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Full Length Article

From Weed to Medicinal Plant: Antioxidant Capacities and Phytochemicals of Various Extracts of *Mikania micrantha*

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Abstract

Mikania micrantha is commonly consumed as traditional medicine in some countries, including Malaysia. Little is known about the antioxidant properties and phytochemicals of *M. micrantha*. This study was aimed to investigate the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacities of the leaves and stems of *M. micrantha* of hot water, cold water, 70% ethanol, ethyl acetate, and hexane extracts. Folin-Ciocalteu and aluminium chloride colorimetric assays were used to determine the TPC and TFC, respectively. The antioxidant capacities were determined using rapid, inexpensive and small-scale microplate of five different antioxidant assays. Gas chromatography-mass spectrometry (GC-MS) was used to chemically profile and characterize the phytochemicals. In comparison with different solvents, the ethyl acetate stems (EAS) and leaves (EAL) extracts of *M. micrantha* had the significantly greatest TPC (141 ± 0.51 mg gallic acid equivalent/g) and TFC (70.1 ± 0.92 catechin equivalent/g), respectively. Moreover, EAS extract had the significantly greatest antioxidant capacities using DPPH (EC_{50} = 324 ± 61.4 µg/mL), ABTS (0.53 ± 0.01 mmol trolox equivalent/g), FRAP (1.28 ± 0.05 mmol Fe^{2+}/g), phosphomolybdenum antioxidative power (219 ± 7.03 mg ascorbic acid equivalent/g), and β-carotene bleaching (108 ± 2.23%) assays. GC-MS analysis of EAS showed the presence of sesquiterpenes (30.46%), phenol (16.38%), and alkane hydrocarbons (10.45%), which may contribute to its antioxidant capacities. These findings suggest the stems extract of *M. micrantha* using ethyl acetate as the potential source of natural antioxidant agents and its utilization to prevent oxidative damage-related diseases could be further explored.

Keywords: Antioxidant; GC-MS; *Mikania micrantha*; Phenolic; Phytochemicals; Sesquiterpenes

Introduction

Herbal medications have gained much attention for their use in relieving symptoms of disease (Shayganni *et al*., 2015) and important in health care especially in developing countries. Studies have reported various plants, fruits and vegetables to contain a high amount of antioxidants that can be utilized to prevent oxidative damage-related diseases (Bordoloi *et al*., 2016; Ozkan *et al*., 2016). Naturally, antioxidants can be found in parts of plants, *i.e.*, flowers, leaves, stems, and roots. However, many factors including genetics, geographical region, climate/season, storage, and even the processing condition can affect the antioxidant capacities and amount of bioactive phytochemicals in plants (Li *et al*., 2012; Arena and Radice, 2016; Rai *et al*., 2017).

Mikania micrantha Kunth (Asteraceae or Compositae) is a perennial creeping vine and widely distributed in South and North America and can also be found in Africa, Pacific Islands and Southeast Asia, including Southern China and Malaysia (Tripathi *et al*., 2012; Day *et al*., 2016; Ishak *et al*., 2016). This plant is known as American rope, Chinese creeper, mile-a-minute, 'Chhagalbati' or 'Japanilata' (West Bengal), 'Selaput tunggul' (Malaysia) and 'Sembung rambat' (Indonesia) (Haisya *et al*., 2013; Nornasuha and Ismail, 2013; Saha *et al*., 2015). In agriculture, *M. micrantha* is a weed plant that can reduces the growth and productivity of several crops such as rubber, oil palm, and cocoa plantation in Malaysia which cost 8–10 million dollars per annum to control its growth (Sankaran, 2008). This is due to its fast-growing habit and production of allelopathic substances (Sankaran, 2008; Nornasuha and Ismail, 2013; Day *et al*., 2016). However, this plant is used traditionally to treat insect bites and stop minor external bleeding or consumed as a juice as an alternative to reduce glucose, cholesterol, and high blood pressure (Facey *et al*., 2010; Ishak *et al*., 2016).

