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Antibacterial and Phytochemical Investigations of *Mikania micrantha* H.B.K. (Asteraceae) From Sabah, Malaysia

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Abstract

Previous study on *Mikania micrantha* had unveiled its importance as protein phosphatase-1 (PP1) inhibitor and cytotoxic agent against HL60 cells. The present study was carried out to investigate the antibacterial properties and to determine the phytochemicals content of *M. micrantha*. Crude methanolic extracts from powdered dry samples were partitioned using liquid-liquid separation technique and further fractionated using silica gel column chromatography to yield six partitionates and 5 fractions. All partitionates and fractions were challenged with Gram positive and Gram negative bacteria and the performances are compared with standard antibiotics. The results revealed that four partitionates (ME, CE, EAE and CME) possessed good antibacterial properties. While, fraction F1 from column chromatography is showing convincing activities towards tested bacteria. Phytochemical tests of the crude extracts, partitionates and fractions had detected the presence of tannins, polyphenols, alkaloids, saponins and triterpenoids. This result supports the potential of this plant species used as a new chemotherapeutic drug.

Introduction

Bacterial infection is acknowledged as one of the major public health issues. Antimicrobial drug resistances however, possess additional problems for the infection control. These lead to the increasing demands of new antimicrobial agents. Plant-derived medicines produced a variety of phyotochemical constituents of known therapeutics properties (Vukovic *et al.*, 2007). Several species from Asteraceae family had been reported to be not just actively inhibiting cancer cells but also harboring antimicrobial properties (Ooi *et al.*, 2004; Jayaraman *et al.*, 2008; Kasim *et al.*, 2011). These were supported by vastly occurrence of secondary metabolites within these plants. *Mikania* and *Chromolaena* genus for instance were widely known for its flavonoid, phenolics and terpenes compound (Lobitz *et al.*, 1997; Rungeler *et al.*, 2001; Bighetti e*t al.*, 2005; Krishanti *et al.*, 2010). Those classes of compound especially sesquiterpene lactones had been closely related to various biological activities including anticancer and antibacterial (Chaturvedi, 2011).

Mikania genus is known as one of the best-selling natural products in the world (Sathi *et al.*, 2015). *Mikania micrantha* or locally known as Selaput Tunggul is medicinally important plants that widely used by local practitioners for treatment of various maladies (Lentz *et al.*, 1998; Laurella *et al.*, 2012). In our previous studies, *M. micrantha* had showed significant potential as protein phosphatase inhibitors, particularly for Protein Phosphatase-1 (PP1) inhibitor (Matawali *et al.*, 2016). However, to date there is no local data on antibacterial properties of *M. micrantha* is available. Thus, the objectives of this study are to identify the potential of *M. micrantha* against gram positive and gram negative bacteria. Secondly, to investigate the secondary metabolites that present on the samples through series of phytochemical tests.

Materials and methods

Plant material

The plant materials of *M. micrantha* (H.B.K) were collected from Membakut, Sabah. Voucher specimen (BORH 0962) was deposited at the BORNEENSIS, Institute of Tropical Biology and Conservation (ITBC), University Malaysia Sabah.

Preparation of plant extracts

The plants were examined to remove any dirt, fungus-infested and twigs. Pieces of the plants were dried at control temperature, grinded and stored at room temperature for future usage.

Fractionation of plant extracts

Powdered samples were soaked three-times in 100% (v/v) methanol overnight at ratio 1:10. The crude methanolic extracts (ME) were than partitioned by using liquid-liquid extraction methods following Harborne (1998) with a slight modification. Partitiones (hexane extract (HE), ethyl acetate extract (EAE), chloroform extract (CE), chloroform:methanol extract (CME), butanol extract (BE) and aqueous extract (AE)) were evaporated under vacuum for drying, while the aqueous extracts were freeze-dried. Potential partitionates were further subjected to open column chromatography using silica gel 60 (0.040-0.063mm with 230-400 mesh ASTM, Merck) and eluted successfully with methanol:chloroform (1:19) yielding 5 fractions (F1 to F5) (Harborne, 1998).

Test Microorganisms

Five bacterial strains were used namely as *Escherichia coli, Pseudomonas aeroginosa, Salmonella typhii, Staphylococcus aureus and Streptococcus pneumonia*. The microorganisms were obtained from Biochemical Lab, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Kota Kinabalu, Sabah.

Antimicrobial susceptibility test

The agar disc diffusion method was applied following to Mbata (2008). Nutrient agar (NA) is used as culture medium. The plates were prepared by pipetting approximately 100µL of the bacterial broth stock from the Nutrient Broth media into 25mL NA medium. Sterile 6mm diameter of Whatman filter

paper was impregnated with 20 µL of samples from stocks (1 mg/mL) and placed at the top of NA medium. The plates then were incubated for overnight at 37^0C. Ampicillin (0.25 µg/µL) was used as positive control, and extraction solvents used as negative control. All tests were performed in triplicates, and the mean and standard deviation of the inhibition zones recorded were calculated by using SPSS ver. 20 (SPSS Inc., USA).

