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Phytochemicals and antimicrobial analysis of selected medicinal plants from Brunei Darussalam

ZULHAMIZAN AWANG-JAMIL[1], MUHAMMAD FAIRUZ AMINUDDIN[1], BARIRAH QURATULAIN ZAIDI[1], AIDA MARYAM BASRI[2], NORHAYATI AHMAD[1], HUSSEIN TAHA[1,♥]

[1]Environmental and Life Sciences Program, Faculty of Science, Universiti Brunei Darussalam. Jl. Tungku Link BE1410, Brunei Darussalam, ♥email: hussein.taha@ubd.edu.bn

[2]Food Science and Technology Department, School of Applied Sciences and Mathematics, Universiti Teknologi Brunei. Jl. Tungku Link BE1410, Brunei Darussalam

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Abstract. *Awang-Jamil Z, Aminuddin MF, Zaidi BQ, Basri AM, Ahmad N, Taha H. 2021. Phytochemicals and antimicrobial analysis of selected medicinal plants from Brunei Darussalam. Biodiversitas 22: 601-606.* Medicinal plants have been widely used but their pharmacological properties are yet to be fully explored. This study was aimed to determine the phytochemicals and their antimicrobial activity of four selected medicinal plants: *Melastoma malabathricum*, *Merremia borneensis*, *Pandanus amaryllifolius*, and *Senna alata* from Brunei Darussalam. Phytochemical constituents were evaluated by using gas chromatography-mass spectrometry (GC-MS) and antimicrobial screening was carried out using agar well diffusion method. A number of phytochemicals were identified in the plant extracts, of which the following compounds were present in higher amounts (>10 %): coumaran, hentriacontane, hexatriacontane, 3-hydroxy benzyl alcohol, α-linolenic acid, palmitic acid, catechol, squalene, and α-tocopherol-β-D-mannoside. A concentration-dependent antimicrobial activity was observed in some extracts against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae*. Results of this study showed medicinal potential of the four selected plants.

Keywords: Antimicrobial, *Melastoma malabathricum*, *Merremia borneensis*, *Pandanus amaryllifolius*, phytochemicals, *Senna alata*

INTRODUCTION

Medicinal plants have been traditionally used to treat many ailments and for maintaining health. In Southeast Asia, numerous plants have been described to have traditional medicinal properties. In Brunei Darussalam, more than a hundred plant species from different plant families have been recorded to have various medicinal properties (Department of Agriculture 2000; Universiti Brunei Darussalam 2018). Although medicinal plants have been used since ancient times, scientific studies have now been actively carried out to support and discover their evidence-based medicinal properties and mechanism of action, as medicines from natural sources have been gaining wide popularity. The plant species like *Melastoma malabathricum* L., *Merremia borneensis* Merr., *Pandanus amaryllifolius* Roxb. and *Senna alata* (L.) Roxb are found in abundance in Brunei Darussalam.

Ethnomedicinal properties of all four medicinal plants in Brunei Darussalam have been well documented (Department of Agriculture 2000; Universiti Brunei Darussalam 2018). Traditionally, *M. malabathricum* is used to treat nausea, reduce scars, as well as, in herbal baths after childbirth, whereas *M. borneensis* is used for hair treatment. On the other hand, *P. amaryllifolius* is a popular cooking ingredient, and is used traditionally to relieve gout, whereas *S. alata* is used to treat skin infection, constipation, and worm infestation.

A number of scientific studies have reported the bioactivities of these plants. The methanol leaf extract of *M. malabathricum* from Brunei Darussalam was reported to have antibacterial activity (Diris et al. 2017), whereas the aqueous leaf extract of *S. alata* was reported to have antifungal activity against yeast and has been used as an active ingredient in natural skincare products (Aminuddin et al. 2016). Other pharmacological properties, such as antinociceptive, anti-inflammatory, wound healing, and antidiarrheal, were also reported for *M. malabathricum* (Mohd-Joffry et al. 2012), whereas anticancer, anti-diabetic, anti-allergenic, and antioxidant properties were reported for *S. alata* (Goh et al. 2017). No dose-dependent antibacterial activity was reported in *M. borneensis* from Malaysia but its essential oils were found to consist mainly mono and sesquiterpene hydrocarbons (Hossain et al. 2012). Four antibacterial alkaloids have been isolated from the leaves of *P. amaryllifolius* in the Philippines (Laluces et al. 2015).

