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Editors :

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Hilda Ismail
Ronny Martien
Triana Hertiani
Ritmaleni

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Antioxidant Properties and Phenolics Content of *Mikania scandens* L.(Wild)

Sumi Wijaya*, Ting Kang Nee, Khoo Teng Jin & Christophe Wiart
School of Pharmacy, The University of Nottingham Malaysia Campus
Corresponding author:_khyx8swi@exmail.nottingham.edu.my

Abstract

Mikania scandens, belongs to the Asteraceae family, is a fast growing perennial creeper found in Malaysia. Traditionally, this plant has been used for treatments of diarrhoae, cancer and wound healing. The main objective of the study was to examine the antioxidant activities of *Mikania scandens* extracts. Whole plants were collected, dried and macerated sequentially in four different solvents, starting from Hexane (H), Ethyl acetate (EA), Ethanol (E) and Water (W). Ferric Reducing-Antioxidant Power (FRAP) and β-carotene bleaching methods were employed. Total phenolics and flavonoids content of the plant extracts were also determined. All *Mikania scandens* extracts, exhibited relatively good antioxidant activities in both assays. For the FRAP assay, the rank order of antioxidant activity is E > trolox = quercetin > W > EA > H. For the β-carotene bleaching assay, ethanol extracts remained the most potent extracts followed by H > EA > W. Ethanol extracts possessed the highest activities with potency as good as quercetin and trolox as exhibited in both assays. There is a positive correlation between phenolics content and antioxidant activity of the extracts based on the FRAP assay.

Key words: *Mikania scandens*, FRAP, β-carotene bleaching, Total phenolics content

Introduction

For centuries, plants have been used throughout the world to treat various diseases. In fact, approximately 60-80% of the world population still rely on traditional medicines derived from plants for treatment of ailments and about 50% of the drugs used in the clinical treatment are derived from plant sources (WHO, 2004; Zhang, X., 2004).

One interesting hyphothesis that many researchers is working on, is diseases purported to be caused by free radicals. It is thought that free radicals e.g reactive oxygen species are responsible for various degenerative diseases, e.g heart diseases, stroke, arteriosclerosis and cancer, as well as aging process (Willcox, *et al.*, 2004).

The aim of this study was to examine the antioxidant activities of *Mikania scandens* (L.) Wild. Asteraceae is the largest family after Orchidaceae, with almost 24,000 species (Steven, P.F., 2001). The member of the family are easily identified through their flowers. Most Asteraceae species grow well in moist soils and can be found along roads and foot trails (Stone, B.C., 1970; Cronquist, A., 1980; Holm, *et al.*, 1997; Rahman, *et al.*, 2008).

Mikania scandens is a herbaceous, perennial twinning herb with saggitate, hastate or cordate leaf bases. This plant is native to South America, useful for treatments of diarrhoae, cancer and wound healing (Hasan, *et al.*, 2009). The plant is reported to have antimicrobial, antiinflamatory, antipyretic, analgesic, ulcerprotective, and anticarcinogenic activities (Ysrael, *et al.*, 1990; Bishayee, A. and Chatterjee, M., 1994; Mosaddik, M.A., and Alam, K.M., 2000; Hasan, *et al.*, 2009). Three deterpenic acids known as kaurenic acid, butyryloxykaurenic acid and beta-sitosterin have also been isolated from this plant (Ghani, A., 2003). Mikania genus reduces growth and productivity of several crops such as oil palm, rubber, citrus, cassava, teak, eucalyptus, acacia, albizia, pineapple, and coconut in Malaysia (Sankaran, K.V., 2007). As a weed and in abundant supply, *Mikania scandens* could be exploited to be a powerful source of antioxidant agent.

Methodology

Sample Preparation

Whole plants of *Mikania scandens* (L.) Wild was collected from areas around Semenyih and Broga. The plant was dried at room temperature and ground into powder. The plant powder was soaked sequentially in four different solvents: Hexane, Ethyl acetate, Ethanol

and Water. The solvents were evaporated using rotary evaporator under reduced pressure at 40°C. Dried extracts were kept at -20°C until tested.

Antioxidant Assay

Ferric-Reducing Antioxidant Power

The FRAP method adopted was based on Benzie & Strain (1996) with slight modifications. The working FRAP reagent was prepared freshly by mixing 300 mM Acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio. Briefly, 180 µl of FRAP reagent was mixed with 20 µl of test sample, so the final dilution of the test sample in the reaction mixture was 1/10. Readings were taken at 90 minutes (at 600 nm) instead of 4 minutes using spectrophotometer (Dynex MRX-Revelation). We have chosen to take the readings at 90 min because some polyphenols take a longer time to react and a longer reaction times is required for detection of any antioxidant activity (Phipps, *et al.*, 2007). Fe(II) concentrations in the range 1 µM – 125 µM/l ($FeSO_4.7H_2O$) were used as standard. Trolox and quercetin were used as a positive control with the same concentration range as the plant extracts.