Phytochemicals test

Phytochemicals profiling were conducted for all the extracts as per the typical methods to verify the presence of chemical constituents in the samples such as alkaloids (Wagner's test), flavonoids (Wilstatter-Sianidin, Batesmith and Metcalf test), tannins (Gelatin test), polyphenols (Ferric chloride test), saponins (Frothing test) and terpenoids (Salkowski test) (Fasihuddin & Hasmah, 1993; Edeoga *et al.*, 2005). All tests were done in triplicate.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and means were compared by the Duncan's multiple range test at $p<0.05$ using the SPSS ver. 20 (SPSS Inc., USA).

Results and discussion

Mikania micrantha is a creepy weed that found native to Central and South America and currently being widely distributed in India, Southeast Asia, Pacific Islands and China (Jyothilakshmi *et al.*, 2015). It is also known as mile-a-minute weed because of its fast-growing characteristic. Thus, this plant had been listed as among world's 100 worst invasive alien species by Invasive Species Specialist Group of IUCN (Li *et al.*, 2013). In recent year, more studies had been reported on the healing properties of this plant; for instance as antidermatophytic, anti inflammatory, antimicrobial and anti stress (Li *et al.*, 2013; Haisya *et al.*, 2013; Ittiyavirah & Sajid, 2013; Jyothilakshmi *et al.*, 2015).

According to the results given in Table 1, ME, CE, EAE and CME partitionates exhibited potential antibacterial activities. Meanwhile, HE, BE and AE partitionates were found as inactive against all bacteria tested. Fractions of CE (F1-F5) using silica gel column that been previously reported possess as anti-phosphatase and cytotoxic activities (Matawali *et al.*, 2016), is also possess for antibacterial properties. Both F1 and F2 were found constantly active to resist the activity of all tested bacteria. The diameters of inhibition zone ranged from 6.67 to 17.33 mm. F1 fraction exhibited the highest inhibition zone against *P. aeruginosa* (15.67±1.15), *S. pneumonia* (17.33±1.15) and *S. typhii* (9.67±1.53). On the other hand, F2 is found to be active against *E. coli* (7.33±7.51) and *S. aureus* (16.67±1.15). These findings were also found in line with the report by Perez-Amador *et al.*, (2010) and Haisya *et al.*, (2013) that *M. micrantha* demonstrate antibacterial activities against *E. coli, B. subtili, P. aeruginosa* and *S. aureus* as well as being active as anti-inflammatory agent.

By referring to the statistical data, the inhibition zones observed from F1 fraction is significantly higher against *P. aeruginosa* than standard antibiotics. This might give indications that *M. micrantha*

extracts were quite effective in combating diseases cause by *P. aeruginosa* infections. *P. aeruginosa* is an aerobic gram negative bacterium that able to grow in almost any environment. This bacteria is an opportunistic pathogen that usually responsible for nosocomial pneumonia cases, wound or burn infections, and chronic lung infection in cystic fibrosis patients. Few antibiotics that currently used in treating *P. aeruginosa* infections are such as gentamicin, amikacin and ciprofloxacin. However, *P. aeruginosa* showed resistance to most antibiotics through few mechanisms such as low outer membrane permeability, the presence of antibiotic modifying enzymes, protective effect of alginate and the existence of genetic capacity to express wide repertoire of resistance mechanisms upon exposure to antibiotics (Van Delden & Iglewski, 1998; Lambert, 2002). Thus, *M.micrantha* might be a good alternative as new chemotherapeutic agent against one of the most problematic human pathogen, *P. aeruginosa*.

Table 1. Antibacterial activities of *M. micrantha* extracts.

Sample	Inhibition zones of antibacterial screening (mm)				
	E. coli	*P. aeruginosa*	*S. aureus*	*S. pneumonia*	*S. typhii*
Ampicillin	13.21±0.81 [a]	3.75±0.79 [c]	28.25±1.4 [a]	32.67±6.43 [a]	16.71±0.70 [a]
ME	0	11.33±3.78 [a]	10.00±2.64 [d]	10.33±1.10 [c]	2.33±2.04 [d]
HE	0	0	0	0	0
EAE	0	10.33±2.08 [b]	8.67±1.15 [e]	9.67±1.53 [c]	2.67±2.61 [d]
CE	0	11.00±2.64 [b]	14.00±0.00 [c]	14.33±2.31 [b]	8.67±0.58 [c]
CME	0	5.00±4.36 [c]	5.00±4.36 [f]	5.00±2.36 [d]	0
BE	0	0	0	0	0
AE	0	0	0	0	0
F1	6.67±6.51 [b]	15.67±1.15 [a]	16.00±2.64 [bc]	17.33±1.15 [b]	9.67±0.53 [b]
F2	7.33±7.51 [b]	15.00±0.00 [a]	16.67±1.15 [b]	16.67±1.15 [b]	9.33±0.58 [b]
F3	0	0	0	0	0
F4	0	0	0	0	0
F5	0	0	0	0	0