A study has revealed that *P. amaryllifolius* collected from different locations in Malaysia contained different levels of compounds and activities, potentially due to various factors (Ghasemzadeh and Jaafar 2013). Furthermore, various extraction methods, solvents and different parts of a plant species can influence the pharmacological activities. For example, the use of different extraction solvents had led to different observations in *Alpinia officinarum* Hance (Basri et al. 2017). Thus, this study aimed to investigate the phytochemicals and antimicrobial activity of *M. malabathricum* fruits, leaves of *M. borneensis*, *P. amaryllifolius* and *S. alata*.

MATERIALS AND METHODS

Sample collection

All samples were collected from several locations in Brunei Darussalam. Fruits of *M. malabathricum* were obtained from Labi and Universiti Brunei Darussalam, leaves of *M. borneensis* from Madang, leaves of *P. amaryllifolius* from Lambak Kanan, and Mentiri, and *S. alata* from Madang. The samples were air-dried in the laboratory for several weeks before being ground into powder with a grinder.

Preparation of aqueous extracts

Powdered sample was mixed with distilled water in the ratio of 1 g to 10 ml, and sonicated (SONICA Ultrasonic Cleaner) for 30 minutes at 25°C. It was then filtered (Whatman filter papers) to remove solid residues, and the filtrate was collected. For *S. alata*, the filtrate was directly used for testing. However, for the other plants, the filtrates were lyophilized using freeze dryer. Prior to phytochemical and antimicrobial testing, each solid extract was re-dissolved at specific concentrations in ethyl acetate and distilled water, respectively.

Preparation of methanol and hexane extracts

Powdered sample was mixed with either methanol (for each plant species) or hexane (for *S. alata* only). Soxhlet extractor was used to extract the sample for few hours per day, and the extraction was stopped when the solvent in the Soxhlet chamber turned pale in color, indicating that most compounds had been extracted. The extract was filtered (Whatman filter paper) to remove any solid residues and the solvent was removed under vacuum by a rotary evaporator. Prior to phytochemical and antimicrobial testing, each solid extract was re-dissolved in its respective solvent at specific concentrations.

GC-MS analysis of plant extracts

Shimadzu QP-2010 gas chromatography-mass spectrometry (GC-MS) was used with DB-5ms column and column flow rate of 1.69 ml/min. The operating conditions used for the GC-MS program were as follows: oven temperature of 50°C for 1 min initially, subsequently raised to 140°C at a rate of 20°C/min, and finally at 300°C for 10 min at a rate of 10°C/min. The injector and detector temperatures were 200 and 250°C, respectively, and a sample volume of 1 µl (at 15 mg/mL) was injected in splitless mode. The relative content of each phytochemical was calculated by peak area normalization. Phytochemical identification was carried out by comparison of retention time and mass spectral data with a GC-MS spectral library, National Institute of Standards and Technology (NIST) library. In this study, the only compound with at least 90 % similarity with the database was considered.

Antimicrobial screening

Agar well diffusion method was used to test for antibacterial and antifungal activity. Four bacterial strains were tested: two Gram-positive bacteria (*Bacillus subtilis* ATCC-6633 and *Staphylococcus aureus* ATCC-29213) and two Gram-negative bacteria (*Escherichia coli* ATCC-25922 and *Pseudomonas aeruginosa* ATCC-27853). Two yeast strains (*Candida albicans* and *Saccharomyces cerevisiae*) and one mold strain (*Aspergillus brasiliensis*) were also used in this study. Bacteria and *S. cerevisiae* were incubated in nutrient broth. A Mueller-Hinton agar plate was then uniformly inoculated with the culture. Similarly, potato dextrose agar (PDA) medium and Sabouraud dextrose agar (SDA) medium were used instead for *C. albicans* and *A. brasiliensis*, respectively. A cork borer was used to create an agar well of 4 mm in diameter, in which a sample volume of 40 µl (at various concentrations) was loaded. For negative control, distilled water, methanol, and hexane were loaded. For positive control, antibiotic (2 mg/mL streptomycin sulfate) was loaded. The plate was incubated before the diameter of inhibition zone, where microbial growth was inhibited, was measured the next day. The incubation was 24 h at 37°C for each bacterium, 48 h at 26°C for *S. cerevisiae*, and 72 h at 26°C for *A. brasiliensis*.

