathricum*. Hence, plant tissue culture has become a valuable tool to produce large number of progeny in order to study their antibacterial and antifungal properties which beneficial to pharmaceutical industries. For example, *in vitro* techniques of medicinal plants such as callus culture could be used to maximize bioactive compounds production [8]. However, the study on *in vitro* plant as antimicrobial potential is still lacking compared to the wild plant.

Therefore, it is worthwhile to scientifically evaluate the antimicrobial activity of wild and *in vitro* extracts of *M. decemfidum* plant against selected human pathogen.

II. Materials and Methods

A. Preparation of Plant Extracts

The methanol extract of leaves was prepared according to Erdogan [9] with a slight modification. Crude extracts of the following amount: 400 mg, 300 mg, 200 mg and 100 mg were dissolved in 1 ml of methanol. Then all the dissolved extracts were filtered through a 0.22 µm membrane. A total of 20 µl of each sample extracts were loaded onto a sterile 6 mm diameter paper disc. The discs were allowed to air dry prior to firm placement onto inoculated plates.

B. Preparation of Inoculum

The bacterial strains were grown in the nutrient broth at 35 ℃ for 24 hours. The fungal inoculum was prepared from 3 to 7 days old culture grown on Sabouraud dextrose agar at 28 ℃. The petri dish was flooded with 10 ml of distilled water and the conidia were scrapped using sterile spatula. The concentration of cultures was adjusted to match 0.5 McFarland standards. The stock cultures were maintained at 4 ℃ for 7 days.

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C. Disc Diffusion Assay

The antibacterial and antifungal activity was measured by the disc diffusion method. A Mueller Hinton sterile agar plate was swabbed with 100 µl of bacterial (approximately 2 x 10^8 CFU/ml) or fungal strain (approximately 2 x 10^6 CFU/ml). The sterile 6 mm diameter paper discs impregnated with 20 µl extracts were placed on the agar surface. A disc impregnated with methanol was used as negative control. The commercial tetracycline 30 and nystatin 100 discs were used as positive controls for bacteria and fungi, respectively. The inhibition zone around the disc was measured after 24 hours of incubation at 35 ℃ for bacteria and 48 to 96 hours for fungi at 28 ℃. All tests were performed in triplicates.

D. Minimum Inhibitory Concentrations (MIC)

Minimum Inhibitory Concentration (MIC) was determined only for bacteria and fungi species which show inhibition zones in the disc diffusion method that described above. MIC was determined according to Mourouge Saadi [10] with two-fold serial dilutions. To each round bottom 96-well, 100 µL of standardized tested pathogen (10^8 CFU/mL) was added to Mueller-Hinton broth containing various concentrations of the above samples to yield a final volume of 200 µL/well. The MIC was recorded as the lowest concentration that produced a complete suppression of visible growth after 24h incubation at 35 ℃.

E. Minimum Microbiocidal Concentration (MMC)

The MMC was defined as the lowest concentration preventing bacterial growth [10]. For the determination of MMC, 100 µl of the content from each well in the MIC assay that show no turbidity changes was used to spread onto fresh nutrient agar without antibiotic. The plates were incubated for 24 h at 35 ℃. All tests were performed in triplicates.

F. Statistical Analysis

All the experiments of antimicrobial susceptibility testing were performed in triplicate. The results were expressed as the mean ± standard error of mean (SE). Data was statistically analyzed by one way analysis of variance (ANOVA). The significance of the differences was determined by Tukey test at $P < 0.05$.

III. Results and Discussion

The results from disc diffusion assay revealed that the extracts had different degrees of bacterial and fungal growth inhibition. All the bacterial and fungal were sensitive to the positive control (tetracycline for bacteria and nystatin for fungi) and resistant to the negative control (methanol). Hence, shows the validity of testing. Example of antimicrobial activity that exhibited positive reaction shown in Fig. 1.

The results were varied based on the concentrations of the extracts used. Results show that *S. aureus* was the most susceptible to the *in vitro* leaves extract compared to *P. aeruginosa*, *E. coli* and *S. typhimurium*. The inhibition zone against *S. aureus* was recorded ranging from 8.6 to 12.3 mm, 7.1 to 12.0 mm for *P. aeruginosa*, *E. coli* (8.0 to 10.1 mm) and *S. typhimurium* (7.7 to 11.3 mm). However, the results for *S. aureus* and *P. aeruginosa* shows no significant different at $p<0.05$ at all tested concentration. As for the wild leaves extract, the results show not much different from *in vitro* leaves extract. *Pseudomonas aeruginosa* recorded 8.3 to 12.6 mm inhibition zone, 12.4 to 13.2 mm for *S. aureus*, 9.8 to 13.2 mm for *E. coli* and 10.0 to 10.3 mm for *S. typhimurium* (Table I). Furthermore, only *in vitro* leaves extract successfully inhibited growth of *C. albicans* with inhibition zone ranging from 8.3 to 12.9 mm. Unfortunately, *A. brasiliensis* was resistant against both leaves extract at all concentrations (Table II).

