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Determination of antioxidant property of commercially selling Borneo plants by 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid) free radical scavenging assay

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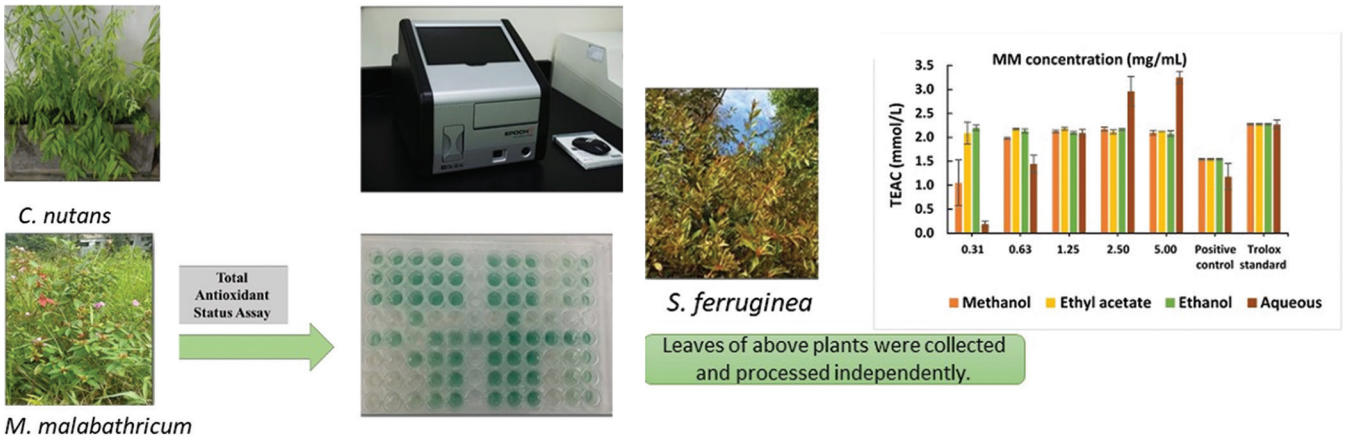
ABSTRACT

Background: Antioxidants from natural products are important to counter the effect of reactive oxygen species occur in chronic diseases and metabolic disorders. **Objective:** This study evaluated the antioxidant activities of the leaf extracts from three plants found in Brunei: *Melastoma malabathricum* (MM), *Scurrula ferruginea* (SF), and *Clinacanthus nutans* (CN). **Methods:** The plant leaves were collected from in Brunei Darussalam air-dried and pulverized into powder. The extracts obtained from Soxhlet, fractionation, and maceration were freeze-dried and the total antioxidant status was evaluated using 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid) scavenging radical activities *in vitro* assay. Both MM and SF extracts exhibited promising antioxidant capabilities. **Results:** The recorded antioxidant activities of MM with 5 mg/mL of methanolic, ethyl acetate, ethanolic, and aqueous extracts were 2.10, 2.12, 2.16, and 3.25 mmol/L, respectively. The antioxidant activities of the same concentration of SF aqueous, ethyl acetate, and methanolic extracts were 2.23, 2.35, and 2.46 mmol/L, respectively. Conversely, CN extracts used in the study did not show significant antioxidant activity. **Conclusion:** The present study demonstrated that MM and SF extracts possess excellent antioxidant activities. The results suggest that these plants have therapeutic potential for metabolic syndrome and inflammatory diseases. Thus, further investigations for anti-inflammatory and analgesic activities are recommended.

Key words: *Melastoma malabathricum*, *Scurrula ferruginea*, *Clinacanthus nutans*, Antioxidative activity, Ethyl acetate fraction

