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Phytochemical Analysis and Biological Activities of *Melastoma malabathricum* and *Dissochaeta gracilis*

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This study is on *Melastoma malabathricum* and *Dissochaeta gracilis* which are from the Melastomataceae family. The two samples were extracted using the cold extraction method with different polarity of solvent. Phytochemical analysis was done qualitatively to determine the presence of alkaloids, flavonoids, saponins, phenols, tannins, terpenoids and steroids compounds. Antibacterial activity was analysed by disc diffusion method using Staphylococcus aureus, Bacillus subtilis, Salmonella typhi and Escherichia coli. In addition, antioxidant activity was conducted to determine the percentage of DPPH scavenging of the crude extract. The study found that methanol crude extract has the highest percentage yield extraction. *M. malabathricum* and *D. gracilis* crude extracts found the presence of several phytochemicals such as steroid, flavonoid, terpenoid, tannin, saponin and phenols compounds with the absence in alkaloid compounds. Apart from that, the antibacterial activites showed ethyl acetate and methanol crude extract gave the higher inhibition of zone against all tested bacteria. This study shows both samples *M. malabathricum* and *D. gracilis* have a potential to be an antibacterial agent. Methanol crude extract for *M. malabathricum* and *D. gracilis* also exhibited free radical scavenging activity with an IC_{50} value 111.90 µg/ml and 54.24 µg/ml. This study revealed that *M. malabathricum* and *D. gracilis* leave extracts are potential antimicrobial and antioxidant agents.

Keywords: *Melastoma malabathricum*; *Dissochaeta gracilis*; phytochemical; antimicrobial; antioxidant

I. INTRODUCTION

Naturally occurring biologically active compounds can be found in plants which can inhibit numerous diseases (Jabeen *et al.,* 2014) and they are also rich sources of antimicrobial agents (Jindal and Vashist, 2012). Throughout our evolution, the potential of natural products for medicine and health has been enormous. Traditionally, it is believed that natural products extracted from several Melastomataceae sp. give various medicinal benefits and can be used for the treatment of many ailments (Nozlena *et al.*, 2018). The local names for Melastomataceae sp. vary such as Senduduk or Keduduk in Malaysia, Senggani in Indonesia, Rhododendron in Singapore, Yeh Mu Tan in China and Malatungaw in the Philippines. In this study, two species, namely, *Melastoma malabathricum* and *Dissochaeta gracilis* are recognized by the locals as senduduk ungu and senduduk hutan, respectively. *M. malabathricum* have been used to treat various illness such as diarrhoea, cuts and wounds, toothache, and stomachache (Joffry *et al.,* 2012). In scientific research, it also acts as antinociceptive, anti-inflammatory, wound healing, antidiarrheal, cytotoxic, and antioxidant (Joffry *et al.,* 2012). However, scientific investigation on *D. gracilis* are still less in number and it is open for further investigation. Therefore, this study is aimed to obtain the leaves crude extracts of *M. malabathricum* and *D. gracilis* by using maceration techniques, to analyse phytochemical on the crude extracts and to investigate the biological activity of the crude extract of *M. malabathricum* and *D. gracilis*.

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II. MATERIALS AND METHOD

The leaves of *M. malabathricum and D. gracilis* were collected from Kuala Pilah, Negeri Sembilan. The plant samples were cleaned using distilled water, air-dried at room temperature until constant weigh and the leaves were grounded into powder for optimum surface area (Faparusi *et al.,* 2012).

A. Extraction of plant samples

150 g of *M. malabathricum* and *D. gracilis* in powdered form was soaked in 2000 ml of three difference polarity of solvents which are hexane, ethyl acetate and methanol for 72 hours at room temperature to accomplish cold extraction method. The crude extracts of *M. malabathricum* and *D. gracilis* were obtained by using vacuum rotary evaporator (Faparusi *et al.,* 2012) and kept in vial for further analysis. The percentage yield of the crude sample was determined by using the Equation 1 (Ewansiha *et al.,* 2016).

$$\% \, yield = \frac{Weight \, of \, the \, crude \, extract \, (g)}{weight \, of \, ground \, sample \, (g)} \times 100 \qquad (1)$$