Statistical analysis

Antimicrobial results were expressed as mean ± standard deviation (SD) of four replicates. Where applicable, the data were subjected to two-tailed *t*-test for assessing significant differences between samples. *P* values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Extraction yields of *M. malabathricum* aqueous and methanol fruit extracts were 18% and 9%, respectively while, 15% and 14% for aqueous and methanol extractions of *M. borneensis* leaves, respectively. Extraction yields of *P. amaryllifolius* aqueous and methanol leaf extracts were 22% and 13%, respectively, whereas *Senna alata* leaves yielded 22% and 6% for methanol and hexane extracts, respectively.

GC-MS analysis resulted in the identification of a total of 10 phytochemicals in *M. malabathricum* methanol fruit extract, accounting for 46.43% of the total peak area observed (Table 1). More phytochemicals were identified in *M. borneensis* methanol extract (14 phytochemicals) compared to its aqueous leaf extract (6 phytochemicals), accounting for 62.09% and 67.95% of the total peak area observed, respectively (Table 2). A total of 22 phytochemicals were identified in *P. amaryllifolius* methanol leaf extract, accounting for 57.56 % of the total peak area observed (Table 3). A total of 15 and 18 phytochemicals were identified in *S. alata* methanol and hexane leaf extract, accounting for 70.90 and 85.79% of the total peak area observed (Table 4).

Based on the relative peak area, the notable compound (>10%) in *M. malabathricum* methanol fruit extract was palmitic acid (15.32%). The notable compound in *M. borneensis* methanol extract was also palmitic acid (14.34 %), whereas, in its aqueous extract, these were 3-hydroxy benzyl alcohol (30.87%), coumaran (14.43%) and catechol (11.5%). In *P. amaryllifolius* methanol leaf extract, the

notable compounds were palmitic acid (16.07%) and squalene (13.73%). In *S. alata* methanol leaf extract, the notable compounds were α-tocopherol-β-D-mannoside (17.12%), α-linolenic acid (16.09%) and palmitic acid (12.31%), whereas in its hexane extract, the notable compounds were hentriacontane (20.29 %), α-tocopherol-β-D-mannoside (19.76%) and hexatriacontane isomer (17.08%).

Concentration-dependent antibacterial activity was determined for each extract at various concentrations (60 to 400 mg/mL) against four bacterial strains i.e. *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. aureus*. Concentration-dependent antifungal activity was only determined in some extracts (*M. borneensis* and *S. alata*) at various concentrations (60 to 400 mg/mL) against *A. brasiliensis*, *C. albicans* or *S. cerevisiae*.

M. malabathricum aqueous fruit extract did not show any antibacterial activity against the four bacterial strains. Similarly, its methanol fruit extract did not inhibit the growth of *E. coli*. However, it was able to inhibit *B. subtilis*, *P. aeruginosa* and *S. aureus* at higher concentrations starting from 100 mg/mL (Table 5), indicating that the antibacterial activity was concentration-dependent. The results also suggest that this extract was significantly ($p < 0.01$) more potent against *S. aureus* due to the larger inhibition zones (11.3 ± 0.2 mm) at 400 mg/mL, compared to those observed in the other strains.

M. borneensis aqueous leaf extract did not show any antibacterial activity against the four bacterial strains. It also did not show any antifungal activity against the three fungal strains. Similarly, no antimicrobial activity was observed for its methanol leaf extract against the four bacterial strains and two fungal strains. However, it showed significant ($p < 0.01$) antifungal activity against *S. cerevisiae*, as the concentration increases, with the inhibition zones ranging from 5.5 ± 0.6 mm to 15.5 ± 1.0 mm, as shown in Table 5.

P. amaryllifolius aqueous leaf extract did not show any antibacterial activity against the four bacterial strains. In contrast, its methanol leaf extract showed antibacterial activity against all four bacterial strains (Table 5). However, the results suggest that this extract was less potent against *E. coli* as inhibition zone was only detected at the highest concentration tested (400 mg/mL).

Table 1. Phytochemicals identified in *M. malabathricum* methanol fruit extract.