GRAPHICAL ABSTRACT



INTRODUCTION

Reactive oxygen species (ROS) can be in a form of free radical or non-radical molecules with high oxidizing nature that could damage cellular systems when present at high levels. A free radical contains one or more unpaired electrons in the atomic or molecular orbitals. Oxygen-centered free radicals such as superoxide ($O_2^{\bullet}$-), hydroxyl radicals ($^{\bullet}OH$), hydroxyl ion (OH-), nitric oxide radical ($NO^{\bullet}$) and hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) are naturally generated as by-products of normal metabolism in the biological systems of any organisms inhabiting the aerobic environment. High levels of ROS in the cells could be damaging as they are responsible for cell death through oxidation of cellular components such as DNA, lipids in cell membranes, carbohydrates, proteins, and enzymes in tissues.[1] There have been many evidences showing that ROS play a major role in the initiation and progression of a number of human diseases such as cancer, cardiovascular diseases, neurodegenerative diseases, gastrointestinal diseases, and diabetes.[1] Various studies have associated diabetes with imbalance of antioxidative natures in the systems due to increased formation of free radicals and impaired antioxidant defenses. The increased in the formation of free radicals is majorly due to hyperglycemia that accelerates auto-oxidation of glucose in glycolysis to produce pyruvate and thus generates ROS through complex II in the electron transfer chain.[2] Whereas in cancer, high concentration of ROS can lead to modifications of DNA coding especially in transversions of thymine (T) to guanine (G) of critical genes such as tumor-suppressing genes and oncogenes that could result in the progression and advancement of cancer.[3]

Table 1: Vernacular names and ethnopharmacological uses of *M. malabathricum, S. ferruginea,* and *C. nutans* plants

Scientific names	Vernacular names	Uses
M. malabathricum Family: Melastomaceae Order: Myrtales	• *Kuduk-kuduk* • *Senduduk*	Stomach problems, diarrhea, as an astringent, postpartum treatment, wounds, hemorrhoids dysentery, leukorrhea, flatulence, sore legs, and thrush[7,8]
S. ferruginea Family: Loranthaceae Order: Santalales	• *Akar naloe* • *Benalu* • *Dalu-dalu* • *Dedalu api merah* • *Dedalu api gajah* • *Menalu asap* • *Suridan*	Skin infection, smallpox, cough, diabetes, cancer, high blood pressure, and gastrointestinal malfunctions[9]
C. nutans Family: Acanthaceae Order: Lamiales	• *Belalai gajah* • *Sabah snake grass* • *Dandang Gendis* • *Ki tajam*	Cancer, inflammatory disorders, diabetes, insect bites, skin problems and rashes, insects and snake bites, sores by herpes simplex infection, diabetes mellitus, fever, and diuretics[10-12]

M. malabathricum: Melastoma malabathricum, S. ferruginea: Scurrula ferruginea, C. nutans: Clinacanthus nutans

The removal of excess reactive molecules formation in the cells could help to slow down the progression of ROS-associated diseases to prevent oxidative stress and its downstream complications. Antioxidants can inhibit the formation of ROS and act as radical scavengers as well as increased the antioxidant defense enzyme in the biological systems.[2] Borneo island is well-known for its flora and fauna biodiversity with the highest diversity of any place on Earth as it is estimated around 15,000 plant species present on the island.[4,5] Over the past decades, there have been anticipating interests and evidences that plant is a good source of antioxidants, particularly polyphenols. Polyphenols which include phenols, phenolic acid, and flavonoids are important natural plant antioxidants and commonly found at high levels.[2,6] Consumption of plants and herbs for disease treatment has been long practiced by the folklore in the Borneo. Our laboratory has a particular interest in studying *Melastoma malabathricum* (MM), *Scurrula ferruginea* (SF), and *Clinacanthus nutans* (CN). These plants were believed to possess antioxidant components and have been used traditionally by the community to treat various diseases and disorders such as skin problems, inflammatory disorders, cancer, diabetes, diarrhea, and insect bites, as summarized in Table 1.

