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Research Article
Phytochemicals and Antimicrobial Activities of *Melastoma malabathricum* and *Melastoma beccarianum* Leaf Crude Extracts

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Abstract

Background: *Melastoma malabathricum* is an important ethnomedicinal plant that has been rigorously studied for its medicinal properties, however, its closely related species, *M. beccarianum* has not been studied. **Methodology:** The phytochemical constituents of *M. malabathricum* and *M. beccarianum* were determined by gas chromatography mass spectrometry (GC/MS) analysis and their antimicrobial activities by agar-well diffusion method. **Results:** Similar chemical compositions were identified between the two *Melastoma* species, where three compounds were only detected in *M. malabathricum* methanol leaf crude extract, i.e., 8,11-octadecadienoic acid methyl ester, stearic acid methyl ester and tocopherol, whereas α-tocopherol-β-D-mannoside was only detected in *M. beccarianum*. The former was predominantly characterised by trans-squalene (17.02%) and the latter was by 5-hydroxymethylfurfural (15.76%). Both methanol extracts were found to exhibit antibacterial activities against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. However, the extracts did not display antifungal activity against *Saccharomyces cerevisiae* under the conditions tested. Likewise, both aqueous leaf crude extracts did not show any detectable antibacterial activities. **Conclusion:** This study not only supported the close relatedness of *M. malabathricum* and *M. beccarianum* in terms of their phytochemical constituents and antimicrobial properties but also showed that these species were still uniquely different from each other.

Key words: Antibacterial, antifungal, antimicrobial, ethnomedicine, medicinal plant, *Melastoma malabathricum*, *Melastoma beccarianum*, melastomataceae, phytochemicals, GC/MS

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Melastoma malabathricum (L.) and *M. beccarianum* (Cogn.) are monoecious flowering plant species in the family Melastomataceae, which is a large angiosperm family with a mainly pantropical distribution[1]. The former is a common and well-studied ethnomedicinal plant in Southeast Asia[2], whereas the latter has not been very well studied yet, potentially due to it being more difficult to find. Both plant species are pioneer shrubs that can be found growing in wastelands, degraded lands and secondary forests in Brunei Darussalam[3]. As the plants are closely related, both have similar morphological traits albeit with a few distinctive differences.

Melastoma malabathricum has been used traditionally for the treatment of wounds, diarrhea, dysentery, scars and many others[4]. Various scientific studies have been previously carried out to show that this plant species has antibacterial, antifungal and antiviral activities[5-10], while being non-cytotoxic to cell lines[7]. Furthermore, it has also been reported that *M. malabathricum* has antinociceptic[11,12] and gastroprotective effects[13,14], anti-inflammatory and antipyretic properties[12], antioxidant[15] and antidiarrheal activities[7]. Although, more exhaustive studies are still required, the previous studies have nicely validated and complemented the ethnomedicinal claims of *M. malabathricum*. The identification of chemical components of the plant species has also been carried out[15-19] but undoubtedly, more efforts are still required to elucidate biologically active chemical components.

To the best of our knowledge, no similar studies have been reported on *M. beccarianum*. Due to its close relation with *M. malabathricum*, *M. beccarianum* should as well have a promising potential as a medicinal plant for natural herbal products, as medicinal plants are currently being considered to be the significant contributors in modern drug discovery. Additionally, *M. beccarianum* might also be more potent in its medicinal benefits when compared to *M. malabathricum,* even though both plant species are almost morphologically similar. Therefore, the main aim of this study was to investigate the phytochemical and antimicrobial properties of two *Melastoma* species, *Melastoma malabathricum* and *Melastoma becarrianum*.

MATERIALS AND METHODS

Sample collection and preparation: Leaves of *M. malabathricum* and *M. beccarianum* were collected from Jalan Labi, Brunei Darussalam between June and September, 2015. The samples were subsequently air-dried for 2 weeks before being ground into powdered form.

Preparation of methanol crude extract: Powdered leaf samples (100 g) were extracted with methanol solvent for 6 h day^{-1} at 50°C in a period of 4 days, as described elsewhere[20]. The resulting extract was then filtered to remove solid residues and the solvent was evaporated under reduced pressure at 40°C. The dried extract was stored in the dark at 4°C until further analyses. For GC/MS analysis and antimicrobial assays, the crude extract was re-dissolved in methanol at specific concentrations.