B. Phytochemical Screening of Extracted Samples

For the alkaloids screening test, crude extracts were treated with methanol and a few drops of Wagner's reagent were added to the test tube. A reddish-brown precipitate indicated the presence of alkaloids (Olabinri *et al.,* 2014). For the test of flavonoids, crude extracts were mixed with 2 ml of 2 % sodium hydroxide solution in a test tube. The formation of the colour yellow indicated the presence of flavonoids withe the adding of a few drops of diluted HCl (Cheng *et al.,* 2017). Further, test for saponins was done by mixed the crude extract into 3 ml of distilled water. The mixture was shaken vigorously in the test tube for 5 minutes. The layer of foam shows the presence of saponins (Tiwari *et al.,* 2011). For the terpenoids screening test, crude extracts were mixed with 2 ml of chloroform in the test tube. A few drops of concentrated sulphuric acid were added to the mixture. The reddish-brown colour at the interfaces indicated the presence of terpenoids (Edeoga *et al.,* 2005). As for the tannin test, the crude extracts were mixed with few drops of ferric chloride solution in a test tube. The blue-black or dark green colour that form indicated the presence of tannins (Bassey *et al.,* 2016). Test for steroids was done by mixing crude extract with 2 ml of acetic acid in the test tube. A few drops of concentrated sulphuric acid were added to the mixture. The green bluish colour indicated the presence of steroids (Nath *et al.,* 2014). For the test of phenol, the crude extracts were treated with methanol and mixed with 2 ml of 2% ferric chloride solution in a test tube. The formation of bluish green colour indicated the presence of phenols (Cheng *et al.,* 2017).

C. Antibacterial Activity

Antibacterial assay was tested using disc diffusion method. The tested bacteria were spread on the agar plate with the help of sterile cotton swab. Four tested bacteria such as *S. aureus*, *B. subtilis*, *S. typhi* and *E. coli* were sub-cultured onto nutrient broth (NB) by using sterile wire loop. The mixture was stored in 37◦C incubator for 24 hours. The mixture was introduced onto the upper layer of agar plate. The plates with upside-down position were incubated overnight at 37◦C. The test was performed on disc diffusion method for all bacterial strains. The inhibition zone was measured in mm unit.

D. Antioxidant Activity

The difference extracts of *M. malabathricum* and *D. gracilis* were evaluated for 2,2-diphenyl-2picrylhydrazyl (DPPH) radical scavenging activity. Ascorbic acid was used as positive control. Crude extracts of *M. malabathricum and D. gracilis* were dissolved with methanol. Each different solvent had different concentration between 7.81 µg/ml to 1000 µg/ml and was added with DPPH. The absorbance was measured at 517 nm by using an Ultraviolet-Visible (UV-Vis) spectrophotometer. The percentage of DPPH scavenging effect was calculated using Equation 2 (Abu *et al.,* 2017).

$$\% \, Inhibition \, = \frac{Abs \, of \, blank - Abs \, of \, sample}{Abs \, of \, sample} \times 100 \qquad (2)$$

III. RESULT AND DISCUSSION

The crude extracts, phytochemical analysis and biological

activities of plants extracts were successfully done, and it is reported in this section.

A. Extraction of Plant Samples

The difference polarity of solvents was chosen to extract the plant samples. According to Wong *et al.* (2006), various solvents of differing polarities must be used to extract different chemicals compounds from plants with a high degree of accuracy. Percentage yields on the extraction process were calculated and tabulated as in Table 1.

Table 1. Percentage yields of *M. malabathricum* and *D. gracilis* crude extracts

Crude extracts	Weight of samples (g)	Weight of crude extract (g)	Yield (%)
M. malabathricum			
Hexane	83.53	2.07	2.48
Ethyl acetate	80.60	2.58	3.21
Methanol	79.79	6.27	7.85
D. gracilis			
Hexane	150.07	3.07	2.05
Ethyl acetate	148.26	7.26	4.90
Methanol	*144.43*	*13.85*	*9.59*

Methanol crude extract of both samples shows the highest percentage yield which are 7.85% and 9.59%, respectively. A study by Awang *et al.* (2016) found that the highest percentage yield of *M. malabathricum* crude extract was extracted by polar solvent which are water, ethanol, ethyl acetate and hexane. Moreover, scientists have discovered that polar solvents, such as methanol and ethanol, have a high effectiveness to extract more polar compounds such as phenolics and flavonoids. (Anokwuru *et al.*, 2011; Koffi *et al.*, 2010).