Readings were obtained from three independent experiments, each performed with triplicate measurements. FRAP value was calculated as Ferrous Equivalents, the concentration of trolox/quercetin or extracts which produced an absorbance value equal to that of 1 mM Fe_2SO_4.

Inhibition of β-carotene Bleaching

β-carotene bleaching assay was conducted according to Barreira, *et al* (2008) with some modifications. A solution of β-carotene was prepared by dissolving two mg of β-carotene in 10 ml chloroform. Two ml of this solution was pipetted into 100 ml round-bottom flask. After the chloroform was removed at 40°C under vacuum, 40 mg of linoleic acid, 400 mg of tween 80, and 100 ml of distilled water were added to the flask with vigorous shaking. 96-wells microtiter plate was used for this method, that giving better reproducibility and higher sample throughput (Tsao,*et al.*, 2003). As soon as the emulsion was added to each tube, the zero time absorbance was measured at 490 nm using spectrophotometer (Dynex MRX-Revelation). Absorbance readings were recorded at 20 min intervals for 240 minutes. A blank, devoid of β-carotene, was prepared for background subtraction. % Antioxidant activity (AA) was calculated using the following equation: % AA = ((DR contol-DR sample) / DR control) x 100, where DR is degradation rate of sample (DR = ln (initial absorbance (470 nm) at time zero) / (absorbance at 240 minutes) / t (time in minutes)). EC_{50} of the plant extracts or controls (quercetin and trolox) were calculated from the graph of antioxidant activity percentage against concentration.

Total Phenolics Content

Total Phenolics content of *Mikania scandens* was determined using Folin-Ciocalteau assay, as described by Slinkard and Singleton (1977). Basically 20 µl of diluted *Mikania scandens* extracts were mixed with 1,58 ml of water and 100 µl of Folin-Ciocalteau's reagent. After standing for 5 minutes at room temperature, 300 µl of sodium carbonate (20% w/v) were added. The solutions were mixed and allowed to stand 30 minutes at 40°C. Changes in absorbance were determined at 765 nm against the blank (the "0 ml" solution) using a UV-Vis Spectrophotometer (Biochrom Libra S12). Gallic acid (50, 100, 150, 250 mg/ml) were used as a calibration standard curve. Results were expressed on fresh weight basis of g gallic acid equivalents/g of sample.

Total Flavonoids Content

Estimation of Total flavonoids content in the plant extracts was carried out using the method, described by Froehlicher, *et al.* (2009). A series of methanolic dilution of Quercetin was prepared and used as a standard curve. Flavonoids amount in the plant extracts were expressed in mg of quercetin/g of plant extracts.

Results and Discussions

Research in recent years has implied the role of oxidative and free-radical mediated reactions in degenerative processes in ageing and progression of diseases such as cancer,

coronary heart disease and other neurodegenerative disorders such as Alzheimer's disease (Ames, B.N., 1983; Gey, K.F., 1990; Ames, *et al.*, 1993; Harman, D., 1995; Diaz, *et al.*, 1997). There is an increasing interest in discovering natural plant products that possess potential anti-ageing or cytoprotective properties as preventative agents against the degenerative processes.

Antioxidant can be chemically grouped into two groups, based on their solubility, hydrophilicity and lipophilicity (Huang, *et al.*, 2002). FRAP assay is usually employed to test for hydrophilic antioxidants and β-carotene bleaching method for lipophilic molecules. Two different assays were used for the study to anticipate the possibility that *Mikania scandens* containing both types of antioxidants.

Results obtained in this present study revealed that the reducing ability of the extracts were in the range of 1.14 – 13 mg/ml (Table 1). The activity of all the plant extracts was comparable to quercetin and trolox and the rank order antioxidant activity is E > trolox = quercetin > W > EA > H extracts. Ethanol extracts possessed the highest reducing ability with potency as good as quercetin and trolox (Table 1).

In the β-carotene bleaching test, β-carotene undergoes rapid discoloration in the absence of antioxidant (Cao, *et al.*, 2009). The rank order of potency observed in the β-carotene bleaching assay was quercetin > trolox > E > H > EA > W (Table 1). In this assay, Hexane extracts appeared to be more active when compared to both Ethyl acetate and Water extracts. It is tought that Hexane extracts comprised mainly lipophilic compounds would dissolve in the lipid phase of the reaction media thus exhibiting a good antioxidant activity.