Preparation of aqueous crude extract: Aqueous crude extract was obtained by ultrasound extraction, as described elsewhere[21]. In the ratio of 1 g to 10 mL, powdered samples (30 g) were suspended in distilled water and was subsequently ultrasonicated (Sonica Ultrasonic Cleaner, Soltec, Italy) for 30 min at 25°C. To eliminate solid residues, the resulting aqueous extract was filtered and the filtrate was directly used for antimicrobial assays.

Phytochemical analysis by GC/MS: Phytochemical identification by gas chromatography mass spectrometry (GC/MS) was performed using Shimadzu QP-2010 GC/MS instrument (Shimadzu Corporation, Japan). A DB-5ms GC column (30 mx0.25 mm, film thickness 0.25 µm) was used. The column temperature was programmed from 50-300°C at an initial rate of 20°C min^{-1} and after reaching 140°C, at a rate of 10°C min^{-1}, with the lowest and highest temperatures being held for 1 and 10 min, respectively. The GC injector and MS detector were set at 250 and 200°C, respectively. Helium was used as a carrier gas at a flow rate of 1.69 mL min^{-1}. By using a splitless mode, 1 µL of the sample was injected into the GC. For MS detection, the electron ionisation mode (70 eV) was used. Compounds were identified by their retention times and mass spectra using NIST library for data of standards and only the compounds with at least 90% similarity with the database were considered. Relative percentage of compound was calculated by peak area normalisation.

Antimicrobial assays: Agar-well diffusion technique was employed to determine the antibacterial activities of crude extracts, as described elsewhere[22]. Four bacterial strains were tested, *Bacillus subtilis* ATCC-11774, *Escherichia coli* ATCC-11775, *Pseudomonas aeruginosa* ATCC-27853 and *Staphylococcus aureus* ATCC-29213 and one fungal strain, *Saccharomyces cerevisiae*. A Mueller-Hinton agar plate was inoculated with an overnight nutrient broth culture of the microorganism. The agar was bored to produce wells of 4 mm diameter, in which the crude extract was introduced for testing. For positive control against the bacterial strains,

2 mg mL^{-1} streptomycin sulfate was used and for negative control, depending on the crude extract tested, either methanol or distilled water was used. The plate was incubated at 37°C for 24 h. Zone of inhibition around the well indicates the presence of antibacterial activity and the diameter of this zone was measured.

Statistical analysis: The means of zone of inhibition of *M. malabathricum* and *M. beccarianum* methanol extracts on four bacterial strains were analysed using a two-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Extraction yields: Soxhlet extraction of methanol crude extracts yielded 32% (*M. malabathricum*) and 8% (*M. beccarianum*) of extractable components relative to the weight of dried leaf material, suggesting *M. malabathricum* may have more methanol-soluble compounds compared to the other *Melastoma* species. Both crude extracts were sticky and brownish in color and produced a tea-like aroma.

Identification of phytochemicals by GC/MS: The GC/MS analysis of the methanol crude extracts of *M. malabathricum* and *M. beccarianum* resulted in the identification of 10 and 7 compounds, accounting for 59.2 and 52.2% of their total relative area, respectively (Fig. 1, Table 1). The compounds were identified in comparison with the standard mass spectral in the NIST library with more than 90% similarity. Other compounds were also detected from the appearance of peaks in the spectra, however were not considered as they only show at least 50% similarity with the standard mass spectral in the NIST library.

The comparison between *M. malabathricum* and *M. beccarianum* phytochemical components (Table 1) showed 6 out of 7 compounds identified in *M. beccarianum*

