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ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *MIKANIA MICRANTHA* LEAVES

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ABSTRACT

MIKANIA MICRANTHA is a very fast growing, perennial creeping weed. It has been attracted the attention of natural products chemists because of its antibacterial, antitumor, cytotoxic, analgesic, inflammatory, antiproliferative and phytotoxix activities. The antioxidant activity of the leaves of *MIKANIA MICRANTHA* was evaluated. The DPPH, FRAP and total Phenolic content of the leaves were 31.97±1.03 mg TE, 18.47±1.15 mg TE and 4.63±0.37 mg GAE/ gm dry leaves respectively. The antioxidant content of the plant is high therefore this plant will have many positive effects on the human health as it can scaveng free radicals and reactive oxygen species which are responsible for number of human disorders.

KEYWORDS: *MIKANIA MICRANTHA*, antioxidant activity, DPPH, FRAP, Total phenolic.

INTRODUCTION

MIKANIA MICRANTHA is an extremely fast- growing perennial creeping weed commonly known as mile-a-minute weed.[1] It is one of the 100 worst alien species,[2] is among the ten worst exotic species in South-east and South Asia, and one of the 16 exotic species in China.[3] Its native distribution is in Central and South America.[4,5] The weed first invaded Hong Kong via sea route at the beginning of the last century, and from there it spread to the cost of Guangdong province in the 1980s.[6] It has now spread to Mauritius, India, Sri Lanka,

Bangladesh, South East Asia and the Pacific.[7] It was introduced into India during World War II to camouflage airfields or as ground cover for tea plantations. It has spread to moist tropical and subtropical regions and the north-east of India.[8] It is used to treat fever, rheumatism, influenza and respiratory diseases.[9,10] The leaves of *MIKANIA MICRANTHA* are used to make a poultice for snake bites and scorpion sting, decoction of the leaves is used to bath rashes and skin itches.[11] It has been reported to have antibacterial, antitumor, cytotoxic, analgesic, inflammatory, antiproliferative and phytotoxix activities.[12-20] In the present study, the antioxidant activity of the methanolic extract of leaves of *MIKANIA MICRANTHA* was estimated by three in vitro assay methods namely (1) DPPH free radical scavenging activity, (2) Ferric reducing antioxidant potential (FRAP) assay and (3) Total Phenolic content, spectrophotometerically.

MATERIALS AND METHODS

Plant Material

The plant *MIKANIA MICRANTHA* was collected from the campus of the College of Veterinary Sciences & Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram and submitted the herbarium specimens for authentication / identification to the Regional Office, Botanical Survey of India (BSI), Shillong. The BSI, Shillong has authenticated the plants and communicated the identification / authentication reports vide letter reference No.BSI/ERC/Tech/2010/052, dated 27.04.2010.

The fresh leaves of the plant were collected, washed and air dried in shade. On complete drying, the dried plant material was ground to powder with Willey / Laboratory Mill and sifted through sieve number 22. The powdered leaves were then subjected to cold maceration using methanol as solvent following the procedure of Manjunatha et al.[21] and Harborne [22] with slight modifications. Briefly, five hundred (500g) grams of powder was soaked in 2.5 L of methanol (1:5 w/v) in a conical flask for a period of 3 days with intermittent stirring and at the end of 3rd day the content was filtered with muslin cloth followed by Whatmann filter paper No. 1. For complete extraction of the active principles, this process was repeated three times using fresh solvent on each occasion or until the colour of the methanol becomes light. The filtrate obtained was pooled and further subjected to rotary vacuum evaporator. The material was stored at -40^{o}C in deep freezer in air tight containers till further use.

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8–tetramethyl chromane-2-carboxylic acid (Trolox), Gallic acid were purchased from Sigma Chemicals Co. (St. Louis, USA); Methanol, Ethanol, Sodium acetate trihydrate, ferric chloride hexahydrate (FeCl3. 6H2O), Folin-Ciocalteu Phenolic reagent, Sodium carbonate were obtained from Merck (Darmstall, Germany). Trichloroacetic acid (TCA) was obtained from Sisco Research Laboratories (SRL), Mumbai. All the chemicals used were of analytical grade.