M. micrantha has demonstrated many health benefits, such as antimicrobial (Facey *et al*., 2010; Chetan *et al*., 2012; Chetia *et al*., 2014), anti-diabetic (Wan Nurhayati *et*

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al., 2013), anti-dermatophytic (Jyothilakshmi *et al*., 2015), anti-stress (Ittiyavirah and Sajid, 2014), anti-inflammatory (Pérez Amador *et al*., 2010), anti-proliferative (Ríos *et al*., 2014), and anti-cancer (Dou *et al*., 2014; Matawali *et al*., 2016) activities. In fact, *M. micrantha* is rich in phytochemicals such as terpenoids (sesquiterpene lactones), alkaloids, flavonoids, steroids, reducing sugars, saponins, phenolics and tannins (Dev *et al*., 2015; Dong *et al*., 2017).

In spite of its medicinal benefits as aforementioned, the information of the potential antioxidant agent in this plant is still scarce especially the comparison between different parts and the effect of different solvents which can affect the antioxidant capacities and bioactive compounds in this plant. Antioxidant agents is important to prevent oxidative damage-related diseases *i.e*., cancer, cardiovascular diseases and diabetes etc., (Sharma *et al*., 2014) thus potential health benefits of this plant should be further investigated. We hypothesized that the effectiveness and efficiency of secondary metabolites are significantly affected by the extraction solvents (Kong *et al*., 2012). Therefore, this study was aimed to determine and compare the antioxidant properties of various solvent polarities, *i.e*., hot water, cold water, 70% ethanol, ethyl acetate, and hexane extracts of the leaves and stems of *M. micrantha* using rapid and small-scale microplate assays of the antioxidant procedures. The GC-MS analysis was performed to chemically profile and characterize phytochemicals of *M. micrantha* and hence, to confirm its medicinal values.

Materials and Methods

Sample Collection and Preparation

Mikania micrantha was collected in August 2015 from Penang, Malaysia, and identified by the Forest Research Institute Malaysia (FRIM), Kepong, Selangor, Malaysia (Voucher no: SBID 051/15). The leaves and stems of *M. micrantha* were separated, washed and dried in a ventilated drying oven at 35°C for 72 h.

Extraction of *M. micrantha*

Powdered leaves and stems of *M. micrantha* were extracted separately using solvents of different polarities, *i.e*., hot water and cold water (highly polar), 70% ethanol (polar), ethyl acetate (semi-polar) and hexane (non-polar). For hot water extraction, 25 g of the samples were immersed in 250 mL of distilled water (dH_2O) and incubated at 70°C for 18 h. Cold water, 70% ethanol, ethyl acetate and hexane extracts were prepared by homogenizing 25 g of the samples with 250 mL of the solvent on an orbital shaker at 100 rpm for 72 h at room temperature. The organic solvents (70% ethanol, ethyl acetate and hexane) were evaporated at 48°C, while the filtrate for water extracts was freeze-dried.

Determination of Antioxidant Contents and Capacities

Total phenolic content (TPC): Determination of TPC was carried out according to Lee *et al*. (2014). Samples or a standard (20 µL) were mixed with 100 µL of diluted Folin-Ciocalteu reagent (1:10, v/v in distilled water) in a 96-well plate. After 5 min, 80 µL of 7.5% sodium carbonate (Na_2CO_3) were added to each well. The plate was covered and left in the dark for 30 min on a Stovall belly dancer (Greensboro, NC, USA). The absorbance was measured at 765 nm against a reagent blank. A standard calibration curve using gallic acid (0.98–1000 µg/mL) was plotted, and the results were expressed as mg gallic acid equivalent (GAE)/g extract using the following formula: TPC per 1 g extract = [(TPC per mL sample x dilution factor x total sample volume used)/sample weight].

Total flavonoid content (TFC): The TFC was carried out according to Belguith-Hadriche *et al.* (2013) with modifications. Samples or a standard (25 µL) were pipetted into the 96-well plate. Then, 100 µL of distilled water (dH_2O) and 7.5 µL of 5% sodium nitrite ($NaNO_2$) were added and incubated for 5 min. After that, 7.5 µL of 10% aluminium chloride hexahydrate ($AlCl_3.6H_2O$) was added and incubated for another 5 min. Then, 50 µL of 1 M sodium hydroxide (NaOH) and 60 µL of dH_2O were added and the absorbance was read using a microplate reader at 510 nm. A standard calibration curve using catechin (1.95–250 µg/mL) was plotted, and TFC were expressed as mg catechin equivalent (CE)/g extract using the formula: TFC per 1 g extract = [(TFC per mL sample x dilution factor x total sample volume used)/sample weight].