B. Phytochemical Analysis of Extracted Samples

The analysis and characterization of bioactive compounds from plants is important to ascertain their medicinal value (Sasidharan *et al.*, 2011). This study found that pharmacologically active compounds such as phenolics, flavonoids, steroids, terpenoids, tannins and saponins compounds were present in ethyl acetate and methanol extracts of *M. malabathricum* and *D. gracilis*. However, alkaloids were absent in all plant samples (Table 1). The finding is similar to a previous study by Nath *et al.* (2014) on the diversity of different classes of secondary metabolites such as flavonoids, steroids, saponins and tannins in the plant extracts.

Phyto-chemical tests	Leaves crude extract					
	MH	ME	MM	DH	DE	DM
Alkaloids	-	-	-	-	-	-
Steroids	+	+	+	+	+	+
Flavanoids	+	+	+	+	+	+
Tannins	-	+	+	-	+	+
Saponins	-	+	+	-	+	+
Phenols	-	+	+	-	+	+
Terpenoids	+	+	+	+	+	+

Key:
MH: *M.malabathricum* hexane crude extract
ME: *M.malabathricum* ethyl acetate crude extract
MM: *M. malabathricum* methanol crude extract
DH: *D. gracilis* hexane crude extract
DE: *D. gracilis* ethyl acetate crude extract
DM: *D. gracilis* methanol crude extract
(+): presence
(-): absence

This study found that the plant contained more active compounds which are valuable for medicinal applications. Traditionally, phytochemicals extracted from the Melastomataceae family can be used for the treatment of ailments such as diarrhea, puerperal infection, dysentery, leucorrhoea, wound healing and haemorrhoids (Anbu *et al.*, 2010; Sari *et al.,* 2018; Zakaria *et al.*, 2016). Interestingly, the isolated chemical compounds from this plant exhibited many biological activities such as anti-lipid peroxidation, radical scavenger, antioxidant, antibacterial and anti-inflammatory. (Diris *et al.,* 2016; Ismail *et al.,* 2017; Hamid *et al.*, 2018; Hanafiah *et al.*, 2018).

C. Antibacterial Activity

The antibacterial activity assay was done by using four selected bacteria including two Gram-positive (*S. aureus*, *B. subtilis*) and two Gram-negative (*S. typhi* and *E. coli)* bacteria. Disc diffusion method was used to measure the resistance of antibiotic of these crude extract against

selected bacteria. The positive control that were used in this study is chloramphenicol and the negative control used is dimethyl sulfoxide (DMSO). The result of antibacterial activities of *M. malabathricum* and *D. gracilis* are shown in Table 3 and Table 4, respectively.

Table 3. The inhibition zone of each *M. malabathricum* crude extract against bacteria

Types of bacteria	Zone of inhibition (mm)				
	MH	ME	MM	Chloram phenicol	DMSO
S. aureus	7.0	9.0	8.0	20.0	6.0
B. subtilis	7.0	10.0	7.0	19.0	6.0
S. typhi	7.0	8.0	7.0	26.0	6.0
E. coli	7.0	9.0	8.0	22.0	6.0

Note: Diameter of disc (6.0 mm)

Table 4. The inhibition zone of each D. Gracilis crude extract against bacteria

Types of bacteria	Zone of inhibition (mm)				
	DH	DE	DM	Chloram phenicol	DMSO
S. aureus	7.0	10.0	15.0	17.0	6.0
B. subtilis	7.0	12.0	17.0	16.0	6.0
S. typhi	7.0	16.0	17.0	25.0	6.0
E. coli	7.0	10.0	8.0	25.0	6.0

Note: Diameter of disc (6.0 mm)

As shown in Table 3, ethyl acetate extract of *M. malabathricum* has a good antibacterial activity against all bacterial strains compared to hexane and methanol extract. This might be due to the present of bioactive semi-polar compound in *M. malabathricum*. Ethyl acetate crude extract for *M. malabathricum* shows the highest antibacterial activity when against gram positive bacteria *B. subtillis* which diameter inhibition zone is 10 mm. According to Wong *et al.* (2012), ethyl acetate leaves extract of *M. malabathricum* consist of terpenoids, steroids, phenolics and flavonoids compound which are important for antibacterial activity.