Rt (min)	Compound	Molecular weight	Peak area (%)
6.23	5-Hydrxoymethylfurfural	126	4.00
7.51	Pyrogallol	126	6.12
13.14	Palmitic acid methyl ester	270	3.60
13.52	Palmitic acid	256	15.32
14.76	Linoleic acid methyl ester	294	1.64
15.12	Linoleic acid	280	4.84
15.17	Z-7-Tetradecenal	210	5.22
15.38	Stearic acid	284	2.96
16.60	Heneicosane	296	1.81
18.25	Hexatriacontane	506	0.92

Note: Rt: Retention time

Table 2. Phytochemicals identified in *M. borneensis* methanol and aqueous leaf extracts

Rt (min)	Compound	Molecular weight	Peak area (%)	
			Methanol extract	Aqueous extract
5.62	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	144	3.27	-
5.93	Catechol	110	1.25	11.5
6.14	Coumaran	120	3.15	14.43
7.28	3-Hydroxybenzyl alcohol	124	-	30.87
7.40	4-Hydroxy-benzaldehyde	122	-	5.58
7.84	3-Hydroxy-4-methoxy-benzaldehyde	152	-	1.91
9.62	3,4-Dihydroxy-benzaldehyde	138	-	3.66
12.16	Phytol	296	1.48	-
13.14	Palmitic acid methyl ester	270	2.81	-
13.53	Palmitic acid	256	14.34	-
14.90	Phytol	296	2.02	-
15.20	α-Linolenic acid	278	6.60	-
15.38	Stearic acid	284	1.81	-
20.62	Squalene	410	5.91	-
22.97	α-Tocopherol	430	2.74	-
24.21	Stigmasterol	412	3.46	-
24.93	Clionasterol	414	7.05	-
28.06	Friedelin	426	6.20	-

Note: Rt: Retention time

Table 3. Phytochemicals identified in *P. amaryllifolius* methanol leaf extract.

Rt (min)	Compound	Molecular weight	Peak area (%)
6.18	Coumaran	120	2.77
6.31	5-hydroxymethylfurfural	126	1.19
7.43	Capric acid	172	0.16
9.41	Lauric acid	200	0.34
11.50	Myristic acid	228	0.34
12.34	Hexahydrofarnesyl acetone	268	0.14
12.53	Pentadecaonic acid	242	0.97
13.18	Palmitic acid methyl ester	270	1.54
13.69	Palmitic acid	256	16.07
14.17	Margaric acid methyl ester	284	0.07
14.52	Margaric acid	270	0.96
14.80	Linoleic acid methyl ester	294	1.15
14.96	Phytol	296	2.68
15.10	Stearic acid methyl ester	298	0.35
15.40	Linolenic acid	278	5.39
15.53	Stearic acid	284	2.35
17.50	Tetracosane	338	0.21
18.32	Pentacosane	352	0.21
20.73	Squalene	410	13.73
22.40	γ-Tocopherol	416	0.85
23.08	α-Tocopherol	430	2.12
24.38	Stigmasterol	412	3.97

Note: Rt: Retention time

Table 4. Phytochemicals identified in *S. alata* methanol and hexane leaf extracts

Rt (min)	Compound	Molecular weight	Peak area (%) Methanol extract	Hexane extract
9.44	Lauric acid	120	0.60	-
10.13	Methyl β-D-glucopyranoside	194	3.07	-
11.03	Tetradecanal	212	-	1.21
11.46	Tetradecanoic acid	228	0.54	-
12.27	Phytol	296	0.75	-
12.48	Pentadecanoic acid	242	1.01	-
12.85	α-Linolenic acid	278	-	1.30
13.15	Palmitic acid methyl ester	270	2.46	-
13.55	Palmitic acid	256	12.31	3.74
14.23	α-Linolenic acid	278	1.39	-
14.83	Linolenic acid methyl ester	292	3.85	-
14.91	Phytol	296	2.15	2.07
15.23	α-Linolenic acid	278	16.09	2.55
15.41	Stearic acid	284	2.66	-
16.60	Heneicosane (isomer)	296	-	0.57
17.44	Heneicosane (isomer)	296	-	1.05
18.26	Heneicosane (isomer)	296	-	1.05
19.03	Hexatriacontane (isomer)	506	-	1.12
19.79	Tetratetracontane	618	-	1.44
20.52	Hexatriacontane (isomer)	506	-	1.37
21.23	Hexacosyl heptafluorobutyrate	578	1.61	-
21.24	Hexatriacontane (isomer)	506	-	17.08
21.93	Hexatriacontane (isomer)	506	-	2.92
22.77	Hentriacontane	436	-	20.29
23.01	α-Tocopherol-β-D-mannoside	592	17.12	19.76
23.62	Dotriacontane	450	-	1.43
24.24	Stigmasterol	412	5.29	-
24.73	Tritriacontane	464	-	4.23
24.83	1-Heptacosanol	396	-	2.61