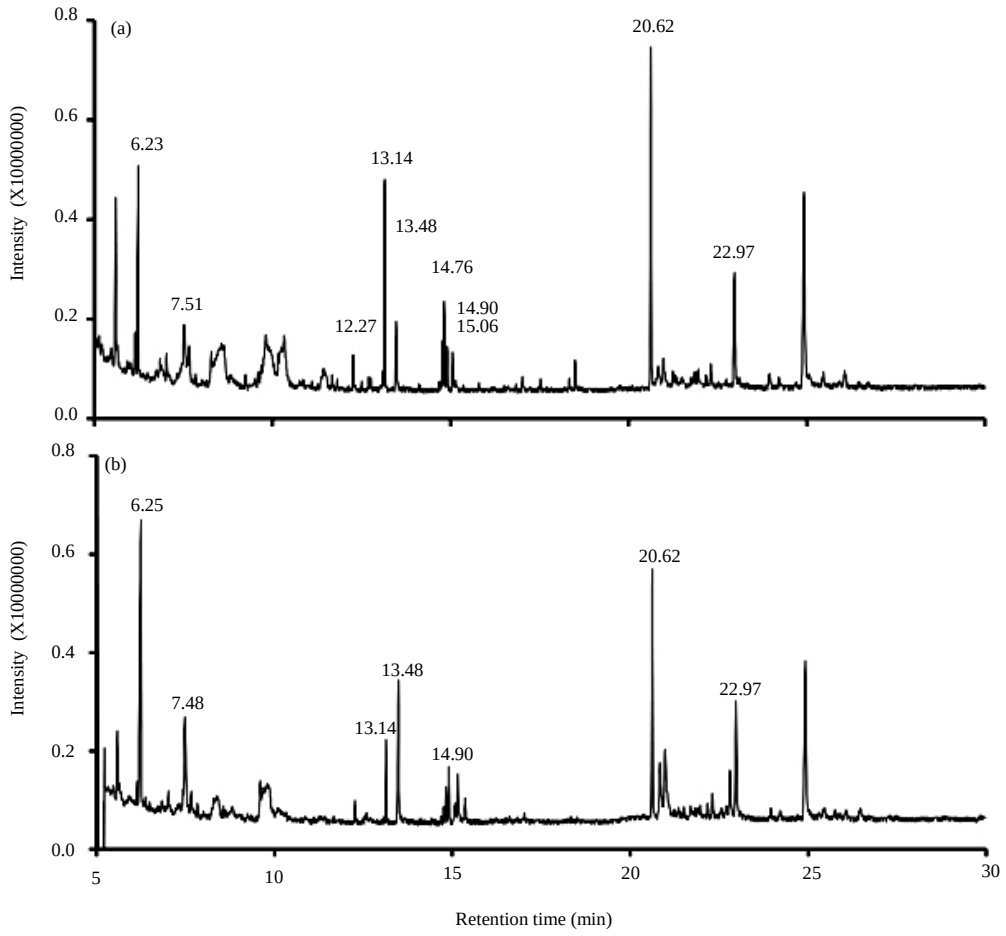

Fig. 1(a-b): (a) GC/MS spectra of *Melastoma malabathricum* and (b) *Melastoma beccarianum* methanol leaf crude extracts. Numbers shown represent the retention times of the peaks analysed in this study

Table 1: Phytochemicals detected by GC/MS analysis in *Melastoma malabathricum* and *Melastoma beccarianum* methanol leaf crude extracts

			M. malabathricum		*M. beccarianum*	
Compound name	MW	Molecular formula	R_t (min)	Peak area (%)	R_t (min)	Peak area (%)
5-hydroxymethylfurfural	126	$C_6H_6O_3$	6.23	9.78	6.25	15.76
Pyrogallol	126	$C_6H_6O_3$	7.51	4.97	7.48	6.47
Phytol	296	$C_{20}H_{40}O$	12.27	1.45	-	-
Palmitic acid methyl ester	270	$C_{17}H_{34}O_2$	13.14	8.52	13.14	2.80
Palmitic acid	256	$C_{16}H_{32}O_2$	13.48	3.88	13.48	7.29
8,11-octadecadienoic acid methyl ester	294	$C_{19}H_{34}O_2$	14.76	2.11	-	-
Phytol	296	$C_{20}H_{40}O$	14.90	2.02	14.90	2.32
Stearic acid methyl ester	298	$C_{19}H_{38}O_2$	15.06	1.70	-	-
Trans-squalene	410	$C_{30}H_{50}$	20.62	17.02	20.62	10.67
Tocopherol	430	$C_{29}H_{50}O_2$	22.97	7.73	-	-
α-Tocopherol-β-D-mannoside	592	$C_{35}H_{60}O_7$	-	-	22.97	6.90

Compounds are listed in order of retention time (R_t), peak area represents relative percentage of detected compound in the crude extract, Molecular Weight (MW) and formula of the compound are also shown

that were also found in *M. malabathricum* except for α-tocopherol-β-D-mannoside, which might be unique to this particular species. The high similarity in phytochemical constituents should not be surprising, considering both are of closely related species. Interestingly, three compounds were only identified in *M. malabathricum* and might be unique to this species, which were 8,11-octadecadienoic acid methyl ester, stearic acid methyl ester and tocopherol. The methanol extract of *M. malabathricum* was predominantly characterised by trans-squalene (17.02%), whereas *M. beccarianum* was by 5-hydrxoymethylfurfural (15.76%). Phytochemical constituents of any plant can be affected not only by its genetic makeup but also by environmental factors. It is suspected that the slight but unique phytochemical variations between the 2 species observed in this study could mostly be genetically contributed, as both plants had been collected from the same locality.

