PHYTOCHEMICAL OF INVASIVE PLANT: *MIKANIA MICRANTHA*

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ABSTRACT

Mikania micrantha is an invasive plant that has become a weed pest, grows aggressively, spreads, and displaces other plants in agriculture corps and generally consumed as traditional medicine purposes by local practitioners. The study on the phytochemical for these species rather scanty. This study was conducted with the aim to identify phytochemical in M. micrantha and its potential usage for midecinal purposes. Phytochemical of methanolic and petroleum ether extract obtained from leaf and stem of M. micrantha was analyzed using Gas Chromatography – Mass Spectrometry (GC-MS) for the identification of biochemical components present. Phytochemical analysis of the M. micrantha extract by GCMS revealed the presence of various compound such as phenol, fatty acid, sesquiterpenes, diterpenes, alkane hydrocarbon and others which have diverse use. The occurrence of Ascorbic acid in all extract for the present study may be the reason for the use of the extract from M. micrantha in the treatment of skin diseases such as wounds, heal cuts and stop minor external bleeding. α-Bisabolol known as Sesquiterpenes, were found abundant in Mikania genus could also act as antioxidant compounds. Phytol which also have Sesquiterpenes nature is one among the compounds appear in leaves for M. micrantha methanolic extract of the present study. Most of the compound lies under phenolic compound possess perfect structural chemistry for free radical scavenging activities as they have phenolic hydroxyl groups that are susceptible to contributing a hydrogen atom or electron to a free radical, expanded conjugated aromatic system to delocalize an unpaired electron. A wide range of phytochemical which are having antimicrobial and antioxidant activity were identified so that it can be recommended as a plant of phytopharmaceutical importance. Futher research should be done on different type of extraction for better result in phytochemical analysis.

Key words: *Mikania micrantha*, phytochemical, phenolic compound.

INTRODUCTION

Invasive legally defined as an organism that is not native to the ecosystem under consideration and whose introduction causes or likely to cause harm to environment, economy or human health (Esham, 2012). This includes microorganism, fungi, animal and plant. Invasive plant is a name for a species that has become a weed pest, a plant which grows aggressively, spreads, and displaces other plants. Invasive plants tend to appear on disturbed ground, and the most aggressive can invade existing ecosystems such as young plantation and agriculture corps (Cock *et al*., 2000). Invasive plants are generally undesirable because they are difficult to control, can escape from cultivation, and can dominate whole areas. These invasive plants also can affect soil quality (Weidenhamer *et al*, 2010). An aggressive plant freed from its environmental, pest, and disease limits, can become an invader of other ecosystems (Pérez-Amador, Muñoz Ocotero, Ibarra Balcazar, & García Jiménez, 2010). There is increasing evidence that allelopathy may play a key role in the successful of plant invaders (Murrell *et al*., 2011).

Allelopathy refers to chemically mediated interference between plants, whereby secondary compound produced directly or indirectly suppress the growth and fitness of other species (Inderjit and del Moral, 1997). They can cause interference and result in losses for the cultivation of plants to absorb nutrients and water from the soil and reception of light for photosynthesis. Allelopathic effects likely contribute to the success of several important plant invaders, such as in agriculture plantation area in Malaysia; *Mikania micrantha* (*M. micrantha)* (RISDA, 2011).

M.micrantha also known as selaput tunggul in Malaysia (Ishak *et al*, 2016), Bitter vine or Climbing Hemp vine or Amarican rope (Dev *et al*, 2015), Chinese creeper or mile a minute (Werren, 2001) or Sembung rambat (Tantra, 2014) (**Figure 1**) belonging to *Asteraceae* family (Zhang, Chen, & Wen, 2016) that is the largest family of flowering plants and its has the nature of climbers. This climber with heart- shaped leave can be found growing along the roadside, swampy woods, bushes of moist places, forest borders, and also along streams and rivers (Saha, Mandal, & Chowdhury, 2015). It is called mile-a-minute weed because of its fast-growing properties.These weeds have a capacity of being vegetative and its sexual reproduction is high and has a very fast growth. The seed dissemination of this plant relies on the wind power and might successfully germinate within 8 days (Li *et al.*, 2015). Various methods have been developed to control invasive plants to restore native plant communities. However, restoration

in habitats invaded by *M. micrantha* is sometimes difficult even though they have been controlled or removed. These invaders have the tendency to strike back. *M. micrantha* is a noxious weed that causes damage to agricultural land and plantations. This is caused by allelopathic chemical contained in *M. micrantha* which compound allelochemical that can inhibit the growth of other plants.