Note: Rt: Retention time

Senna alata methanol and hexane extracts showed antibacterial activity against the four bacterial strains, with both extracts showing significantly ($p < 0.05$) the highest antibacterial activity against *E. coli* with an inhibition zone of 7.0 ± 1.0 mm and 5.9 ± 0.5 mm, respectively (Table 6). Both extracts also showed antifungal activity against *S. cerevisiae*. Methanol extract showed significantly ($p < 0.01$) higher inhibition zone of 24.3 ± 0.9 mm, compared to hexane extract with inhibition zone of 13.0 ± 1.0 mm. *S. alata* aqueous leaf extract, however, did not show any antibacterial activity. Still, it was able to inhibit the growth of *S. cerevisiae* with inhibition zone of 21.0 ± 1.0 mm. The other two fungal strains, *A. brasiliensis* and *C. albicans*, were not tested for this plant.

In this study, four selected medicinal plants (*M. malabathricum*, *M. borneensis*, *P. amaryllifolius* and *S. alata*) from Brunei Darussalam were analyzed for their concentration-dependent antimicrobial activity. The aqueous extracts of these four plants did not show any antibacterial activity against the four bacterial strains. Similar work on the aqueous leaf extract of *Aidia borneensis* Ridsdale from Brunei Darussalam also did not show encouraging antibacterial activity when compared to its methanol extract (Awang-Jamil et al. 2019). The present study also showed that antibacterial activity was observed in methanol and hexane extracts. This could suggest that the antimicrobial compounds are non-polar; hence aqueous extraction may be less suitable for the isolation of antimicrobial compounds as compared to extraction using organic solvents. Furthermore, a previous study on *Dillenia suffruticosa* (Griff) Martelli identified its organic extracts as better sources of bioactive compounds as compared to its aqueous extract (Yakop et al. 2020).

Antifungal activity was only tested in *M. borneensis* and *S. alata*. This study showed that the two plants had antifungal activity against *S. cerevisiae*. This antifungal activity was only observed in the methanol extract of *M. borneensis* and not in its aqueous extract. Similarly, in *A. borneensis*, antifungal activity was only observed in its methanol extract and not aqueous extract (Awang-Jamil et al. 2019). This further suggests that organic extraction is perhaps more suitable for the isolation of antimicrobial compounds as compared to in aqueous. However, in *S. alata*, the antifungal activity was observed in all extracts including aqueous extract. This is perhaps not surprising considering that this plant has been traditionally used to treat fungal infections such as ringworm (Department of Agriculture 2000; Universiti Brunei Darussalam 2018).

Table 6. Antimicrobial activities of *S. alata* leaf extracts at 100 mg/mL. Hyphen (-) means inhibition zone was not detected.

Microbe	Zone of inhibition (mm) Methanol extract	Hexane extract	Aqueous extract
B. subtilis	6.0 ± 0.4	5.6 ± 0.8	-
E. coli	7.0 ± 1.0	5.9 ± 0.5	-
P. aeruginosa	6.1 ± 0.6	5.0 ± 0.0	-
S. aureus	5.6 ± 0.3	5.4 ± 0.5	-
S. cerevisiae	24.3 ± 0.9	13.0 ± 1.0	21.0 ± 1.0

Table 5. Antimicrobial activities of *M. malabathricum* methanol fruit extract, *M. borneensis* methanol leaf extract and *P. amaryllifolius* methanol leaf extract. Hyphen (-) means inhibition zone was not detected.