DPPH free radical scavenging assay

The free radical scavenging activity was measured by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method proposed by Leong and Shui.[23] DPPH solution of 0.1 mM was prepared in methanol and the initial absorbance was measured at 517 nm in a UV-Visible Spectrophotometer (Thermo- Evolution 201). An aliquot (20 µl) of extract was added to 3 ml of DPPH solution and the decrease in absorbance was measured at different time intervals at 517 nm until the absorbance remained constant. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and vice versa. A standard curve was prepared using trolox (250 -1250µg/ml) and the free radical scavenging ability of the extracts were calculated from the decreased in the absorbance. The free radical scavenging ability of the extracts were expressed as mg Trolox equivalent (TE) per gram of dry leaves.

Ferric Reducing Antioxidant Potential (FRAP) assay

The ferric reducing antioxidant potential (FRAP) assay was carried out according to the procedure described by Benzie and Strain.[24] Briefly, 30µl of extract was added to 3 ml of FRAP reagents (10 parts of 300 mM sodium acetate buffer of pH 3.6, 1 part of TPTZ and 1 part of 20 mM Ferric chloride solution). The reaction mixture was incubated at 37ºC for 30 min and the increase in absorbance was measured at 593 nm using a UV-Visible Spectrophotometer (Thermo-Evolution 201). The standard curve was prepared using trolox (250 -1000µg/ml) and the value of FRAP was calculated from the standard curve. The results were expressed as mg Trolox equivalent (TE) per gram of dry leaves.

Total phenolic content (TPC)

The total phenolic content of the extracts were estimated by the Folin-Ciocalteau method described by Singleton and Rossi.[25] Briefly, one hundred (30) microlitres of extract was added to 1ml of 1:10 Folin-Ciocalteau's reagent and incubated at room temperature for 5 min

followed by addition of 970 µl of sodium carbonate (7.5%) solution. After 1 hr incubation at room temperature, the absorbance was measured at 640 nm using a UV/Visible Spectrophotometer (Thermo-Evolution 201). Different volume (20-100µl) of Gallic acid (100µg/ml) was used for calibration of a standard curve. The results were expressed as mg Gallic acid equivalent (GAE) /gm of dry leaves.

RESULT AND DISCUSSION

The antioxidant capacity is widely used as a parameter for medicinal bioactive components. In the present study the antioxidant activity of the leaves of *MIKANIA MICRANTHA* were evaluated by three in vitro assay methods viz. DPPH free radical scavenging assay, Ferric reducing antioxidant potential assay and determination of total phenolic content. Table 1 gives the amount of antioxidant activity evaluated by the three methods.

Table 1: Antioxidant content of *MIKANIA MICRANTHA* leaves

Sl. No.	Methods of estimation	Antioxidant content/ g of dry leaves
01	DPPH free radical scavenging method	31.97±1.03 mg TE
02	FRAP assay	18.47±1.15 mg TE
03	Total phenolic content	4.63±0.37 mg GAE

The DPPH free radical scavenging activity of *MIKANIA MICRANTHA* leaves was 31.97±1.03 mg TE /g dry weight of the leaves while the ferric reducing antioxidant potential activity was 18.47±1.15 mg TE/ g of dry leaves. The DPPH free radical scavenging is one of the generally accepted mechanisms against lipid oxidation. Difference between DPPH free radical binding method and other method is the short run time allowing rapid determination of the radical scavenging. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability while the ferric reducing antioxidant potential assay is based on the reducing power of a compound (antioxidant). It measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron). As the ferric to ferrous ion reduction occurs rapidly with all reductants with half reaction reduction potentials above that of Fe^{3+}/ Fe^{2+}, the values in the FRAP assay expresses the corresponding concentration of electron donating antioxidants. In general, the reducing power of plant extract was reported to be directly correlated with its antioxidant activity and is based on the presence of reductants which exert antioxidant activity by breaking the free radical chain and donating a hydrogen atom. [26,27] It is well documented that the antioxidant activity of putative antioxidants have