In spite of that, *M. Micrantha* is traditionally known for its medicinal properties and has been used as a tradisional healers (Bhardwaj & Gakhar, 2005; King, Vital & Rivera, 2009). These medicinal properties come from phytochemical in *M. Micrantha*. Phytochemicals are plant chemicals that are produced to protect themselves against microorganisms and diseases. Recent research shows that phytochemicals can protect humans from most diseases as well. Phytochemicals can be classified as polyphenols, carotenoids, alkaloids, terpenes, glucosinolates, polysaccharides, lectins, polyacetylenes, allium compounds, capsaicinoids, betalains, and chlorophyll (Tiwari *et al.*, 2013). However, there is only limited research on the phytochemicals of *M. Micrantha* as a scientific proof of its traditional uses. This paper shall include details on the phytochemical or bioactive compound in *M. Micrantha* extract, which will provide reference knowledge for potential analysis.

Figure 1: The plant *Mikania micrantha*

METHODOLOGY

M. micrantha leaf and stem were collected from idle plantation area in Meru, Klang Selangor. Authentication of the plant was carried out at Herbarium, Faculty of Forestry, UPM, where the specimen of the voucher was housed under reference number H030. The fresh samples were washed thoroughly using water to remove dirt, separated leaves and stems and dried under shades at room temperature for 4 days. The dried samples were grinded to fine powder using grinder, sieved (40-60 mesh size) and stored 4°C ±1 for further usage.

The extraction was conducted using the Soxhlet apparatus followed by a slight tweak (Ahmad *et al.*, 2009) with methanol and petroleum ether as a solvent. Five grams powdered leaves were extracted with refluxing methanol and petroleum ether in soxhlet apparatus for 8 hours. The filtrates were concentrated in rotary evaporator until all solvent removed and oven dry to constant weight. The crude extracts were stored in the refrigerator at 4°C ±1 for further used.

Gas Chromatography- Mass Spectroscopy (GC-MS) Analysis

GC-MS analysis was carried out on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859, and USA) which includes a Perkin Elmer Auto sampler XLGC. The column used was Perkin Elmer Elite - 5 capillary column measuring 30m × 0.25mm with a film thickness of 0.25mm composed of 95% Dimethyl polysiloxane. The carrier gas used was Helium at a flow rate of 0.5ml/min. 1µl sample injection volume was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed initially at 110°C for 4 min, then increase to 240°C. And then programmed to increase to 280°C at a rate of 20°C ending with a 5 min. Total run time was 90 min. The MS transfer line was maintained at a temperature of 200°C. The source temperature was maintained at 180°C. GCMS was analyzed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library. Measurement of peak areas and data processing were carried out.

Most components extracted from *M. Micrantha* were identified by comparing the retention indices (RIs) and comparing the obtained mass spectra of the analytes with those of authentic standards from the National Institute Standard and Technology (NIST) libraries and with the mass spectra published previously.

RESULTS

Analysis of the gas chromatography mass spectrometry was performed for leaves and stems of *M. micrantha* extracted from Methanol and petroleum ether. The peaks were integrated into the chromatogram and compared with the spectrum database of known components stored in the GC-MS library. Detailed GC-MS analysis tabulations of the extracts are given in Tables 1, 2, 3 and 4.

Table 1: Phytocomponents Identified in the Methanol Extract of *M. micrantha* Leaf by GC-MS Peak Report TIC.