Extract	Microbe	Zone of inhibition (mm) 60 mg/mL	80 mg/mL	100 mg/mL	200 mg/mL	400 mg/mL
M. malabathricum	*B. subtilis*	-	-	6.5 ± 0.0	6.7 ± 0.1	9.5 ± 0.1
	P. aeruginosa	-	-	-	7.5 ± 0.0	10.2 ± 0.1
	S. aureus	-	-	8.7 ± 0.4	9.2 ± 0.1	11.3 ± 0.2
M. borneensis	*S. cerevisiae*	5.5 ± 0.6	6.5 ± 0.6	8.0 ± 0.8	13.8 ± 1.0	15.5 ± 1.0
P. amaryllifolius	*B. subtilis*	-	-	6.3 ± 0.1	6.4 ± 0.1	6.9 ± 0.1
	E. coli	-	-	-	-	6.3 ± 0.0
	P. aeruginosa	-	-	6.1 ± 0.1	6.1 ± 0.0	6.4 ± 0.1
	S. aureus	-	-	6.2 ± 0.0	6.2 ± 0.0	6.5 ± 0.1

A number of phytochemicals were successfully identified from the four selected plants, and nine of the phytochemicals were considered as notable because they were present in higher amount (>10 %) i.e. coumaran, hentriacontane, hexatriacontane, 3-hydroxybenzyl alcohol, α-linolenic acid, palmitic acid, catechol, squalene and α-tocopherol-β-D-mannoside.

Interestingly, palmitic acid was present notably in all methanol extracts. This suggests that methanol is perhaps a suitable solvent for extracting this saturated fatty acid, as this compound was not observed in the aqueous and hexane extracts. The results also suggested that this phytochemical might be responsible for the antibacterial activity observed in all methanol extracts. However, other phytochemicals should not be ruled out yet, as antibacterial activity could arise from multiple compounds. Palmitic acid and other fatty acids have been reported to inhibit the growth of many oral microbes (Huang et al. 2011). Antifungal activity is also reported for palmitic acid against several fungal strains (Altieri et al. 2007; Liu et al. 2008). A derivative of vitamin E, α-tocopherol-β-D-mannoside was present notably in both methanol and hexane extracts of *S. alata*. This suggests that *S. alata* was rich in vitamin E. This vitamin, which can exist in different forms, is a well-known antioxidant agent, with α-tocopherol being the most important for human physiology (Rigotti 2007).

The other seven notable compounds, on the other hand, were only present in one of the extracts. This indicates that phytochemical constituents were unique in different extracts and plants. Coumaran has previously been reported to have antioxidant activity (Murakami et al. 2000), anti-inflammatory effect (Inagaki et al. 2003), and can inhibit acetylcholinesterase activity (Rajashekar et al. 2014). Hentriacontane and hexatriacontane are hydrocarbon lipid molecules, which were both found in *S. alata* hexane leaf extract. Hexane is a commonly used solvent for lipid extraction. Hentriacontane has also been reported to possess anti-inflammatory effects and antitumor activity (Kim et al. 2011; Takahashi et al. 1995). Hexatriacontane is reported as one of the major components in *Rosa damascena* Mill. essential oils that showed stronger antioxidant activity (Yassa et al. 2009). It is reported that 3-hydroxy benzyl alcohol from fungus showed antimicrobial, antibiofilm, antioxidant and anti-inflammatory activities (Kumar et al. 2017). However, in the present study, antimicrobial activity was not detected in the aqueous extract containing this phytochemical. This is perhaps due to it being bacterial strain selective or due to differences in the methods used. The essential fatty acid, α-linolenic acid, is essential in the human diet, likely because it is required to synthesize other compounds that are important for tissue function (Burdge 2006). Catechol has antibacterial activity (Kim and Lee 2014; Jeong et al. 2009), antifungal (Kocacaliskan et al. 2006) and antioxidant activities (Eshwarappa et al. 2014). Squalene is known to have biological activities such as antioxidant and antitumor properties (Huang et al. 2009).

In conclusion, four selected medicinal plants (*M. malabathricum*, *M. borneensis*, *P. amaryllifolius* and *S. alata*) from Brunei Darussalam were found to have specific antimicrobial activity, and were also identified to have some phytochemicals that are known to be bioactive. Further investigation on the biological activities of the isolated compounds needs to be carried out to determine which ones are responsible for the acclaimed medicinal properties. More research is still needed to elucidate the medicinal potential of the four selected plants, especially that several factors, such as age of plant, plant variety, and environmental factors, may influence the bioactivities and phytochemical constituent of any plants.

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