A few of the identified phytochemicals have been previously reported to be biologically active. For example, phytol has been reported to demonstrate antimicrobial, anticancer, anti-inflammatory, anti-diuretic and anti-diabetic activities and squalene has anti-inflammatory, anti-atherosclerotic and antineoplastic activities, whereas tocopherol has antioxidant, anti-inflammatory and antimicrobial activities[23]. The presence of three pentacyclic triterpenoids in *M. malabathricum* leaf methanol extract has previously been reported[17], which were not detected in this study. This could probably be due to environmental factors and/or the use of different varieties of *M. malabathricum*, as several varieties have been reported[4]. Phytochemical constituents of any plant could also depend on the extracting solvents used, as different solvents would extract different compounds. It has been reported that *M. malabathricum* leaf hexane fraction contained β-sitosterol, α-amyrin and uvaol, while the ethyl acetate fraction contained sitosterol 3-O-β-D-glucopyranoside[17].

Antimicrobial activities of leaf extracts: Antibacterial activities of *M. malabathricum* and *M. beccarianum* methanol and aqueous extracts were evaluated against Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria.

Under the conditions tested, antibacterial activities were detected in all concentrations (100-800 mg mL^{-1}) for both *M. malabathricum* and *M. beccarianum* methanol extracts, indicating the presence of antibacterial compound(s) in both plant species (Fig. 2). Statistical comparison between *M. malabathricum* and *M. beccarianum* methanol extracts using two-way ANOVA showed the means of zone of inhibition were significantly different between the two extracts for *B. subtilis* and *E. coli* ($p<0.05$), but not for *S. aureus* and *P. aeruginosa* ($p>0.05$). This suggests that the methanol extracts might have differential effectiveness against *B. subtilis* and *E. coli* but might be equally potent against *S. aureus* and *P. aeruginosa*. However, this needs to be interpreted with caution because although the size of the inhibition zone could indicate the antimicrobial potency, the test itself is considered as a qualitative technique.

The observed antibacterial activities could possibly be due to the presence of phytol and tocopherol that were identified in the methanol extracts, as these 2 compounds have been reported to possess antimicrobial properties[23]. However, other compounds have also been reported. For example, a few phytochemicals namely ursolic acid, 2α-hydroxyursolic acid, asiatic acid, β-sitosterol 3-O-β-D-glucopyranoside, kaempferol, quercetin and ellagic acid, which were found either in the chloroform or ethyl acetate fraction of *M. malabathricum* had exhibited antibacterial activities[10].