DPPH radical scavenging assay: The DPPH radical scavenging capacity was determined using the method described by Kong *et al*. (2012). Samples or a standard (50 µL) at various concentrations (0.98–1000 µg/mL) and 195 µL of 100 µM DPPH solution were mixed in a 96-well plate and left in the dark at room temperature for 30 min. The absorbance of the reaction mixture was read at 515 nm. Butylated hydroxytoluene (BHT) and gallic acid were used as standards.

ABTS radical scavenging assay: ABTS radical scavenging capacity was determined according to the method described by Othman *et al*. (2016) with some modifications. The samples or a standard (20 µL) were mixed with 200 µL of the $ABTS^{\bullet+}$ solution in a 96-well plate, and incubated at 30°C for 6 min. The results were expressed as mmol trolox equivalent (TE)/g extract from the trolox calibration curve (0.02–0.31 mM).

Ferric reducing antioxidant power (FRAP) assay: The FRAP assay was carried out to determine the iron-reducing capacity of each extract according to the method by Benzie and Strain (1996), with modifications. Firstly, 300 mM acetate buffer (pH 3.6) and 40 mM HCl were prepared. FRAP reagent was freshly prepared by mixing the acetate buffer, 10 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ at a ratio of 10:1:1

(v:v:v), and pre-warmed in a water bath at 37°C. Then, 20 µL of samples/standard/blank were mixed with 180 µL FRAP reagent in a 96-well plate and incubated at 37°C for 30 min. The absorbance was read at 593 nm. A standard calibration curve was plotted using iron (II) sulphate heptahydrate ($FeSO_4.7H_2O$) (0.1 – 1.0 mM) and the final results were expressed as mmol Fe^{2+}/g extract.

Phosphomolybdenum antioxidative power (PAP) assay: The total antioxidant capacity of the samples was determined by green phosphomolybdenum complex formation (Prieto *et al*., 1999). Reagent solution was prepared by mixing of sulphuric acid (0.6 M), sodium phosphate (28 mM) and ammonium molybdate (4 mM) in 50 mL distilled water. Sample (100 µL) and 1 mL reagent solution were mixed in a microcentrifuge tube and incubated at 95°C for 90 min. The sample mixture was allowed to cool to room temperature prior pipetting 200 µL of the mixture into a 96-well plate. The absorbance was measured at 695 nm against the reagent blank. The antioxidant capacity was expressed as mg ascorbic acid equivalent (AAE)/g extract from the standard calibration curve of ascorbic acid (0.001–0.25 mg/mL) using the formula: Antioxidant capacity (AC) = [(AC per mL sample × dilution factor × total sample volume used) / sample weight].

β-carotene bleaching (BCB) assay: The β-carotene bleaching assay was performed using the procedure described by Othman *et al*. (2016) with some modifications. The β-carotene (BC) working reagent was prepared by mixing 1 mL of BC (0.2 mg/mL in chloroform) with 20 µL linoleic acid and 200 µL Tween 40 in a 100 mL round bottom flask, and the mixture was evaporated using a rotary evaporator at 30°C for 1 min. Next, 50 mL of ultrapure water was added to the mixture and shaken vigorously to form an emulsion. An aliquot of 200 µL of the preheated emulsion (50°C) was transferred into a 96-well plate containing 20 µL of the sample/standard/blank. The zero-time absorbance was measured at 470 nm immediately, and the mixture was incubated at 50°C for 2 h. Blank samples (in the absence of β-carotene) were prepared for background subtraction, and BHT was used as the standard. The capacity of the extracts to protect against the oxidation of β-carotene was determined according to Othman *et al*. (2016).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC–MS analysis was used for phytochemicals identification of the ethyl acetate leaves (EAL) and stems (EAS) extracts of *M. micrantha* according to Painuli *et al*. (2016). The samples (100 µg/mL) were prepared in methanol (HPLC grade) and filtered through a 0.2 µm nylon membrane filter prior to analysis. Compounds were identified based on the retention time (RT) for GC, and interpretation of mass spectrum was done by comparing spectral fragmentation obtained to the database provided by the National Institute Standards and Technology (NIST08.LIB).

