Asian Journal of Chemistry; Vol. 23, No. 10 (2011), 4525-4527

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Evaluation of *in vitro* Antioxidant Activity of the Methanolic Extract of the Leaves of *Mikania micrantha* Kunth.

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(*Received*: 13 December 2010; *Accepted*: 27 June 2011) AJC-10091

Free radicals and reactive oxygen species (ROS) are well known inducer of cellular tissue pathogenesis leading to numerous human diseases such as cancer, inflammatory disorder as well as in aging process. Antioxidants are the compounds which prevent the oxidative damage caused by free radicals and hence ward off the occurrence of the disease, cancer and aging. In present study the free radical scavenging activity of methanolic extract of the leaves of *Mikania micrantha* Kunth. was carried out using five *in vitro* methods such as, DPPH scavenging activity, reducing power, nitric oxide scavenging activity, super oxide anion scavenging activity and hydrogen peroxide scavenging activity. The extract showed maximum activity in DPPH method. The result showed that the plant have antioxidant activity.

Key Words: Antioxidant, DPPH scavenging, Mikania micrantha, Reducing power.

INTRODUCTION

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. Free radicals are formed continuously as normal byproducts of oxygen metabolism during mitochondrial oxidation, phosphorylation and it is also involved in normal physiology of living organisms. They act as messengers for signal transduction and also affect gene expression[1,2]. However, reactive oxygen species (ROS) that form during metabolism may contribute to pathogenesis of many diseases such as diabetes, cancer, cardiovascular, stoke and arteriosclerosis[3-6]. Excess production of reactive oxygen species can cause tissue injuries. However tissue injury can itself cause reactive oxygen species generation[7]. Antioxidants are the compounds which can scavenge the free radicals and provide the protection against various diseases[8]. Now-a-days natural antioxidants are receiving increasing attention because, there are some toxicological suspicions on synthetic antioxidants due to their adverse side effects and people are much more concerned about food safety and quality[9]. The medicinal properties of plants have been investigated in the recent scientific development throughout the world due to their potential antioxidant activities[10]. Therefore, the aim of present work is to evaluation of *in vitro* antioxidant activity of methanolic extract of the leaves of *Mikania micrantha* kunth. For this purpose total phenolic content, total flavonoid content, DPPH scavenging activity, reducing power, nitric oxide scavenging activity, super oxide anion scavenging activity and hydrogen peroxide scavenging activity of the methanolic extract of the leaves were evaluated.

EXPERIMENTAL

1,1-Diphenyl-2-picryl hydrazyl (DPPH) were purchased from Sigma-Aldrich. Sodium nitroprusside, potassium ferric cyanide, ferric chloride, sodium carbonate and naphthyl ethylene diamine dihydrochloride were purchased from Rankem Fine Chemicals Ltd., India. L-ascorbic acid, gallic acid and auercetin were purchased from S.D Fine Chemicals Ltd., India. Aluminium chloride, trichloroacetic acid and sulphanilic acid were purchased from Merck Ltd., Mumbai, India. Nitroblue tetrazolium chloride, phenazine methosulphate and nicotinamide adenine dinnucleotide (NADH) were purchased from Sisco Research Laboratories, India.

Plant materials and extraction procedures: Leaves of *Mikania micrantha* was collected from botanical garden of Dibrugarh University, Assam, India during March, 2010 and was authenticated at Department of Life Sciences, Dibrugarh University. The leaves were cleaned and dried in the shade, then powdered and stored in an air tight container at 25 ºC. The powdered leaves (250 g) were first defatted with petroleum ether and then extracted with methanol to obtain the methanolic extract of the leaves. The solvents were evaporated under reduced pressure in a rotary evaporator. This methanolic extract was subjected to evaluate the antioxidant activity of plant.

Total phenol content: Total phenol content of extracts was determined by the folin ciocalteau colourimetric method[11] with a slight modification and result was expressed as gallic acid equivalents.

Total flavonoid content: Estimation of flavonoid concentration of the extract was based on the aluminium chloride colourimetric method[12] with a slight modification and result was expressed as quercetin equivalents.