In support of traditional medicine, scientific evidences of these plants' functions in health have been well documented through animal testing in the pharmacological studies, *in vitro* and *in vivo* analysis of the biological compounds which include antioxidant, antiproliferative, cytotoxic, antidiabetic, antihyperlipidemic, and hepatoprotective activities.[5,13] In MM studies, phytochemical screening conducted on various MM extracts showed that the aqueous extract contained only flavonoids, whereas flavonoids and steroids were detected in the chloroform extract, and the methanol extract contained saponins, flavonoids, condensed tannin, and steroids.[14] Pharmacological studies performed on alloxan-induced diabetic Wistar albino rats demonstrated that ethanol extract of MM exerted positive results on several biochemical parameters tested such as lipid profiles, urea and creatinine levels, HbA1c concentration, and protein albumin and globulin levels. All these parameters were significantly reduced and restored to normal levels compared to diabetic control rats which demonstrated its antidiabetic properties.[8] In another study, the methanol extract of MM leaf was shown to possess a hepatoprotective activity in carbon tetrachloride-induced liver toxicity rats.[15] Whereas in SF studies, methanol extracts of SF leaves, stems, and flowers contained bioactive compounds, flavonoids and condensed tannins, and terpenoids. SF was shown to have antiviral activity against poliovirus and antihypertensive properties.[12] Antimicrobial activity of SF aqueous extract was confirmed by minimum inhibitory concentration test against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli,* and *Pseudomonas putida.*[12] In CN scientific studies, petroleum ether leaves extracts of CN were reported to exhibit high cytotoxicity against HeLa cells (IC_{50} of 18.0 μg/mL) and proliferation of K-562 cells (IC_{50} of 20.0 μg/mL) was reduced after 72 h incubation.[16] Microbial and fungal studies conducted on the same experiment demonstrated its excellent antimicrobial and antifungal activities against *Bacillus cereus* and *Candida albicans,* respectively, at 1.39 mg/mL.[17]

Table 2: Details of plant leaf extract solvents and methods used for the experiment

Plants	Solvents	Extraction methods
M. malabathricum	Methanol	Soxhlet
	Ethyl acetate	Fractionation
	Ethanol	Soxhlet
	Aqueous	Maceration
S. ferruginea	Aqueous	Maceration
	Methanol	Soxhlet
	Ethyl acetate	Fractionation
C. nutans	Aqueous	Maceration
	Chloroform	Soxhlet
	Acetone	Maceration
	Aqueous	Fractionation

M. malabathricum: Melastoma malabathricum, S. ferruginea: Scurrula ferruginea, C. nutans: Clinacanthus nutans

To the best of our knowledge, the previous antioxidant studies reported were performed mostly using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays and hydrogen peroxide (H_2O_2) scavenging assays but there have not been any antioxidant studies conducted using 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) free radical scavenging assays on any of our plants of interest. It has been found that plants from different regions have different amounts of constituents due to the regional and climatic differences. Thus, it is important to understand the amounts of constituents present in the extracts. The measurement of antioxidant capacity showed a stronger positive correlation of the oxygen radical absorbance capacity with ABTS than DPPH. ABTS assay better reflects the high-pigmented and hydrophilic antioxidants than DPPH assay, suggesting that ABTS is better for testing antioxidant activity.[18]

As there is increasing evidence of antioxidant activities and promising pharmacological properties of MM, SF, and CN, we are taking the opportunity to evaluate their antioxidant activity using ABTS free radical scavenging assays. The aim of the present study is to determine the antioxidant activity of various leaf extracts of MM, SF, and CN, as listed in Table 2.

MATERIALS AND METHODS

Plants processing and preparation of extracts

The fresh plant leaves of MM, SF, and CN were collected from Brunei-Muara district in Brunei Darussalam and air-dried at room temperature. The dried leaves were pulverized into powder. For the preparation of aqueous and acetone extract, 10 g of powdered leaves, individually, were soaked in 50 mL distilled water and acetone, respectively. They were sonicated for 2 h using a Bransonic® ultrasonic bath (Branson, Danbury, USA) at 20 kHz. The solutions were collected and filtered using Whatman No.1 filter paper (Whatman, Maidstone, England).

However, for the preparation of methanol, ethanol, and chloroform crude extracts, the powdered leaves were extracted by Soxhlet with methanol, ethanol, and chloroform, individually. Meanwhile, ethyl acetate fraction was prepared from methanol extract mixed with distilled water and ethyl acetate and separated to obtain ethyl acetate fraction.

CN aqueous fraction was prepared by Soxhlet extraction with chloroform and the marc was collected and macerated with methanol to obtain methanol extract. The methanol extract was then fractionated with distilled water and ethyl acetate, and separated to obtain CN aqueous fraction.