Statistical Analysis

Results were expressed as mean ± standard error of the mean (SEM) of at least three independent experiments. One-way analysis of variance (ANOVA) and Tuckey's multiple comparison test were used to compare means between solvents. An independent t-test was used to compare means between leaves and stems. Pearson correlation test was used to determine the relationship between antioxidant contents and antioxidant activities. The data were analysed using IBM SPSS Statistics, version 21 and were considered statistically significant when $p < 0.05$.

Results

Total Phenolic and Flavonoid Content in *M. micrantha* Extracts

The TPC ranged between 38.3±4.67 to 104±2.50 and 34.8±1.80 to 141±0.51 mg GAE/g, for the leaves and stems of *M. micrantha*, respectively (Table 1). The ethanol leaves (ETL), hot water leaves (HWL), and ethyl acetate leaves (EAL) extracts had the greatest phenolic contents but not significantly ($p > 0.05$) different when compared with each other. However, ethyl acetate stems (EAS) extract showed significantly ($p < 0.05$) greater TPC (141±0.51 mg GAE/g) when compared to EAL. Hexane extracts contained the lowest TPC and no significant ($p > 0.05$) different (38.3±4.67 mg GAE/g and 34.8±1.80 mg GAE/g) between the leaves and stems, respectively.

The TFC ranged between 8.54±0.69to 70.1±0.92 and 3.47±0.25 to 44.6±4.15 mg catechin equivalent/g (CE/g), for the leaves and stems of *M. micrantha*, respectively (Table 1). The EAL extract had significantly ($p < 0.05$) greater TFC compared to other extraction solvents in the following trend: ethyl acetate > hexane > 70% ethanol > hot water > cold water. A similar trend was observed for TFC of the stems extracts. Between parts, ethyl acetate and hexane extracts of the leaves contained significantly ($p < 0.05$) greater TFC than the stems extracts. Hot water, cold water, and 70% ethanol extracts of the leaves showed greater but not significant ($p > 0.05$) TFC than the stems.

Antioxidant Capacities

DPPH radical scavenging capacity: The EC_{50} of DPPH scavenging capacity of *M. micrantha* extracts ranged between 544±19.1 to 699±21.2 µg/mL and 324±61.4 to 752±77.7 µg/mL, for the leaves and stems, respectively (Table 2). EAS was the most efficient extract which had a significantly ($p < 0.05$) lower EC_{50} value (324 µg/mL) when compared to the other extracts. A low EC_{50} value indicates the potent radical scavenging capacity of the extracts as a low concentration was adequate to inhibit the DPPH radicals. For the standards, the EC_{50} of the gallic acid (2.33±0.14 µg/mL) was lower than BHT (62.27±5.46 µg/mL).

Table 1: Antioxidant contents of the leaves and stems of *M. micrantha* extracted using hot water, cold water, 70% ethanol, ethyl acetate and hexane

	Extraction solvent				
	Hot water	Cold water	70% ethanol	Ethyl acetate	Hexane
Total phenolic content (TPC) (mg GAE/g extract)					
Leaves	$93.7 \pm 0.53^{a*}$	$69.6 \pm 0.64^{b*}$	$104 \pm 2.50^{a*}$	83.4 ± 8.89^{ab}	38.3 ± 4.67^{c}
Stems	56.5 ± 2.25^{b}	48.8 ± 2.00^{bc}	44.2 ± 2.53^{c}	$141 \pm 0.51^{a*}$	34.8 ± 1.80^{d}
Total flavonoid content (TFC) (mg CE/g extract)					
Leaves	11.6 ± 1.05^{c}	8.54 ± 0.69^{c}	12.8 ± 1.81^{c}	$70.1 \pm 0.92^{a*}$	$38.6 \pm 1.70^{b*}$
Stems	5.49 ± 0.46^{c}	3.47 ± 0.25^{c}	10.0 ± 0.89^{c}	44.6 ± 4.15^{a}	25.7 ± 1.74^{b}

Results are expressed as the means ± SEM (n = 3). Values with different letters are significant at $p < 0.05$ between the extraction solvents of the same parts and * indicates significance at $p < 0.05$ between different parts of the same solvent. GAE, gallic acid equivalent; CE, catechin equivalent. The concentration of samples used for TPC and TFC were 1 mg/mL

Table 2: Antioxidant capacities of the leaves and stems of *M. micrantha* extracted using hot water, cold water, 70% ethanol, ethyl acetate, and hexane