DPPH scavenging activity: The DPPH radical scavenging activity was studied accordingly to the method described by Blois[13] with slight modification. In brief, 4 mL DPPH solution (100 µM in methanol) was added to 1 mL of methanolic extract. The mixture was shaken and allowed to stand at room temperature in the dark for 20 min. The absorbance was measured at 517 nm against a corresponding blank. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

Reducing power assay: The reducing power of extract was determined by the potassium ferricyanide method[14] with some modifications. Sample with different concentrations was mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1 % (w/v) potassium ferricyanide. The mixture was incubated for 20 min at 50 ºC. After incubation, 2.5 mL of 10 % trichloroacetic acid was added to the mixture followed by centrifugation at 3000 rpm for 10 min. The upper layer (5 mL) was mixed with 5 mL of distilled water and 1 mL of 0.1 % (w/v) ferric chloride and the absorbance of the resultant solution was measured at 700 nm. Ascorbic acid and quercetin was used as reference compound.

Nitric oxide scavenging activity: Nitric oxide scavenging can be estimated by the method described by Marcocci[15] with some modification. The reaction mixture (6 mL) containing sodium nitroprusside (10 mM, 4 mL), phosphate buffer saline (1 mL) and the extract /standard solutions (1 mL) were incubated at 25 ºC for 150 min. After incubation, 0.5 mL of the reaction mixture was removed and 1 mL of sulphanilic acid reagent (0.33 in 20% glacial acetic acid) was mixed and allowed to stand for 5 min for completion of diazotization reaction and then 1mL of naphthyl ethylene diamine dihydrochloride (0.1 % w/v) was added, mixed and allowed to stand for another 30 min in diffused light. The absorbance was measured at 540 nm against the corresponding blank solutions.

Super oxide anion scavenging activity: Measurement of super oxide anion scavenging activity of extract was carried out based on the method described by Nishikimi[16] with slight modification. One milliliter of nitroblue tetrazolium (NBT) solution (156 µM NBT in 100 mM phosphate buffer, pH 7.4), 1 mL NADH solution (468 µM in 100 mM phosphate buffer, pH 7.4) and 0.1 mL of sample solution of melatonin in methanol were mixed. The reaction was initiated by adding 1 mL of a phenazine methosulphate (PMS) solution (60 µM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25 ºC for 5 min and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased super oxide anion scavenging activity. All data are an average of triplicate analyses.

Hydrogen peroxide scavenging activity: The capability of the extracts to scavenge hydrogen peroxide (H_2O_2) was determined by the reported method[17]. A solution of hydrogen peroxide (20 mM) was prepared in phosphate-buffered saline (pH 7.4). Various concentrations of the extract/standard in methanol (1 mL) were added to 2 mL of hydrogen peroxide solution in phosphate buffer saline. After 10 min the absorbance was measured at 230 nm.

RESULTS AND DISCUSSION

Total flavonoid content: The antioxidant activity of plant materials is well correlated with their content in phenolic compounds[18]. Therefore, the content of total phenolic in the extract of *Mikania micrantha* was determined using the foline ciocalteu assay, calculated from regression equation of calibration curve ($Y = 0.03X + 0.066$, $R^2 = 0.998$) expressed as gallic acid equivalents (GAE) and it was found to be 49.67 ± 0.010 mg g^{-1} of dry extract. Different works have reported good correlation between antioxidant activity and folin ciocalteu assay[19]. The phenolic compounds in plants act as antioxidant due to their redox properties, allowing them to act as reducing agents[20].

Total flavonoid content: Flavonoids are very effective antioxidant possess various biological activities such as hepatoprotective, antithrombotic, antiinflammatory and antiviral activity due to their antioxidant and free radicals scavenging activity[21,22]. The content of total flavonoid in the methanolic extract of *Mikania micrantha* was expressed in quercetin equivalent (QUE) and was found to be 17.50 ± 0.028 mg g^{-1} of dry extract. The regression equation of calibration curve was $Y = 0.004X + 0.024$, $R^2 = 0.997$. The result indicates that the leave of plant have low flavonoid as compared to phenolic compound.

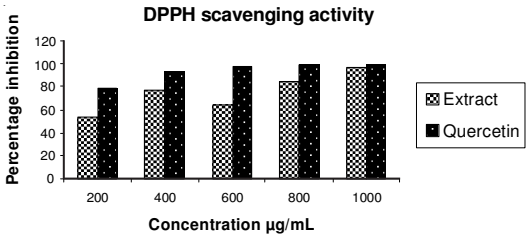

Fig. 1. DPPH scavenging activity of extract and standards

DPPH scavenging activity: DPPH free radical method is a compatible, rapid and sensitive way to evaluate the antioxidant activity of a specific compound or plant extracts[23]. The method is based on reduction of methanolic DPPH solution in the presence of a hydrogen donating compound. The decrease in absorbance is taken as a measure of the extent of radical scavenging. Free radical scavenging capacities of the extract and standard measured by DPPH assay are shown in Fig. 1. It was observed that the extract and standard shown 96.82 ± 0.013 and 99.07 ± 0.156 percentage inhibition at concentration 1000 µg mL^{-1} respectively.