All extracts obtained from Soxhlet and fractionation methods were evaporated using the rotary evaporator and then dried in the oven for 2 days. All extracts including aqueous extract were stored in the −80°C freezer overnight and were subjected to freeze-drying for 2 days. The percentage yield of the maceration was around 30–40 while that of the Soxhlet extraction was around 25–35. The details of the plant leaf extract solvents and methods used for the experiment are mentioned in Table 2 and Figure 1.

Antioxidant activity

Total antioxidant status assay. The antioxidant activity of the selected extracts is based on the scavenging activity of the antioxidants to counter the formation of 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium free radical cations ($ABTS^{\bullet+}$). All freeze-dried extracts were dissolved in 70% ethanol and filtered through 0.22 μm filter paper. The activities of antioxidants were determined using the procedure outlined in the commercial kits (Randox Laboratories Ltd., Antrim, UK) with slight modifications. The assay used was adapted to micro plate method as described by Kirakosyan *et al*.,[19] as opposed to the conventional cuvette method to allow a large number of samples reading simultaneously and to economize the volume of reagents used. ABTS was incubated with metmyoglobin and H_2O_2 to produce $ABTS^{\bullet+}$ which was measured at 600 nm using a Bio-Tek microplate reader (BioTek Instruments Inc., Highland Park, USA). The presence of antioxidants decreases the intensity of the blue-green color produced by $ABTS^{\bullet+}$ resulting in a lower absorbance value.

Each well of 96-well microtiter plate, independently, contained 2 μL of extracts, standards, a blank and controls. The positive and negative controls used in this study were control serum of human origin and 70% ethanol, respectively. Distilled water was used as a blank. Each sample was run in triplicates. To each well, 100 μL of chromogen was added, and the plate was incubated at 37°C in a water-jacketed incubator with 5% CO_2 for 10 min. After 10 min, initial absorbance reading at 600 nm was taken as a baseline for color development. Subsequently, after measurement, 20 μL of H_2O_2 was added to each well using multichannel pipettes, and the plate was reincubated for 3 min in the same conditions to allow color development. Later, the final absorbance reading at 600 nm was recorded. The difference between the initial and final absorbance readings was used for assessing the antioxidant activities. The antioxidant activities were expressed in Trolox units, mmol/L where the Trolox standard and 70% ethanol negative control were used for data transformation which was calculated using a two-step method as given below:

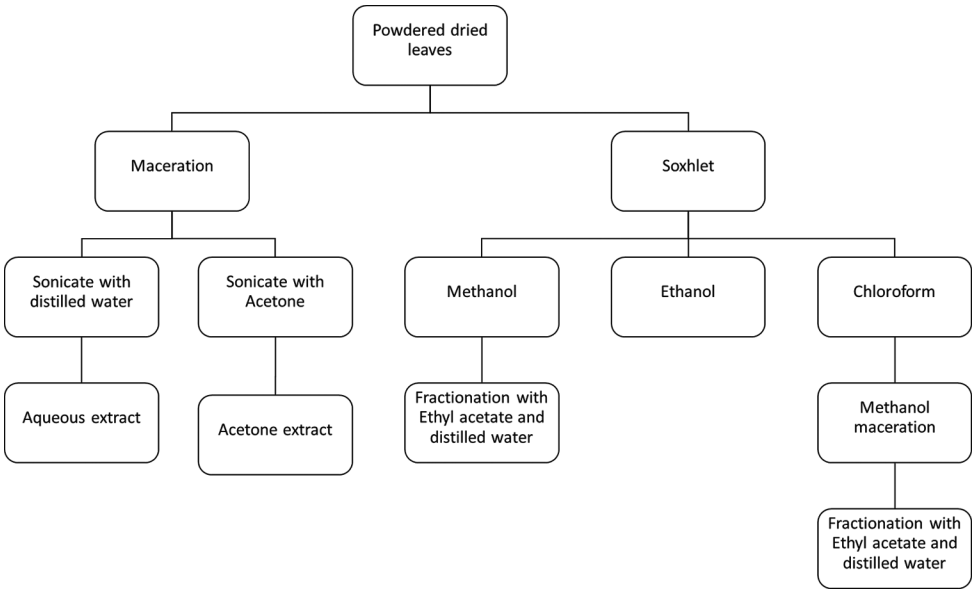


Figure 1: Preparation of extracts

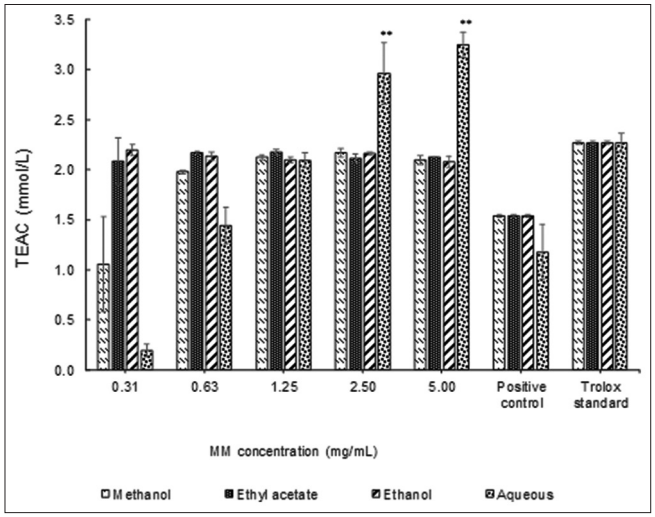


Figure 2: Total antioxidant status of MM extracts. The (mean ± SD) data represent the Trolox equivalent antioxidant capacities (TEACs). Experiments were performed in triplicates. Comparisons were made between standard and samples; significant differences are indicated with * ($P < 0.05$) and ** ($P < 0.01$)

Step 1: Determination of standard factor

$$\text{Standard(Trolox) factor} = \frac{\text{Concentration of Trolox}}{\text{change in absorbance of 70\% EtOH blank} - \text{change in absorbance of Trolox}}$$

Step 2: Calculation of antioxidant status

$$\text{Antioxidant status}\left(\frac{\text{mmol}}{\text{L}}\right) = \text{"Standard (Trolox) Factor"} \times (\text{change in absorbance of 70\% EtOH blank} - \text{change in absorbance of test sample})$$

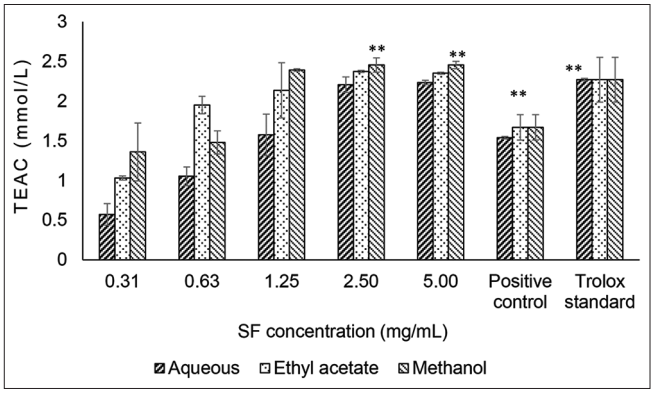


Figure 3: Total antioxidant status of SF extracts. The data (mean ± SD) represent the TEAC. Experiments were performed in triplicates. Comparisons were made between standard and samples; significant differences are indicated with * ($P < 0.05$) and ** ($P < 0.01$)

Statistical analysis

The results are presented as mean standard deviation of samples and analyzed using ANOVA in SPSS with the Scheffe's *post hoc* test where $P < 0.05$ was considered as statistically significant.

RESULTS

The method described gives a measure of the antioxidant activity of the leaf extracts expressed as Trolox equivalent antioxidant capacities (TEACs) determined by the decolorization of $ABTS^{\bullet+}$ through reduction of the absorbance at 600 nm within 3 min due to the reaction between metmyoglobin and H_2O_2. This gives the values of TEAC at a specific time point and the calculated results are tabulated in Table 3. From the graphs shown, different plant extracts demonstrate distinct antioxidant activity profiles. Figure 2 illustrates the antioxidant activity of MM extracts. All concentrations of MM methanol, ethanol, and ethyl acetate extracts, except 0.31 mg/mL methanol and aqueous extracts,

Table 3: Antioxidant activities of *M. malabathricum*, *S. ferruginea*, and *C. nutans* leaf extracts as expressed in Trolox equivalent antioxidant capacity (TEAC) in mmol/L