	Extraction solvent				
	Hot water	Cold water	70% ethanol	Ethyl acetate	Hexane
DPPH assay EC_{50} (µg extract/mL)					
Leaves	$544 \pm 19.1^{a*}$	ND	699 ± 21.2^{a}	583 ± 14.3^{a}	ND
Stems	752 ± 77.7^{b}	ND	640 ± 3.00^{b}	$324 \pm 61.4^{a*}$	ND
ABTS assay (mmol TE/g extract)					
Leaves	0.18 ± 0.01^{b}	0.13 ± 0.00^{c}	$0.26 \pm 0.01^{a*}$	0.22 ± 0.00^{a}	0.10 ± 0.00^{c}
Stems	0.15 ± 0.02^{b}	0.09 ± 0.01^{c}	0.14 ± 0.01^{b}	$0.53 \pm 0.01^{a*}$	0.08 ± 0.01^{c}
FRAP assay (mmol Fe^{2+}/g extract)					
Leaves	0.34 ± 0.04^{b}	$0.25 \pm 0.01^{bc*}$	0.36 ± 0.03^{ab}	0.48 ± 0.04^{a}	0.18 ± 0.03^{c}
Stems	0.21 ± 0.01^{bc}	0.09 ± 0.02^{c}	0.31 ± 0.02^{b}	$1.28 \pm 0.05^{a*}$	0.16 ± 0.04^{c}
PAP assay (mg AAE/g extract)					
Leaves	39.4 ± 1.25^{d}	36.8 ± 1.19^{d}	65.6 ± 3.40^{c}	109 ± 6.89^{a}	84.4 ± 2.64^{b}
Stems	$62.2 \pm 1.60^{c*}$	$64.2 \pm 5.53^{c*}$	54.1 ± 1.09^{c}	$219 \pm 7.03^{a*}$	$124 \pm 3.34^{b*}$
BCB assay (% inhibition of bleaching)					
Leaves	61.2 ± 11.4^{a}	60.1 ± 10.1^{a}	80.6 ± 8.68^{a}	89.6 ± 3.51^{a}	76.6 ± 3.92^{a}
Stems	63.9 ± 10.5^{b}	ND	68.4 ± 6.73^{b}	$108 \pm 2.23^{a*}$	75.8 ± 8.12^{b}

Results are expressed as the means ± SEM (n = 3). Values with different letters are significant at $p < 0.05$ between the extraction solvents of the same parts, and * indicates significance at $p < 0.05$ between different parts of the same solvent; ND, not detected; EC_{50}, concentration of the extracts (µg/mL) required to inhibit 50% of the radicals; the lowest EC_{50} indicates the greatest antioxidant capacity; TE, trolox equivalent; AAE, ascorbic acid equivalent

ABTS radical scavenging capacity: For the leaves, ethanol leaves (ETL) and ethyl acetate leaves (EAL) extracts showed significantly ($p < 0.05$) greater TEAC (trolox equivalent antioxidant capacity) value compared to hot water, cold water, and hexane extracts (Table 2). For the stems, ethyl acetate stems (EAS) extract showed significantly ($p < 0.05$) greatest TEAC value (0.53±0.01 mmol TE/g) compared to the other extracts. Between parts, only ETL and EAS showed a significantly ($p<0.05$) greater TEAC value than the stems and the leaves, respectively.

Ferric reducing antioxidant power (FRAP): The ethyl acetate extracts of both the leaves and stems (0.48±0.04 mmol Fe^{2+}/g, 1.28±0.05 mmol Fe^{2+}/g, respectively) exhibited the greatest iron reducing ability, followed by 70% ethanol and hot water of both parts. However, cold water and hexane extracts showed the lowest antioxidant capacity (Table 2). Similar to other assays, the FRAP assay also demonstrated that EAS exhibited the most significant ($p < 0.05$) and greatest antioxidant capacity (1.28±0.05 mmol Fe^{2+}/g) when compared to the other solvents.