Reducing power assay: The reducing power of a compound may serve as a significant marker of its potential antioxidant activity[24]. For the measurement of the reducing ability of extract the Fe^{3+}-Fe^{2+} transformation was investigated in the existence of extract[25]. In reducing power assay showed reducing activity by scavenging with active oxygen and free radicals to make them stable[26]. The increased absorbance at 700 nm indicates increase in the antioxidant activity/reducing power.

Fig. 2 shows the reducing power of extract and standards. The extract shows the significant reducing power as compared to standards.

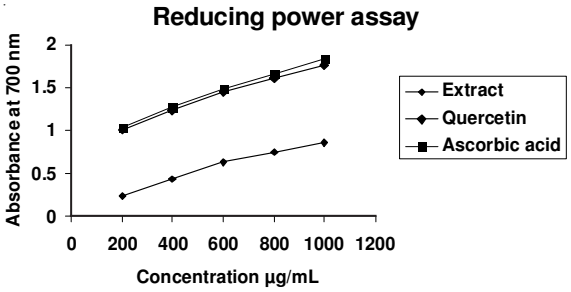

Fig. 2. Reducing power assay

Nitric oxide scavenging activity: Nitric oxide scavenging activity is expressed as percentage inhibition and is presented in Fig. 3. Nitric oxide is an essential bio-regulatory molecule required for several physiological processes[27,28]. Excess concentration of nitric oxide lead to cytotoxic effect observed in various disorder like AIDS, cancer, alzeimer's disease and arthritis[29]. The extract and standard quercetin showed the 47.55 ± 1.063 and 90.41 ± 0.750 % inhibition of nitric oxide radical, respectively at concentration 1000 µg mL^{-1}. Thus found to be below than the standard.

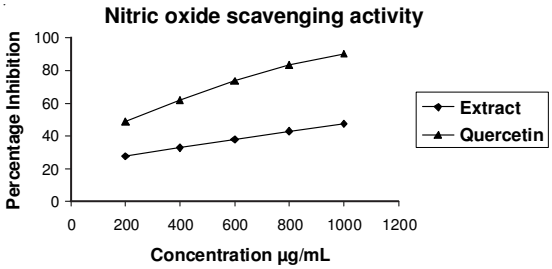

Fig.3. Nitric oxide scavenging activity of extract and standard

Super oxide anion scavenging activity: Super oxide anions are precursor to active free radicals, it is known to be a harmful species to cellular components and have potential of reacting with biological macromolecules inducing tissue damage[30,31]. In PMS-NADH-NBT system, super oxide anions resulting from the dissolved oxygen by PMS/NADH coupling reaction reduce NBT. The extract have antioxidant activity inhibited the blue NBT formation. The extract shows 49.23 ± 0.648 % inhibition at concentration 1000 µg mL^{-1} (Fig. 4.) while in same concentration the standard shows 94.22 ± 0.953 % inhibition. The extract contains flavonoids which are effective to scavenge the super oxide anion[32].

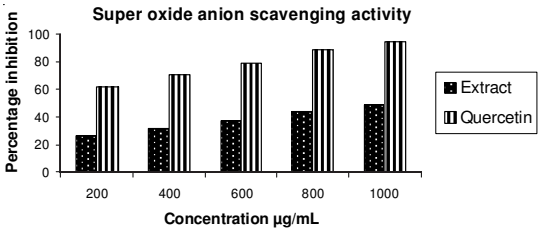

Fig. 4. Super oxide anion scavenging activity of extract and standard

Hydrogen peroxide scavenging activity: Hydrogen peroxide itself is low reactive but sometimes is toxic to cell because it may lead to hydroxyl radicals in the cells[33]. Hydrogen peroxide scavenging activity of extract was compared with ascorbic acid and quercetin, result has been presented in Fig. 5. The result showed the extract was effective in scavenging to hydrogen peroxide. At concentration 1000 µg mL^{-1} extract, ascorbic acid and quercetin showed 43.97 ± 0.536, 90.11 ± 0.411 and 88.39 ± 0.571 % inhibition, respectively.

Conclusion

The results of present study suggest that tested plant material have low antioxidant and free radical scavenging activity. More detailed studies on isolation and characterization of plant extract as well as other *in vivo* assay are essential to established as biological antioxidant which are beyond the scope of this study.

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