Concentration (mg/ml)	*M. malabathricum*				*S. ferruginea*			*C. nutans*			
	Aqueous	Ethyl acetate	Methanol	Ethanol	Aqueous	Ethyl acetate	Methanol	Aqueous	Aqueous fraction	Acetone	Chloroform
0.31	0.19±0.06**	2.09±0.23**	1.05±0.48	2.20±0.06	0.57±0.14**	1.03±0.03**	1.36±0.37	0.27±0.06**	−0.46±0.13**	0.28±0.21**	−0.66±0.11**
0.63	1.44±0.18	2.17±0.02**	1.99±0.02	2.13±0.04	1.05±0.11	1.95±0.11	1.48±0.15	0.02±0.10**	−0.72±0.15**	0.77±0.31**	−1.01±0.34**
1.25	2.09±0.08**	2.18±0.03**	2.12±0.03	2.10±0.03	1.58±0.26	2.14±0.35	2.39±0.02	−0.03±0.08**	−0.89±0.10**	0.71±0.28**	−0.87±0.08**
2.50	2.96±0.31**	2.12±0.04**	2.17±0.04**	2.16±0.02	2.21±0.10**	2.37±0.01	2.46±0.09	−0.01±0.19**	−0.69±0.08**	0.59±0.00**	−0.64±0.10**
5.00	3.25±0.13**	2.12±0.00**	2.10±0.05	2.16±0.02	2.23±0.03**	2.35±0.01	2.46±0.05	0.00±0.36**	−0.46±0.07**	0.94±0.06**	−0.63±0.19**
Positive	1.18±0.28	1.54±0.02	1.54±0.02	1.54±0.02	1.54±0.02	1.67±0.16	1.67±0.16	1.67±0.16	1.18±0.28	1.18±0.28	1.18±±0.28
Standard	2.27±0.09	2.27±0.02	2.27±0.02	2.27±0.02	2.27±0.02	2.27±0.28	2.27±0.28	2.27±0.28	2.27±0.09	2.27±0.09	2.27±0.09

The values are expressed in mean±standard deviation, n=3. Comparisons were made between positive controls and samples; significant differences are indicated with * (P<0.05) and ** (P<0.01)

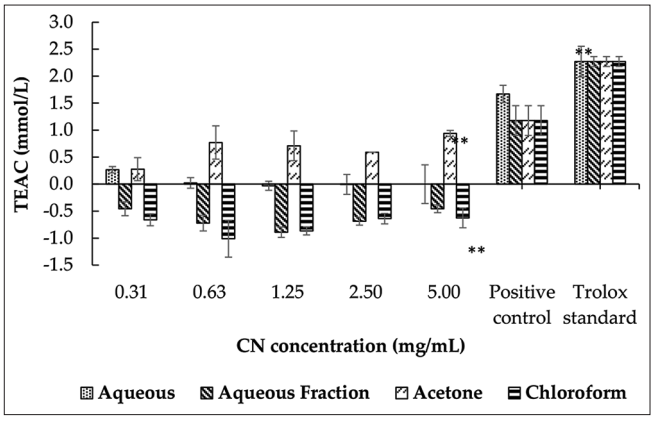


Figure 4: Total antioxidant status of CN extracts. The (mean ± SD) data represent the TEAC. Experiments were performed in triplicates. Comparisons were made between standard and samples; significant differences are indicated with * (P < 0.05) and ** (P < 0.01)

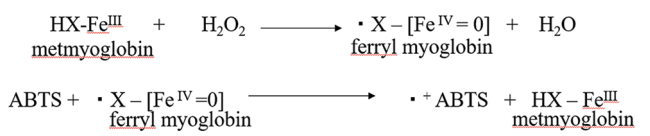


Figure 5: 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium free radical cations ($ABTS^{\bullet+}$) formation reaction. In the first step of the reaction, metmyoglobin reacts with hydrogen peroxide (H_2O_2) to form ferrylmyoglobin radicals and water. Consequently, the ferryl radicals react with 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) to produce $ABTS^{\bullet+}$ and regenerate metmyoglobin

exhibited higher antioxidant capacities than positive control (1.54 mmol/L ± 0.02). Both aqueous and methanol extracts demonstrate a dose-dependent activity whereas the other extracts reached plateau right at the lowest concentration (0.3125 mg/mL). It is interesting to note that MM aqueous extract of 2.5 and 5 mg/mL concentration exhibited significantly high antioxidant activities compared to both standard and control.