Phosphomolybdenum antioxidative power (PAP): Total antioxidant capacity of *M. micrantha* extracts ranged between 36.8±1.19 to 109±6.89 and 54.1±1.09 to 219±7.03 mg ascorbic acid equivalent/g (AAE/g) for the leaves and stems, respectively (Table 2). The ethyl acetate and hexane extracts (semi-polar and non-polar, respectively) had significantly ($p < 0.05$) greater total antioxidant capacities compared to the more polar extracts (hot water, cold water, and 70% ethanol). Between parts, stems extracts had a greater antioxidant capacity than the leaves extracts. However, the ethanol leaves (ETL) extracts showed a significantly ($p < 0.05$) greater antioxidant capacity than the ethanol stems (ETS) extract (65.6±3.40 and 54.1±1.09, respectively).

β-carotene bleaching (BCB): All extracts showed the inhibition of β-carotene bleaching except the cold water stem extract, which had no detectable inhibition (Table 2). EAS had a significantly ($p < 0.05$) greater antioxidant capacity (108±2.23%) when compared to the leaves and other extracts. Interestingly, EAS extract had a greater antioxidant capacity when compared to the standard BHT (94.95%) at the same test concentration of 1000 ppm.

Table 3: Pearson correlation analyses of the antioxidant contents and the antioxidant capacities in the extracts of *M. micrantha*

		DPPH	ABTS	FRAP	PAP	BCB
TPC	r value	-0.685**	0.897**	0.829**	0.490**	0.463*
TFC	r value	-0.459	0.410*	0.485**	0.629**	0.576**

**Correlation is significant at $p < 0.01$; *Correlation is significant at $p < 0.05$. TPC, total phenolic contents; TFC, total flavonoid contents; DDPH, 2,2-Diphenyl-1-picrylhydrazyl; ABTS, 2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid; FRAP, ferric reducing antioxidant power; PAP, phosphomolybdenum antioxidative power; BCB, β-carotene bleaching assay

Correlation between Antioxidant Contents and Antioxidant Capacities

The correlation coefficients (r) between TPC, TFC, and antioxidant capacities of *M. micrantha* extracts are shown in Table 3. TPC revealed a very strong (r >0.8) positive correlation with the ABTS (r = 0.897) and FRAP (r = 0.829) assays, and strong (r = 0.60-0.79) negative correlation with the DPPH assay (r = -0.685). Meanwhile, TFC exhibited a strong positive correlation with the PAP assay (r = 0.629) and moderate (r = 0.40–0.59) positive correlation with the BCB (r = 0.576) assay.

GC-MS Analysis of Ethyl Acetate Extracts of *M. micrantha*

GC-MS analysis was performed to identify the phytochemicals present in the ethyl acetate extracts of the leaves (EAL) and stems (EAS) of *M. micrantha* which had the greatest antioxidant contents and capacities. The EAL extract had a total of 19 identified compounds (Table 4), which are composed primarily of alkane hydrocarbons (26.7%), sesquiterpenes and their derivatives (18.74%), and phenol (14.74%). In EAS, there were a total of 24 compounds identified (Table 4), which included sesquiterpenes and their derivatives (30.46%), phenol (16.38%), and alkane hydrocarbons (10.45%).

Discussion

Extraction is the main step in medicinal plant study for the recovery and isolation of bioactive plant phytochemicals prior to analysis. The choice of extracting solvent is important as various solvent polarities affect the yield and type of compound extracted (Chan *et al*., 2015). Therefore, *M. micrantha* samples were extracted using five different solvents to determine the antioxidant content and capacities. The greatest TPC content in the ethyl acetate, 70% ethanol, and hot water extracts of *M. micrantha* indicate the presence of medium polar to highly polar phenolic compounds in the extracts. Hexane, the non-polar solvent has a reduced ability to extract phenolic compounds from *M. micrantha* compared to the polar solvents (Table 1). However, for TFC, ethyl acetate and hexane were the best solvents to extract flavonoid compounds from *M. micrantha* (Table 1).