Peak	R. time	IUPAC Name/ Compound name/ Common name	Molecular formula
20	7.725	Hexadecenoic acid	$C_{21}H_{42}O_2$
39	10.550	Phenol, 2-methoxy	$C_7H_8O_2$
60	12.758	Phenol, 3-(1methylethyl)	$C_9H_{12}O$
70	13.433	Hydroquinone	$C_6H_6O_2$
83	14.576	Phenol, 2-methoxy-4-(1-propenyl)	$C_{10}H_{12}O_2$
104	16.225	Phenol, 2-(1,1-dimethyl-2-propenyl)-3,6-dimethyl	$C_{13}H_{18}O$
109	16.525	Phenol, 2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$
110	16.600	Phytol	$C_{20}H_{40}O$
143	18.450	α-Bisabolol	$C_{15}H_{26}O$
152	18.958	Astaxanthin	$C_{16}H_{24}O$
156	19.158	Fumaric acid, cyclobutyl heptyl ester	$C_8H_{24}O$
159	19.250	Globulol	$C_{15}H_{26}O_2$
175	20.158	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$
192	21.200	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
193	21.283	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$
195	21.533	Ascorbic acid, 2,6-dihexadecanoate	$C_{38}H_{68}O_8$
213	22.992	Phytol	$C_{20}H_{40}O$
219	23.433	Ascorbic acid 2,6-hihexadecanoate	$C_{38}H_{68}O_8$
264	32.867	Stigmasterol	$C_{29}H_{48}O$
271	36.517	Lup-20(29)-en-3-ol, acetate,	$C_{32}H_{52}O_2$

Table 2: Phytocomponents identified in the water layer separated from methanol extract of *M. micrantha* stems by GC-MS Peak Report TIC.

Peak	R. time	IUPAC Name/ Compound name/ Common name	Molecular formula
13	5.081	Propanoic acid, 2-oxo-, methylester	$C_4H_6O_3$
22	5.975	Phenol, 3-methoxy-2-methyl	$C_8H_{10}O_2$
39	8.183	2-Furoic acid, phenylethyl ester	$C_{13}H_{12}O_3$
43	8.658	Phenol	C_6H_6O
49	9.475	3-Methyl-1,2-cyclopentenedione	$C_6H_8O_2$
55	10.008	3(2H)-Furanone	$C_6H_8O_3$
59	10.343	Palatone	$C_6H_6O_3$
64	11.206	Butanoic acid, 2-methyl-3-oxo-, ethyl ester	$C_7H_{612}O_3$

78	12.583	Coumaran	C_8H_8O
84	13.175	Homocatechol	$C_7H_6O_2$
87	13.417	Hydroquinone	$C_6H_6O_2$
93	13.992	4-Hydroxy-2-methylacetophenone	$C_9H_{10}O_2$
108	15.192	Vanillin	$C_8H_8O_3$
111	15.425	Benzoic acid, 4-methoxy	$C_8H_8O_3$
124	16.525	Phenol, 2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$
126	16.700	Phenol, 4-methoxy-2,3,6-trimethyl	$C_{10}H_{14}O_2$
130	17.067	Hydroquinone, tert-butyl	$C_{10}H_{14}O_2$
133	17.275	Fumaric acid, ethyl 3-heptyl ester	$C_{10}H_{14}O_4$
135	17.633	Octadecyl chloride	$C_{18}H_{37}C_1$
147	18.492	α-Bisabolol	$C_{15}H_{26}O$
153	18.950	Astaxanthin	$C_{40}H_{52}O_4$
160	19.300	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy	$C_{10}H_{12}O_3$
166	19.692	Phytol	$C_{20}H_{40}O$
170	19.867	Benzediol, 2,5-bis(1,1-dimethyletyl)	$C_{14}H_{22}O_2$
177	20.108	Apidic acid, isohexyl methyl ester	$C_{13}H_{24}O_4$
184	20.492	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$
193	21.192	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
197	21.525	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$
213	23.129	Stearic acid, methyl ester	$C_{19}H_{38}O_2$
249	32.858	Stigmasterol	$C_{29}H_{48}O$

Table 3: Phytocomponents identified in the petroleum ether extract of *M. micrantha* leaves by GC-MS Peak Report TIC.