On the other hand, Figure 3 demonstrates a dose-dependent antioxidant activity of SF methanol, ethyl acetate, and aqueous extracts. The antioxidant activities of ethyl acetate and methanol extracts reached the highest and plateau at 1.25 mg/mL concentration of the extracts. At the highest extract concentration, 5 mg/mL, the antioxidant activities of methanol, ethyl acetate, and aqueous extracts showed TEAC values of 2.46 ± 0.05, 2.35 ± 0.01, and 2.23 ± 0.03 mmol/L, respectively. Statistical analysis shows no significant differences between SF samples, the TEAC values indicate that the antioxidant capacity of SF is similar in methanol, ethyl acetate, and aqueous extract. As illustrated in Figure 4, there was no significant antioxidant activity evident in all CN extracts between 0.31 and 5 mg/mL concentrations.

TEAC is calculated by multiplying standard Trolox factor with the difference between changes in absorbance of 70% ethanol and changes in absorbance of the sample.

DISCUSSION

The present study has revealed the presence of antioxidant activities in both MM and SF extracts. The distinct antioxidant

Table 4: Comparison of antioxidant activities from different research studies conducted on M. malabathricum, S. ferruginea, and C. nutans

	Solvent (s)	Part of plant used	Plant processing method	Antioxidant test used	Reference
M. malabathricum	Ethyl acetate and methanol	Flowers	Air-dried and powdered flowers were extracted by Soxhlet extraction	2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay	[7]
	Aqueous, chloroform, and methanol	Leaves	Air-dried and grinded leaves were extracted by soaking in the respective solvents for 72 h	DPPH radical scavenging assay superoxide scavenging assay	[14]
	Chloroform, ethyl acetate, and methanol	Leaves	Air-dried and powdered leaves were extracted by Soxhlet extraction	β-carotene bleaching method	[20]
S. ferruginea	Acetone	Leaves, stems, and flowers	Oven-dried samples were grounded and extracted by soaking in the respective solvents	DPPH radical scavenging assay	[9]
C. nutans	Chloroform, methanol, and aqueous	Leaves	Oven-dried and powdered leaves were extracted by soaking in the respective solvents	DPPH Galvinoxyl Nitric oxide (NO) radicals Hydrogen peroxide (H_2O_2) scavenging assay	[21]
	Petroleum ether, ethyl acetate, and methanol	Leaves and stems barks	Oven-dried and powdered samples were extracted by soaking in the respective solvents	DPPH radical scavenging assay	[17]
	Ethanol	Leaves and stems	Various drying and extraction methods used; with either freeze dry, oven dry, or air dry Sonication or soaking	DPPH radical scavenging assay	[22]

M. malabathricum: Melastoma malabathricum, S. ferruginea: Scurrula ferruginea, C. nutans: Clinacanthus nutans

profiles may indicate the presence of unique antioxidant constituents. The present study shows that MM extracts achieved high antioxidant activities even at low concentration which signifies excellent antioxidant capacities better than the control. Table 4 summarizes antioxidant research studies conducted on MM, SF, and CN plants.

The antioxidant activities of plant can be read through several radical scavenging assays. In this ABTS principle assay, the generation of $ABTS^{\bullet+}$, as outline in Figure 5, involves the reaction between metmyoglobin, an oxidized form of the oxygen carrying hemeprotein myoglobin, and H_2O_2 to form myoglobin radicals and water.[23] Ferrylmyoglobin radicals oxidize ABTS to form $ABTS^{\bullet+}$ and regenerate metmyoglobin. The formation of $ABTS^{\bullet+}$ results in a blue-green color development, which is measured at 600 nm. Therefore, an increased in the color intensity means lesser antioxidant activities. Antioxidants inhibit the oxidation of ABTS by electron transfer radical scavenging to a degree which is proportional to the antioxidant concentration.[24]