Table 4: Phytochemicals identified in the leaves and stems of *M. micrantha* extracted using ethyl acetate

Peak	Compounds	CAS	RT (min)	MW	Area (%)
Ethyl acetate leaves					
1	2,2-Dimethoxybutane	3453-99-4	3.358	118	15.64
2	Nonane	111-84-2	5.465	128	2.65
3	Undecane	1120-21-4	7.172	156	4.06
4	Cyclohexyl(dimethoxy)methylsilane	17865-32-6	9.748	188	0.90
5	α-Cubebene	17699-14-8	12.604	204	0.65
6	α-Longipinene	5989-08-2	12.710	204	0.51
7	Copaene	3856-25-5	13.028	204	0.65
8	Germacrene D	23986-74-5	13.176	204	4.26
9	Caryophyllene	87-44-5	13.650	204	4.38
10	Cedrene	11028-42-5	13.747	204	0.81
11	α-Caryophyllene; Humulene	6753-98-6	14.119	204	0.88
12	Acoradiene	24048-44-0	14.234	204	0.41
13	4,6-Dimethyldodecane	61141-72-8	14.350	198	1.14
14	3,5-bis(1,1-dimethylethyl)phenol	1138-52-9	14.610	206	14.74
15	β-Himachalene	1461-03-6	14.685	204	2.71
16	δ-Cadinene	483-76-1	14.849	204	3.48
17	Hexadecane	544-76-3	14.918	226	1.73
18	2,6,11-Trimethyldodecane	31295-56-4	15.170	212	0.98
19	Eicosane	112-95-8	17.385	282	0.50
Ethyl acetate stems					
1	2,2-Dimethoxybutane	3453-99-4	3.358	118	4.76
2	Undecane	1120-21-4	5.489	156	1.60
3	Decane	124-18-5	7.201	142	2.34
4	Cyclohexyl(dimethoxy)methylsilane	17865-32-6	9.756	188	0.61
5	α-Cubebene	17699-14-8	12.607	204	1.52
6	α-Longipinene	5989-08-2	12.720	204	0.17
7	Thujopsene	470-40-6	12.824	204	0.18
8	Copaene	3856-25-5	13.032	204	1.28
9	Caryophyllene	87-44-5	13.653	204	8.20
10	Cedrene	11028-42-5	13.751	204	1.28
11	cis-β-Farnesene	28973-97-9	13.933	204	0.79
12	α-Caryophyllene; Humulene	6753-98-6	14.123	204	2.77
13	β-Cubebene	13744-15-5	14.225	204	0.96
14	Di-epi-α-cedrene; α-Funebrene	50894-66-1	14.239	204	1.12
15	α-Amorphene	483-75-0	14.324	204	0.52
16	Germacrene D	23986-74-5	14.439	204	3.78
17	α-Zingiberene	495-60-3	14.525	204	0.51
18	3,5-bis(1,1-dimethylethyl)phenol	1138-52-9	14.613	206	16.38
19	β-Himachalene	1461-03-6	14.690	204	1.66
20	trans-α-Bergamotene	13474-59-4	14.750	204	0.61
21	δ-Cadinene	483-76-1	14.854	204	4.35
22	Eicosane	112-95-8	14.919	282	1.28
23	Nonadecane	629-92-5	15.174	370	0.47
24	Cedrol	77-53-2	16.221	222	0.76

CAS = chemical abstract service number; RT = retention time; MW = molecular weight

The high flavonoid content in the ethyl acetate extracts demonstrated the presence of semi-polar flavonoids and less polar flavonoids, such as isoflavones, flavanones, methylated flavones, and flavonols (Martson and Hostettmann, 2005). Moreover, ethyl acetate and hexane promotes the extraction of lipophilic compounds, such as flavonoids and carotenoids (Bae *et al*., 2012). Another study also showed the ability of ethyl acetate to extract more flavonoid compounds from medicinal plant compared to other organic solvents (Mohd Hadzri *et al*., 2014).

Natural antioxidants have various functions and show synergistic interactions. Therefore, it is important to use different types of solvent extractions and various methods to

evaluate the antioxidant capacities of plant extracts to avoid a misleading interpretation (Lin *et al*., 2013). From our data, the ethyl acetate stems (EAS) extract stands out with efficient antioxidant capacities (Table 2), which significantly correlated with the phenolic and flavonoid contents (Table 3). A very strong positive correlation between TPC with ABTS and FRAP assays demonstrated that the phenolic compounds act as hydrogen donors and reducing agents in neutralizing the free radicals due to their hydroxyl groups (Saeed *et al*., 2012). Meanwhile, flavonoids contribute moderately to the total antioxidant capacity and the inhibition of β-carotene bleaching based on the PAP and BCB assays, respectively. Overall, phenolic compounds might act as antioxidant agents in scavenging radicals using the DPPH and ABTS assays, whilst the total flavonoids act as chain-breaking antioxidants by donating a hydrogen atom to lipid radicals using the BCB assay.