Notes: ME=Crude Methanolic Extract, HE=Hexane Extract, EAE=Ethyl Acetate Extract, CE=Chloroform Extract, CME=(Chloroform: Methanol) Extract, BE=Butanol Extract, AE=Aqueous Extract, F1-F5=CC fractions of *M. micrantha*.

n = 0.5kg

Table 2. Summary of phytochemicals constituents of *Mikania micrantha*.

Extract	Alkaloid test	Flavonoid test			Tannin and polyphenol test		Saponin test	Triterpenoids test
	Wagner test (formation of cloudy sediment)	Wilstatter-Sianidin test	Batesmith test	Metcalf test	Gelatin test	$FeCl_3$ test	Foam test (formation of bee dane)	Salkowski test
Mikania micrantha								
CME	(++++)	-	-	-	(+++)	(++++)	-	(+++++)
HE	-	-	-	-	(++)	-	-	-
EAE	-	-	-	-	(+++)	(+++)	(+)	(+++)
CE	-	-	-	-	-	(++++)	(+++++)	(++++)
C:ME	-	-	-	-	-	-	-	(+)
BE	-	-	-	-	-	-	(+++++)	-
AE	(++)	-	-	-	-	-	(+)	-
F1	-	-	-	-	-	-	(++)	(++++)
F2	-	-	-	-	-	-	(++++)	(+++)
F3	-	-	-	-	(+++)	-	(+++)	-
F4	-	-	-	-	(+++)	-	(+++++)	(++)
F5	-	-	-	-	(++)	(++++)	-	(+)

Notes:
CME=Crude Methanolic Extract, HE=Hexane Extract, EAE=EtnylAcetate Extract, CE=Chloroform Extract, CME=(Chloroform:Methanol) Extract, BE=Buthanol Extract, AE=Aqueous Extract. Score: (+++++)= copiously present, (++++)= present, (+++)= moderately present, (++)=weekly present, (+)=, (-)= No activity

Table 2 shows the result of phytochemical tests of *M. micrantha*. Tannins, polyphenols, alkaloids, saponins and triterpenoids were found in crude extracts, partitionates and fractions of *M. micrantha*. Both CME and AE were found to have cloudy sediments during alkaloids test. These results were generally found in line with the data reported by Jyothilakshmi *et al.*, (2015), Haisya *et al.*, (2013) and Ittiyavirah & Sajid (2013) with the exception of flavonoid. Flavonoids also had been reported to be present in *Mikania micrantha*. But, uniquely for Sabah variety, this phytochemical is not detected.

Phytochemical test done had revealed that most of the *M. micrantha* extracts were more pronounced against tannins and polyphenols, saponins and triterpenoids. Triterpenes is commonly found in Asteraceae, especially in *Mikania* genera. *M. micrantha* has been reported contained sesquiterpene lactones, diterpenes, flavonoids and phenolic compounds that mostly responsible for allelopathic response, antibacterial and anticancer activities (Cuenca *et al.*, 1988; Wei *et al.*, 2004; Huang *et al.*, 2009; Perez-Amador *et al.*, 2010; Chaturvedi, 2011). Sesquiterpene lactones such as mikanolide, dihydromikanolide, n-methoxy benzoic acid, deoxymikanolide, scandenolide and dihyrdoscandenolide were reported as among major antimicrobial activity constituents in *Mikania micrantha* (Li *et al.*, 2013). Thus, the antimicrobial potential of the sample in this study might be due to the presence of these phytoconstituents. This study strongly suggests *M. micrantha* as a promising medicinal weed with respects to its antimicrobial potential. However, further work is still needed to find out the inhibitory mechanisms. Isolation of pure compounds is also strongly recommended to specifically identify the metabolites responsible for such bioactivities.

Conclusion

As conclusions, *M. micrantha* indicates significant antibacterial properties with the presence of various phyto-constituents such as alkaloids, tannins and polyphenols, saponins and triterpenoids. It was proven that instead of being potential as Protein Phosphatase-1 (PP1) inhibitor, *M. micrantha* also offered roles as good antibacterial agents. This preliminary study had paved a way in effort to discover new and nobel therapeutic antimicrobial.

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