In another study, the antioxidant activities of the flower extracts of MM revealed that the methanol extract was a stronger free radical scavenger (59.3%) compared to ethyl acetate extract (53.2%).[7] Karupiah and Ismail[20] reported that chloroform extract of MM leaves possessed a stronger antioxidant activity (83.28%) followed by methanol extract (81.74%) and then the ethyl acetate fraction (44.41%). The different solvent extracts of MM used were 0.2 mg/mL concentration which were extracted by Soxhlet. Conversely, a different method of extraction carried out in a different study showed that extraction by soaking revealed that methanol extract of MM possessed a greater antioxidant capacity (97.3%) followed by aqueous extract (69.8%), however, chloroform extract failed to show any antioxidant activities. This discrepancy in results provides an evidence that the choice of extraction methods affects the presence of phytochemical compositions and thus antioxidant activities of the plants extracts.[22,25] Other than exploiting different extraction methods, Karupiah and Ismail[20] and Zakaria team[14] employed different antioxidant assays, that is, DPPH scavenging assay and β-carotene bleaching method, respectively. Different radical species are present in different assays and different phytochemical constituents may react kinetically different on the radicals present which lead to significant discrepancies in their results. It was shown that polyphenolic compounds have not only a wide range of antioxidant activities but react distinctively in DPPH radical scavenging and $ABTS^{\bullet+}$ assays.[26] The rate of scavenging activities is highly dependent on the stereoselectivity of the radicals and the structure of the antioxidant itself. Antioxidant activities of phenolic compounds are related to the arrangement of the hydroxyl groups and the presence of conjugated rings in the compound structures.[27,28] The kinetic rate of reactions is linearly correlated to the number of hydroxyl groups present in the antioxidants.[7,29]

Similar to MM and SF extracts demonstrate excellent antioxidant activities where the SF methanol extract showed the highest activity while SF aqueous had the least, however, it is worth mentioning that the difference is not very high. This is confirmed by the statistical analysis. In par with the only antioxidant study of SF extract to date, all SF acetone extracts from the stems, leaves, and flowers exhibited antioxidant study in a dose-dependent manner.[9] The study reported at 0.5 mg/mL, the highest antioxidant activity of SF leaves acetone extract was recorded (80%). The scavenging activity of SF extracts increases as the concentration increases. This may be attributed to the wide range of phytochemical constituents of the plants such as phenols,

carotenoids, tannins, flavonols, and some other compounds.[9,12]

The present study demonstrated that aqueous and chloroform extracts of CN did not show any significant antioxidant activities whereas the acetone extract exhibits little antioxidant activities (0.94 mmol/L) which was observed at 5 mg/mL. However, a study using DPPH radical scavenging assay had shown that aqueous extract of CN at 10 mg/mL demonstrated 60% radical scavenging activity.[30] The concentrations used in the present experiment might be too low to observe its antioxidant activity. Moreover, between 26.53 and 46.71 mg gallic acid equivalent per gram of phenolic compounds were reported in CN soaked in distilled water in various time points at 0.5, 1, 3, 5, and 24 h.[11] An interesting study utilizing various methods of scavenging assays was conducted by Yong *et al.*[21] comparing the antioxidant activities of various CN extracts which demonstrated that different kind of extracts response differently to different assays. 0.1 mg/mL concentration of aqueous CN extract was shown to be a good NO radical scavenger (30%) and methanol CN extract was the most effective H_2O_2 radical scavengers (34%). Meanwhile, chloroform CN extract exhibited highest DPPH scavenging activity with an antioxidant capacity of 7852.63 μgTeq/g. This provides evidence that different antioxidant molecules may present in each different extract. The highly selective antioxidant molecules may have preferential scavenging activities. Future research can look into the mechanism of action of the activities and to determine the structural conformation of the antioxidant.

CONCLUSION

Medicinal plants such as MM, SF, and CN have been scientifically reported to be useful in treating a variety of diseases and have been traditionally practiced by the people across Borneo. Our findings demonstrated that MM and SF plants are rich in antioxidants, using the ABTS method, which support previous claims on its traditional uses in the treatment of various ailments, and thus, further drug development and formulation of MM and SF plants would be beneficial.

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ETHICS APPROVAL

This study does not involve any animal or human.

COMPETING INTEREST

None was declared.

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