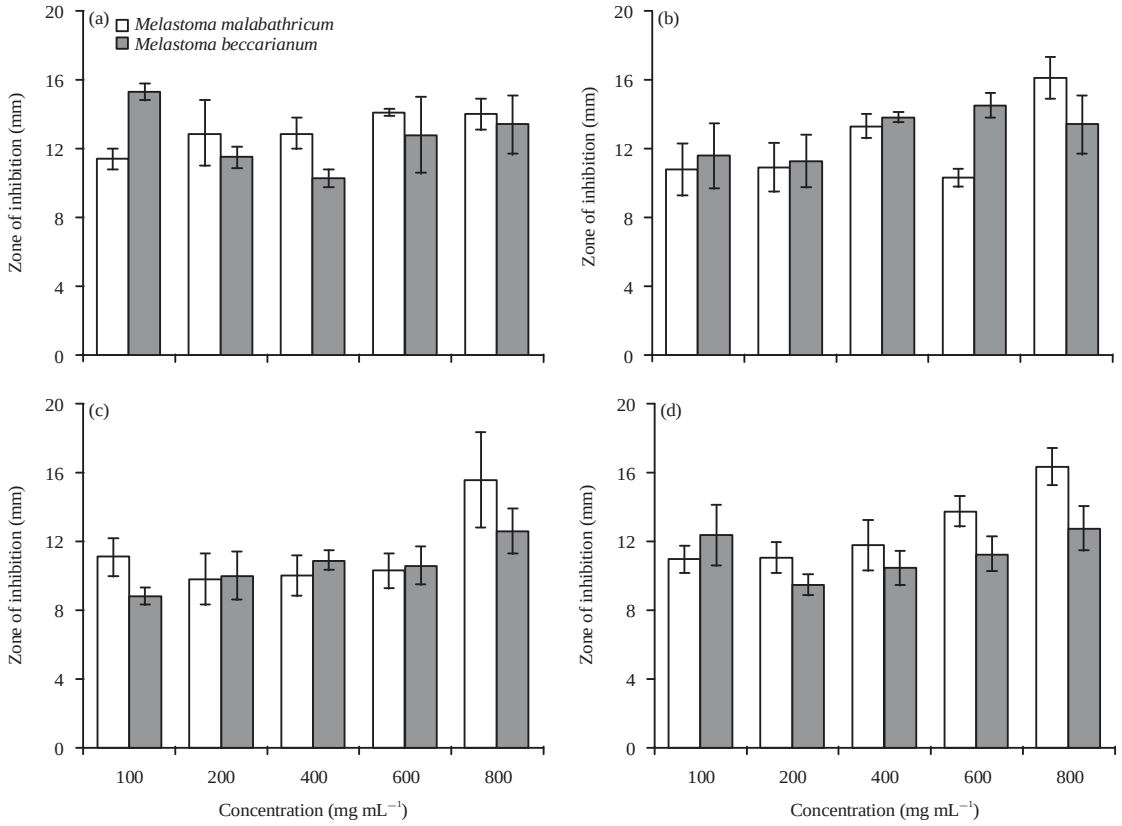

Fig. 2(a-d): Antibacterial activities of *Melastoma malabathricum* and *Melastoma beccarianum* methanol leaf crude extracts against, (a) *Bacillus subtilis*, (b) *Escherichia coli*, (c) *Staphylococcus aureus* and (d) *Pseudomonas aeruginosa*. Mean values±Standard Deviation of four replicates (n = 4) were presented. Negative control (n = 16) did not show any detectable activity as expected. Positive control (n = 16) showed zone of inhibition of 15.1±0.7 mm

The antibacterial properties of *M. malabathricum* leaves have been previously reported[5,8,9], which nicely supported these findings. To the best of our knowledge, this study is the first to document the antibacterial activities of *M. beccarianum*. The antibacterial results are perhaps not surprising due to both species belonging to the same family of plants. The results reported here are of great importance, particularly in the case of *S. aureus* which is well-known for being resistant to a number of antibiotics. However, this study did not use antibiotic-resistant clinical strain of this Gram-positive bacterium but only standard laboratory strain. Nevertheless, the results are encouraging that warrant further investigation. For future study, more bacterial strains could be tested to further determine if *M. malabathricum* and *M. beccarianum* extracts could exert significant antibacterial effect on a wide range of bacterial species. The study could be extended to include extracts from other parts of the plants as well, such as the flower and bark, which in *M. malabathricum*, have been reported to also have antimicrobial activities[5,8,10].

Antifungal activities of both methanol crude extracts were similarly evaluated against unicellular fungus, *S. cerevisiae* at varying concentrations of 100-800 mg mL^{-1}. Under the conditions tested, both plant extracts did not show any detectable antifungal activity. However, this does not necessarily mean antifungal properties were not present in the plants as the outcomes of the test could be condition-dependent. *M. malabathricum* has been previously shown to inhibit the fungal growth of *Candida krusei*[8] but it is also reported that smaller inhibition zones were observed for *S. cerevisiae* and *Fusarium oxysporum*[5], suggesting weak antifungal efficacy.

Unlike the methanol extracts, the aqueous leaf crude extracts of *M. malabathricum* and *M. beccarianum* did not

exhibit, under the conditions tested, any antibacterial activity against the 4 bacterial strains tested. Antifungal activity was not tested in this study for both of the aqueous extracts. The lack of any detectable antibacterial activity could probably be explained by the use of dilute amount of aqueous crude extract. The study could be improved by using a more concentrated aqueous crude extract through freeze-drying or lyophilisation. Other extracting solvents could also be further tested, such as ethanol, ethyl acetate, dichloromethane and petroleum ether to gain more valuable insight into the medicinal properties of these plants.

CONCLUSION

Melastoma malabathricum and *M. beccarianum* methanol leaf crude extracts have almost similar phytochemical constituents and yet both extracts were still relatively different, strongly implying the close relatedness and yet the uniqueness of the two plant species. Both extracts also exhibited antibacterial activities against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*.

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