The result of D. *gracilis* crude extract activity is shown in Table 4. The highest level of antibacterial activity found on methanol crude extract show the highest inhibition zone of 17.0 mm against *B. subtilis* and *S. typhi*. This might be due to the ability of polar compounds in *D. gracilis* to act as antibacterial agent. Further investigation is needed to identify the type of compound needed for good antibacterial activity, especially in *D. gracilis*.

D. Antioxidant Activity

In this study, DPPH scavenging assay was used to determine the antioxidant activities of Melastomaceae sp. According to Proestos *et al.* (2013), the reduction of the radical reaction is followed by a decrease in the absorbance at 517 nm of UV-VIS. Methanol crude extract for both samples were chosen with 8 different concentrations in a range starting from 7.81 µg/ml to 1000 µg/ml. This is because methanol extracts contain more polar compounds and the extracts show highest percentage of DPPH scavenging assay in screening test. Figure 1 shows the percentage of radical scavenging activity against different concentration of crude extracts by using linear regressions.

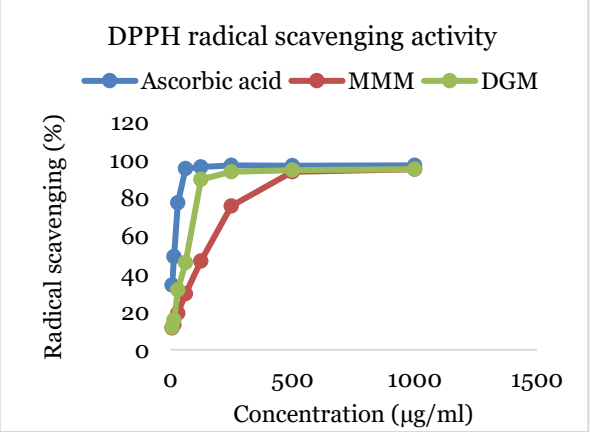

Figure 1. The percentage of radical scavenging activity against different concentration of crude extracts

Based on the graph, the IC_{50} value of ascorbic acid show 13.02 µg/ml while methanol crude extract of *M. malabathricum* and *D. gracilis* were 111.90 µg/ml and 54.24 µg/ml. This shows that, methanol crude extract of *D. gracilis* has better efficiency in antioxidant activity and antibacterial activity. The antioxidant activity is dependent on their phytochemical compound and their derivatives. According to Nuresti *et al.* (2003), flavonoid from the extract of *M. malabathricum* has good antioxidant properties.

IV. CONCLUSION

Phytochemical study on ethyl acetate and methanol extract of these plant samples show the presence of most secondary metabolites such as phenolics, flavonoids, steroids,

terpenoids, tannins and saponins compounds. Nevertheless, it does not the alkaloids compound. Ethyl acetate crude extract of *M. malabathricum* show the highest inhibition zone with diameter 10.0 mm on *B. subtilis* species. Meanwhile methanol crude extract of *D. gracilis* show the highest inhibition zone with diameter 17.0 mm on *B.subtilis* and *S.typhi*. Moreover, methanol crude extract for *D. gracilis* show good free radical scavenging activity with an IC_{50} value 54.24 µg/ml as compared to *M. malabathricum* with an IC_{50} value of 111.90 µg/ml. This study revealed that methanol crude extract of *D. gracilis* is a potential active antimicrobial and antioxidant agent. This is the first study as far as we know on biological activities of *D. gracilis crude extract*. Thus, the application of these plant samples based on our findings may lead to valuable discoveries in various fields such as in the pharmaceutical and biomedical industries. The bioactive compound from *D. gracilis* may open the door to a new range of antibacterial agents.

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