been attributed to various mechanisms among which are : prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging power.[28] The total phenolic content of the *MIKANIA MICRANTHA* was 4.63±0.37 mg GAE / g of dry leaves. The most important phytochemicals in plant foods are phenolics whereas there are more than 8000 phenolic phytochemicals.[29] These phenolic compounds interrupt chain oxidation reactions by donation of a hydrogen atom or chelating metals. Moreover, their bioactivities may be related to their ability to inhibit lipoxygenase and scavenge free radicals. [30, 31] Probably the most important natural phenolics are flavonoids, which contain hydroxyl functional groups, because of their broad spectrum of chemical and biological activities, responsible for antioxidant effect of the plants.[32] So, the true antioxidant potential is often more accurately revealed by expressing antioxidant activity in terms of phenolics and flavonoids content. [33, 34]

CONCLUSION

MIKANIA MICRANTHA is a very fast growing, perennial creeping weed. It has been attracted the attention of natural products chemists because of its antibacterial, antitumor, cytotoxic, analgesic, inflammatory, antiproliferative and phytotoxix activities. The antioxidant content of the plant is high therefore this plant will have many positive effects on the human health as it can scaveng free radicals and reactive oxygen species which are responsible for number of human disorders.

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REFERENCES

1. Li Y, Li J, Li Y, Wang X-x, Cao A-c. Antimicrobial constituents of the leaves of *MIKANIA MICRANTHA* H.B.K. PLoS ONE, 2013; 8(10): e76725. Doi: 10. 1371/ journal.pone. 0076725.
2. Lowe S, Browne M, Boudjelas S, Depoorter M. 100 of the World's Worst Invasive Alien Species. A selection from the Global Invasive Species Database. IUCN/SSC Invasive species specialist Group (ISSG), Auckland, New Zealand. (2001).

3. Zhang LY, Ye WH, Cao HL, Feng HL. *MIKANIA MICRANTHA* H.B.K. in China - an overview. Weed Research, 2004; 44: 42–49.
4. Wirjahar S. Autoecological study of Mikania spp. In: Kataoko, T., Furuya, S., Hyakutake, H., Ishizuka, K. Matsunaka, S. and Takabayashi, M. (eds) Proceedings of the Fifth Asian Pacific Weed Science Society Conference, Tokyo, 1976; 70–73.
5. Holm LG, Plucknett DL, Pancho JV, Herberger JP. The World's Worst Weeds. Distribution and Biology. University Press of Hawaii, Honolulu, Hawaii, 1977.
6. Xie Y, Li Z, William PG, Li D. Invasive species in China - an overview. Biodiversity and Conservation, 2000; 10: 1317–1341.
7. Deng X, Ye WH, Feng HL, Yang QH, Cao HL, Xu KY et al. Gas exchange characteristics of the invasive species *MIKANIA MICRANTHA* and its indigenous congener M. cordata (Asteraceae) in South China. Botanical Bulletin of Academia Sinica, 2004; 45: 213–220.
8. Tripathi RS, Khan ML, Yadav AS. Biology of *MIKANIA MICRANTHA* H.B.K.: a Review CAB International. Invasive Alien Plants: An Ecological Appraisal for the Indian Subcontinent (eds J.R. Bhatt et al.), 2012; 99-107.
9. Cabral LM, dos Santos TC, Alhaique F. Development of a Profitable Procedure for the Extraction of 2-H-1-Benzopyran-2-one (Coumarin) from Mikania glomerata. Drug Development and Industrial Pharmacy, 2001; 27: 103-106.
10. Muceneeki RS, Amorim CM, Cesca TG, Biavatti MW, Bresolin TMB. A Simple and Validated LC method for the simultaneous determination of three compounds in Mikania laevigata extracts. Chromatographia, 2009; 69: 219-223.
11. Ayensu ES. Medicinal Plants of the West Indies. Michigan: Reference Publishing, 1981; 82.
12. Zhuang SH, Hao CQ, Feng JT, Zhang X. Active Antifungal Components of *MIKANIA MICRANTHA* H. B. K. J Zhejiang Uni, 2010; 36: 293–298.
13. Facey PC, Pascoe KO, Porter RB, Jones A. Investigation of Plants used in Jamaican Folk Medicine for Anti-bacterial Activity. J Pharm Pharmacol, 1999; 51: 1555–1560.
14. Bohlmann F, Tsankova E, King RM, Robinson H. Five mikanolide derivatives from mikania cynanchifolia and their biogenetic relationships. Phytochemistry, 1984; 23: 1099–1101.
15. Gutierrez AB, Oberti JC, Kulanthaivel P, Herz W. Sesquiterpene lactones and diterpenes from Mikania periplocifolia. Phytochemistry, 1985; 24: 2967–2971.