Table 1. Antioxidant activities, total phenolics and flavonoids content of *Mikania scandens* extracts

	FRAP [Ferrous Equivalent (mg/ml)]	**β-carotene bleaching assay** [*EC_{50} (µg/ml)]	**Total phenolics content** [Gallic acid Equivalent (g/g)]	**Total flavonoids content** [Quercetin Equivalent (mg/g)]
Hexane (H)	13.00 ± 0.04	10.00 ± 0.64	106.67 ± 0.00	**3.33 ± 0.03**
Ethyl acetate (EA)	2.57 ± 0.01	26.67 ± 0.29	441.56 ± 0.01	2.46 ± 0.03
Ethanol (E)	**1.14 ± 0.03**	**5.87 ± 0.59**	**1066.67 ± 0.02**	2.12 ± 0.01
Water (W)	2.17 ± 0.03	46.87 ± 0.17	347.56 ± 0.02	1.43 ± 0.00
Quercetin	1.31 ± 0.02	1.09 ± 0.30	-	-
Trolox	1.14 ± 0.02	1.68 ± 0.21	-	-

Data were obtained from three independent experiments, each performed in triplicates (n=9) and represented as mean ± SD.
* EC_{50} represents the effective concentration at 50% of total antioxidant activity.

Polyphenols are bioactive compounds believed to be involved in the defence process against harmful oxidative damage, by donating hydrogen to highly reactive radicals (Fresco, *et al.*, 2006; Lapornik, *et al.*, 2005). Expressed in Gallic Acid Equivalent (g/g), Total phenolics content of *Mikania scandens* extracts were in the order of E > EA > W > H. A direct relationship has been found between the content of total phenolics and antioxidant capacity of plants (Ferreira, *et al.*, 2007; Robards, *et al.*, 1999). Data on the phenolics content in our study give a positive correlation with the antioxidant activity measured by the FRAP assay (see Figure 1). Phenolics compounds are usually found in the water or ethanol extracts (Waksmundzka-Hajnos, 2008).

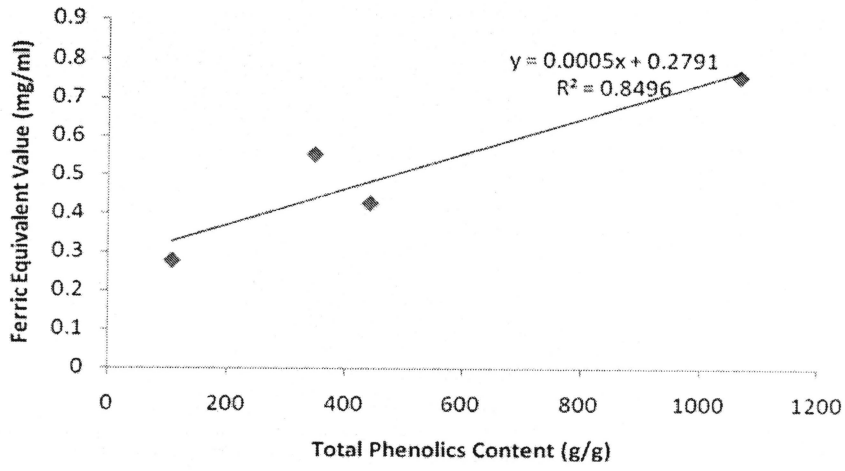

Figure 1. Correlation between FRAP Antioxidant activity and total phenolics content of *Mikania scandens* extracts

Flavonoids are a subgroup of these polyphenolic compounds that possess strong antioxidant activities associated with their capacity to scavenge free radical and terminate radical chain reactions (Bors, *et al.*, 1990). Several studies have highlighted that flavonoids can act as a good antioxidants (Lewis, 1999; Vijayakumar *et al.*, 2008, Vinson, 1995). Total flavonoids content found in *Mikania scandens* extracts were in the range of 1 -3 mg/g quercetin equivalent. In plants, flavonoid aglycones (flavonoid without attached sugars), occurs in variety and tend to be more soluble in non polar organic solvents (Waksmundzka-Hajnos, 2008). Our data showed that the highest quantity of flavonoids was found in the Hexane fractions. This may suggest that lipid soluble flavonoids contributed to the antioxidant activity of Hexane in the β-carotene assay.

Conclusion

The results of this present study demostrated that *Mikania scandens* crude extracts possessed appreciable antioxidant activity. There was a positive correlation between antioxidant activity in FRAP assay and total phenolics content and possibly weak relationship between lipid soluble flavonoids and the antioxidant activities found in the β-carotene assay. Further research to investigate the cytoprotective properties of *Mikania scandens* extract is warranted.

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