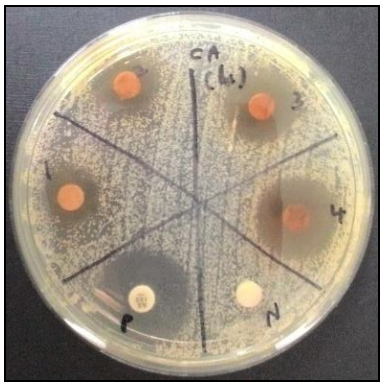

Figure 1. FAntimicrobial *activity of leaves extract against* C. albicans. (1) 100 mg/ml. (2) 200 mg/ml. (3) 300 mg/ml. (4) 400 mg/ml. P: Positive control (Nystatin: 30 µg/disc). N: negative control (Methanol)

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

Bacteria Strain	Inhibition zone (mm)								
	Wild Leave extract (mg/ml)				*In vitro* leaves extract (mg/ml)				
	100	200	300	400	100	200	300	400	[A]Tetracycline 30
S. aureus	12.4 ±0.4^{b}	13.2 ±0.2^{c}	NT	NT	8.6 ± 0.2^{a}	9.8 ±0.1^{b}	10.9±0.2^{c}	12.3 ±0.3^{d}	19.7 ±0.3
E. coli	9.8 ±0.4^{a}	13.2 ±1.2^{b}	10.7±0.2	10.3±0.2	N.D.	8.0 ±0.2^{a}	9.2 ±0.2^{b}	10.1 ±0.3^{c}	21.2 ±0.4
P. aeruginosa	12.6 ±0.8^{ab}	14.2 ±0.7^{b}	8.0 ±0.4	8.3±0.7	7.1 ± 0.1^{a}	9.3 ±0.2^{b}	10.6±0.2^{c}	12.0 ±0.3^{d}	9.4 ±0.5
S. typhimurium	NT	NT	10.3±0.7	10.0±0.3	N.D.	7.7 ±0.2^{a}	10.3±0.2^{b}	11.3 ±0.2^{c}	19.7 ±0.4

N.D.: no activity detected; NT: not tested.
[A]Standard antibiotic (Tetracycline 30 for bacterial species); Negative control (100% methanol) did not show inhibitory activity;
Results are the means of inhibition zone values followed by standard deviations; Different letters within the same row indicate means at different concentrations for the same species are significantly different ($P < 0.05$).

TABLE II. ANTIFUNGAL ACTIVITY OF METHANOLIC LEAF EXTRACT OF *M. DECEMFIDUM*

Fungi Strain	Inhibition zone (mm)								
	Wild Leave extract (mg/ml)				*In vitro* leaves extract (mg/ml)				
	100	200	300	400	100	200	300	400	[A]Nystatin 100
Candida albicans	N.D.	N.D.	N.D.	N.D.	8.3 ±0.2[a]	9.4 ±0.4[a]	11.2 ±0.3[b]	12.9 ±0.4[c]	23.0 ±0.8
Aspergillus brasiliensis	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	20.2 ±0.2

N.D.: no activity detected; [A]Standard antibiotic (Nystatin 100 for fungal species); Negative control (100% methanol) did not show inhibitory activity; Results are the means of inhibition zone values followed by standard deviations; Different letters within the same row indicate means at different concentrations for the same species are significantly different ($P < 0.05$).

The antimicrobial activities of both extracts were further confirmed using the micro-dilution broth assay. The MIC and MMC values were shown in Table III. The MIC values range from 12.5-50 mg/ml for wild leaves extract and 100-200 mg/ml for *in vitro* leaves extract, while the MMC values of both extracts against the tested bacteria and fungi were 100-200 mg/ml as shown in Table III. Overall, the present study showed microbial growth was inhibited and killed at the same concentration used for all bacterial and fungal species tested.

The antibacterial and antifungal effect of *in vitro M. decemfidum* probably due to the high levels of phenols and flavonoids that have been reported as major antimicrobial component in medicinal plants [5]. Many factors have been reported to be responsible for antibacterial activity of flavonoids. Gautam [15] reported that the antimicrobial activity of phenolic groups including flavonoids involves the alteration of the permeability of the cell membrane that could result in the uncoupling of oxidative phosphorylation, active transport inhibition and loss of pool metabolites as a result of cytoplasmic membrane damage. Furthermore, the presence of hydroxyl group in phenolic compound might affect their antimicrobial effectiveness and alter their metabolism. Hence, the lipid solubility and the degree of the steric hindrance of the phenolic substances might also determine their antimicrobial property [10].

TABLE III. MIC AND MMC (MG/ML) OF WILD AND *IN VITRO* LEAVES EXTRACTS OF *MELASTOMA DECEMFIDUM.*

Microbial Strain	Wild leaves extract (mg/ml)		*In vitro* leaves extract (mg/ml)	
	MIC	MMC	MIC	MMC
Staphylococcus aureus	50 ±0.0	100 ±0.0	100 ±0.0	100 ±0.0
Escherichia coli	12.5 ±0.0	N.D.	200 ±0.0	200 ±0.0
Pseudomonas aeruginosa	25.0 ±0.0	100 ±0.0	100 ±0.0	100 ±0.0
Salmonella typhimurium	NT	NT	200 ±0.0	200 ±0.0
Candida albicans	N.D.	N.D.	100 ±0.0	100 ±0.0
Aspergillus brasiliensis	N.D.	N.D.	N.D.	N.D.

Results are the means of MIC and MMC values followed by the standard error of means. All readings are carried out in triplicates. N.D.: no activity detected; NT: not tested.

IV. CONCLUSIONS

The present work has shown that *in vitro M. decemfidum* plant culture was potentially good sources of antimicrobial agents. *Staphylococcus aureus* and *P. aeruginosa* were found as the most susceptible to the *in vitro* and wild leaves extract. Most microbial growth was inhibited and killed at the same concentration used for all bacterial and fungal species tested. This supports the view that traditional use of *M. decemfidum* is important in medicine and in assisting primary health care. However, further studies in determining the bioactive compounds composition, pharmacological action and toxicity of these extracts should be addressed to confirm the therapeutic benefits of the plant prior future applications.

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