Peak	**R. time**	**IUPAC Name/ Compound name/Common name**	**Molecular formula**
15	7.090	Nonane	C_9H_{20}
36	8.827	1-Decane	$C_{10}H_{20}$
38	8.982	Dodecane	$C_{11}H_{24}$
45	9.495	Ethylhexanol	C_8H_{180}
52	9.925	Tolune, m-propyl	$C_{10}H_{14}$
53	9.938	Dodecane, 4,6-dimethyl-	$C_{14}H_{30}$
55	10.035	5-Isobutylnonane	$C_{13}H_{28}$
59	10.306	1-Decane, 2, 4-dimethyl-	$C_{13}H_{280}$
63	10.579	2-Propylheptanol	$C_{10}H_{220}$
64	10690	Dodecane, 4,6-dimethyl-	$C_{14}H_{30}$
65	10.035	Farnesan	$C_{15}H_{32}$
67	1.920	Nonane, 5-butyl	$C_{13}H_{28}$
113	13.303	Heneicosane	$C_{21}H_{44}$
114	13.385	Heptadecane	$C_{17}H_{36}$
115	13.507	Heptadecane	$C_{17}H_{36}$
117	13.591	Eicosane	$C_{20}H_{42}$

118	13.694	Heptadecane	$C_{17}H_{36}$
119	13.757	Dihydrophytol	$C_{20}H_{420}$
120	13.875	Isotridecanol	$C_{13}H_{280}$
123	14.045	Dodecane, 4,6-dimethyl-	$C_{14}H_{30}$
129	14.350	Dodecane, 4,6-dimethyl-	$C_{14}H_{30}$
137	14.616	.alpha.-Longipinene	$C_{15}H_{24}$
167	16.029	Caryphyllene	$C_{15}H_{24}$
171	16.210	Eicosane	$C_{20}H_{42}$
172	16.265	.alpha.-Curcume	$C_{15}H_{22}$
174	16.379	Eicosane	$C_{20}H_{42}$
176	16.530	Phenol, 2, 4-bis(1,1-dimethyl)	$C_{14}H_{220}$
179	16.688	Eicosane	$C_{20}H_{42}$
182	16.831	Heneicosane	$C_{21}H_{44}$
184	16.956	Eicosane	$C_{20}H_{42}$
186	17.075	Heneicosane	$C_{21}H_{44}$

Table 4: Phytocomponents identified in the petroleum ether extract of *M. micrantha* stems by GC-MS Peak Report TIC.

Peak	**R. time**	**IUPAC Name/ Compound name/ Common name**	**Molecular formula**
18	7.089	Nonane	C_9H_{20}
37	8.825	Decane	$C_{10}H_{20}$
39	8.893	Dodecane	$C_{12}H_{26}$
69	10.702	Nonane	$C_{11}H_{22}$
70	10.789	Dodecane, 4,6-dimethyl	$C_{14}H_{30}$
125	13.385	Dodecane, 4,6-dimethyl,	$C_{14}H_{30}$
128	13.589	Heneicosane	$C_{21}H_{44}$
130	13.693	Dodecane, 4,6-dimethyl,	$C_{14}H_{30}$
136	14.046	Dodecane, 4,6-dimethyl	$C_{14}H_{30}$
175	16.205	Eicosane	$C_{20}H_{42}$
177	16.265	Eicosane	$C_{20}H_{42}$
179	16.381	Eicosane	$C_{20}H_{42}$
182	16.527	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$
187	16.808	Heneicosane	$C_{21}H_{44}$
188	16.834	Heneicosane	$C_{21}H_{44}$
189	16.957	Eicosane	$C_{20}H_{42}$
191	17.076	Eicosane	$C_{20}H_{42}$
193	17.209	Eicosane	$C_{20}H_{42}$
200	17.645	Heneicosane	$C_{21}H_{44}$
208	18.036	Eicosane	$C_{20}H_{42}$
209	18.183	Tridecane, 2,5-dimethyl	$C_{15}H_{32}$
214	18.384	Hexadecane, 2-methyl-	$C_{17}H_{36}$
216	18.475	Heptadecane, 2-methyl	$C_{18}H_{38}$
217	18.500	alpha.-Bisabolol	$C_{15}H_{26}O$

DISCUSSIONS

Different solvents can be used for isolation of extractives according to the various purposes. Methanol with the polarity index 5.1 can extract various polar compound hydrophilic compounds, for example phenolic substances and some carbohydrate and certain group of nonpolar compounds while non-polar solvent (petroleum ether) can extract lipophilic compounds, for example terpenoids, alkane and fats (Sjöström, 1993).