GC-MS analysis of the ethyl acetate extracts of *M. micrantha* indicated the presence of three major compounds such as sesquiterpenes, phenol, and alkane hydrocarbons in the extracts (Table 4). The presence of 3,5-bis (1,1-dimethylethyl) phenol in the EAS extract could be the contributor to the high phenolic and antioxidant capacities in the extract, since, in most cases, the inhibitory activity of extracts has been attributed to the most dominant compounds (Das *et al*., 2012). In fact, phenols can act as antioxidants by donating an H-atom to unstable free radicals through the hydrogen atom transfer (HAT) mechanism and can also act as an iron chelator. We have also shown that there was a positive correlation between the total phenolic content as given by the ABTS and FRAP assays (Table 3) that could be attributed by the presence of 3,5-bis (1,1-dimethylethyl) phenol in the ethyl acetate extracts. Moreover, 3,5-bis (1,1-dimethylethyl) phenol has been reported as having antioxidant and anti-cancer properties (Rizvi *et al*., 2014). An alkoxy derivatives of alkane, 2,2-dimethoxybutane found in both stems and leaves of ethyl acetate extracts has been reported to possess anti-dermatophytic activity (Das *et al*., 2012). Other alkanes hydrocarbon compounds detected such as nonane, decane, undecane, 4,6-dimethyldodecane, hexadecane, 2,6,11-trimethyldodecane, eicosane and nonadecane (Table 4) might possess other potentials. Sesquiterpenes, which were found abundantly in the extracts could also act synergistically as the antioxidant compounds. For instance, caryophyllene, one of the bicyclic sesquiterpenes has potential antioxidant, anti-inflammatory, and antimicrobial activities (Sharma *et al*., 2016). Another compounds such as cedrol (a sesquiterpene alcohol), cedrene, and thujopsene possess inhibitory effects against cytochrome P450 (CYP) enzyme (CYP2B6 and CYP3A4) in human liver microsomes *in vitro* as well as anti-inflammatory, antifungal, insecticidal, diuretic, antispasmodic, astringent, sedative and antiseptic properties (Jeong *et al*., 2014). All phytochemicals detected in the ethyl acetate extracts of *M. micrantha* in present study was reported for the first time, except α-Longipinene, β-Himachalene, β-Cubebene, and α-Zingiberene, which were also reported in the previous study of the essential oil composition of *M. micrantha* from China (Zhang *et al*., 2004).

In addition, there are an adequate supply of raw material of this plant for the development of modern medicines in pharmaceutical industry (Jyothilakshmi *et al*., 2015) since it can be found easily in plenty from its natural habitat. Results from this study demonstrated ethyl acetate as the best solvent for extraction of phytochemicals from *M. micrantha* particularly its stems part, which also showed the greatest antioxidant capacities. Therefore, these findings provide scientific evidences for the potential utilization of this plant in the prevention and treatment of oxidative damage-related diseases and many more. In fact, ethyl acetate has been used as an extraction solvent in the production of pharmaceutical which has the lowest toxic potential (Cue and Zhang, 2009). This application could increase the value of *M. micrantha* from weed to medicinal plant.

Conclusion

The present study showed that ethyl acetate stems (EAS) and leaves (EAL) of *M. micrantha* contained the greatest TPC and TFC compared to other extraction solvents, respectively. EAS extract had the greatest radical scavenging activity, hydrogen donating ability, iron reducing ability, total antioxidant capacity, and inhibition of β-carotene bleaching based on DPPH, ABTS, FRAP, phosphomolybdenum, and β-carotene bleaching assays, respectively. The significant relationship between antioxidant contents and antioxidant capacities of *M. micrantha* indicates that total phenolic and flavonoid contribute to the antioxidant capacities. The GC-MS analysis of the ethyl acetate extracts revealed that the bioactive compounds are 3,5-bis (1,1-dimethylethyl) phenol, an alkylphenol, sesquiterpene and their derivatives, and alkane hydrocarbons. In summary, ethyl acetate is the best solvent to extract abundant bioactive compounds with strong antioxidant activity in *M. micrantha*. Hence, utilization of *M. micrantha*, particularly its stem as a source of natural antioxidants should be further explored in future.

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