16. Pickman AK. Biological activity of sesquiterpene lactones. Biochem Syst Ecol, 1986; 14: 255–281.
17. Valdes DA, Bardon A, Catalan CAN, Gedris T, Herz W. Glaucolides, piptocarphins and cadinanolides from Lepidaploa remotiflora. Biochem Syst Ecol, 1998; 26: 685–689.
18. Ahmed M, Rahman MT, Alimuzzaman M. Analgesic sesquiterpene dilactone from Mikania cordata. Fitoterapia, 2001; 72: 919–921.
19. Ysrael MC, Croft KD. Inhibition of leukotriene and platelet activating factor synthesis in leukocytes by the sesquiterpene lactone scandenolide. Planta Med, 1990; 56: 268–270.
20. Li Y, Shen BB, Li J, Li Y, Wang XX, Cao AC. Antimicrobial potential and chemical constituent of *MIKANIA MICRANTHA* H. B. K. Afr J Microbiol Res., 2013; 7: 2409–2415.
21. Manjunatha BK, Vidya SM, Rashmi KV, Mankani KL, Shilpa HJ, Jagadeesh S. Evaluation of wound healing potency of Vernonia arborea HK. Indian J. Pharmacol. 2005; 37: 223-226.
22. Harborne JB. Phytochemical Methods: a guide to modern techniques of plant analysis. 3rd Ed. Spinger international Publication, 1998.
23. Leong LP, Shui G. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chemistry, 2001; 76: 69-75.
24. Benzie IFF, Strain JJ. 'Ferric reducing/ antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power', Methods of Enzymology, 1999; 299: 15-27.
25. Singleton VL, Rossi Jr. JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enology and Viticultur, 1965; 16: 144-158.
26. Hudson BJF. Food antioxidants. Elsevier Applied Science. London, 1-18.
27. Duh PD, Du PC, Yen GC. Action of methanolic extract of mung hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage. Food and Chem. Toxicol., 1999; 37: 1055-1061.
28. Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI. Anatioxidant and analgesic activities of turpentine of Pinus nigra Arn. Sub sp. Pallsiana (Lamb.) Holmboe. Ethnopharmacol, 2003; 86: 51-58.
29. Kuti JO. Antioxidant compounds from four Opuntia cactus pear fruit varities. Food Chem, 2004; 85: 527-533.
30. Decker EA. Phenolics: prooxidants or antioxidants? Nutr. Rev., 1997; 55: 396-407.

31. Kessler M, Ubeaud G, Jung L. Anti- and pro-oxidant activity of rutin and quercetin derivatives. Pharm. Pharmacol, 2003; 55: 131-142.
32. Vundac VB, Brantner HA, Plazibat M. Content of polyphenolic constituents and antioxidant activity of some Stachys taxa. Food Chem, 2007; 104: 1277-1288.
33. Pan Y, He C, Wang, H, Ji X, Wang K, Lui P. Antioxidant activity of microwave assisted extract of Buddleia officinalis and its major active component. Food Chem, 2010; 121: 497-502.
34. Ak T, Gulcin I. Antioxidant and radical scavenging properties of curcumin. Chem Biol Interact, 2008; 174: 27-37.