Phytochemical analysis of the *M. micrantha* by GCMS revealed the presence of various phenolic and hydrocarbon. Some of the above-mentioned compound are lies in phenolic compound. Phenolic compounds possess perfect structural chemistry for free radical scavenging activities as they have phenolic hydroxyl groups that are susceptible to contributing hydrogen atoms or electrons to a free radical, expanded conjugate aromatic system to dislocate unpaired electrons (Dai & Mumper, 2010).

Hexadecaonic acid methyl ester, also widely recognized as palmitic acid methyl ester and linolenic acid, are classified as organic fatty acid compounds (Aji et al., 2015). Okwu & Ighodaro, 2009 found that plant fatty acids and alcohols undergo esterification reactions to form esters that are often sprayed out of the plant as resins / exudates and used in African medicines to treat wounds and ringworms.

α-Bisabolol known as sesquiterpenes, were found abundant in *Mikania* genus (Rufatto, Gower, Schwambach, & Moura, 2012) could also act as antioxidant compounds. Sesquiterpenes, like so many other groups of terpenes and other natural products, have gained tremendous attention due to their functions in biological processes and their human use (Chappell & Coates, 2010). Phytol which also have sesquiterpenes nature is one among the compounds appear in leaves for *M. micrantha* methanolic extract of the present study. Similarly, (Sermakkani & Thangapandian, 2012) observed the presence of phytol in the *Cassia Italica* leaves. Similar result was also observed in the leaves of *Syzygium polyanthum, Monocarpia marginalis and Chromolena odorata* (Jumaat et al., 2017). Sermakkani & Thangapandian, (2012) reported that phytol has antibacterial activities against *Staphylococcus aureus* and have potential anticancer, cancer preventive, diuretic anti-inflammatory.

The presence of l-(+)-ascorbic acid 2,6-dihexadecanoate in all extracts for this study may be the reason for the use of *M. micrantha* extract in the diagnosis of skin diseases such as burns, curing cuts and avoiding minor external bleeding (Dev et al., 2015). Ascorbic Acid is a natural water-soluble vitamin (Vitamin C). Natural ascorbic acid is essential to the efficiency of the body. Particularly the scar tissue, bones, and teeth are needed for connective metabolism and this ascorbic acid function also accounts for its necessity for normal wound healing (Okwu & Ighodaro, 2009). This was also supported by the use of *M. micranth* in the treatment of wounds by the local practitioner.

Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester was present in this extract, and reported to have antifungal and antioxidant activity similarly to Akpuaka et al., 2013.

The GCMS analysis of the concentrated methanol and petroleum ether extract resulted many compounds which have various use. The extract had a total of compounds having antibacterial, antimicrobial and antioxidant properties have been identified. The plant is extensively used as a traditional remedy in Malaysia (Chaturvedi, 2011). This study explores the goodness of the extracts of *M. micrantha* leaves and stems which has a worthy sense of purpose and can be advised as a plant of phytopharmaceutical importance.

CONLUSION

There are more phytochemical with medicinal nature in polar solubility compound compared to non-polar solubility compounds in *M. micrantha* and leaves have more bioactive compounds than stems. In this study, several of these compounds have been identified as highly pharmacological interest in antioxidant activity due to the different chemical compounds that can lead to pharmaceutical use and their extensive use as herbal medicine. It indicates that this plant has a good potential to promote as a natural resource drug. This preliminary study had paved a way in effort to discover new therapeutic antioxidant and antimicrobial. It is suggested that separation and fraction of extract should be done to get accurate phytochemical result.

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