American-Eurasian Journal of Scientific Research 5 (2): 114-119, 2010
ISSN 1818-6785

Free Radical Scavenging and Antioxidant Activity of Leaves from Agathi (*Sesbania grandiflora*) (L.) Pers

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Abstract: *Sesbania grandiflora* (L.) Pers. is a soft-wooded tree belonging to the family Leguminoseae. Leaves are considered to be excellent sources of vitamin C and calcium The antioxidant activity of 70% acetone and 50% ethanol extracts of leaves of Sesbania grandiflora were determined by DPPH radical scavenging method. Among the two different extracts acetone extract was found to be more efficient.

Key words: *Sesbania grandiflora* · DPPH radical scavenging activity · Reducing power

INTRODUCTION

Lipid peroxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids. Formation of free radicals may play an important role in the origin of life and biological evolution, implying their beneficial effects on organisms [1]. Radicals are known to take part in lipid peroxidation, which causes food leterioration, aging of organisms and cancer promotion [2]. However, free radicals and other relatic species can also cause the oxidation of biomolecules (eg. Protein, aminoacids, lipid and DNA) which leads to cell injury and death [3]. Recent development in biomedicals point to the involvement of free radicals in many human diseases. Antioxidants can prevent undesirable oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as O_2 scavengers. Restriction in the use of same synthetic antioxidants is being imposed because of their carcinogenicity.

There is some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), commonly used in processing foods. However, it has been suggested that these compounds have some side effects [4, 5]. In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human disease [6].

Herbal and natural products have been used for centuries in every culture all around the world. The search for natural antioxidants, especially of plant origin, had increased greatly in recent years. Plants have almost limited ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. In many cases, these substances serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive, protect molecular damage and herbivores [7, 8]. Many scientists [9] have investigated the chemical composition and antioxidant and anti microbial property of several tree bark samples. Some tree bark extracts have been used as analgestic, anti fungal and anti-inflammatory medicines. Natural antioxidants can be phenolic compounds (Flavonoids, phenolic acids and tannins), nitrogen containing compounds (Alkaloids, chlorophyll, derivative amino acids, peptides and amino acids, peptides and amines), carotenoids, tocopherols or ascorbic acids and its derivatives [10].

Sesbania grandiflora (L.) Pers. is a soft-wooded tree belonging to the family Leguminoseae [11].This is short lived and quick growing soft-wooded tree up to 6-9m height. The flowers are 6.0-10.0cm long with showy, fleshy, white and pink (or) crimson petals. Flowering and fruiting is almost through out the year chiefly during winter. The *S.grandiflora* flowers, leaves, barks and the roots were used [12].

Leaves are considered to be excellent sources of vitamin C and calcium, the later is utilized to the same extent as the calcium in milk, the utilization factors being 0.74% iodine content of the leaves is reported to be 2.3 g/100g. Pectin present in the leaves (1.5%) is of medium jelly quality. The saponins present in the leaves

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on hydrolysis gave an acid. Besides saponin, the leaves contain an aliphatic alcohol [13]. The leaves are used as aperient, diruretic and tonic in form of poultice and they are applied to bruises. The barks of the plant are used as astringent, febrifuge and tonic and its infusion in small-pox. Besides the root juice along with honey is used as expectorant [12]. Leaves are chewed to disinfect the mouth and throat.

MATERIALS AND METHODS

Chemicals: Folin and Ciocalteu's phenol reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl) methanol, acetone, ethanol, petroleum ether, tannic acid, linoleic acid, absolute ethanol, potassium ferricyanide, sodium carbonate, trichloro acetic acid, ferric chloride, ammonium thiocyanate were procurred from Merck, SRL / S.d.fine chem / Sigma, India.

Plant Material Preparation: Fresh leaves of Sesbania grandiflora (L.) Pers. were collected from Pollachi, Coimbatore, Tamil nadu. They were shadow dried at room temperature (26 ± 2°C) for 5-6 days. Then the dried samples were fine powdered and stored in screw cap bottles until further analysis.

Preparation of Different Solvent Extracts: 10 gm of sample was weighed and added 50 ml of petroleum ether. After 3 hours for occasionally shaking, the sample was centrifuged at 5000 rpm for 20 min. After centrifugation the supernatant was discarded and pellets were allowed for air drying. Then the 100 ml of 70% acetone was added with air dried residues and using shaker, the samples were shaken for one day at room temperature. Then the samples were centrifuged at 5000 rpm for 20 min and the supernatant was allowed to evaporate and the pellets were once again re-extracted with 50 ml of 70% acetone for 4 hours. After 4 hours the sample was centrifuged at 5000 rpm for 7 min. Thus obtained supernatant was added with previous one and the pellets were re-extracted with 50 ml of 70% acetone for 4 hours. The sample was centrifuged at 5000 rpm for 7 min and the combined supernatant were allowed for air drying and the extract was kept in an oven at 40°C for removal of residual moisture and to attain constant weight. The remaining air dried residues (pellets) were extracted with 100 ml of 50% ethanol using the shaker for one day. The residues were centrifuged at 5000 rpm for 20 min.The supernatant was collected in a separate beaker and the left out residue was re-extracted with 50 ml of 50% ethanol using shaker for 4 hours at room temperature. The sample was centrifuged at 5000 rpm for 7 min. The supernatant was combined with previous extract and air dried. Further the residual moisture of extracts was recovered by keeping in an oven at 40°C for 48 hours. Finally both aqueous acetone and ethanol extracts were recovered and stored in a separate screw cap vials.

Determination of Total Phenolic Content: 10 mg of extract was dissolved in 2 ml of respective solvent. From that 10 µl of the extract was added to 490 µl of distilled water. Followed by the 250 µl of Folin - Ciocalteu phenol reagent (1:1) 1N was added. The mixture was allowed to stand for 3 min and 1.25 ml of 20% Sodium carbonate (w/v) solution were added. The contents were vortexed and then left to stand in dark at room temperature for 40 min. The colour was developed and the absorbance was measured at 725 nm using a spectrophotometer (ELICO 177). The amount of total phenolics was calculated in the extract by using tannic acid as standard [14].

Reducing Power Assay: 0.238-6 mg of lyophilised extracts were taken and dissolved in 1 ml of 0.2 M phosphate buffer in a test tube to which was added 5 ml of a 0.1% solution of potassium ferric cyanide [14]. The mixture was incubated in a water bath at 50°C for 20 min. Following this, 5 ml of a 10% trichloro acetic acid (w/v) solution was added and the mixture was then centrifuged at 7000 rpm for 10 min. A 5 ml of aliquot of the upper layer was combined with 5 ml of distilled water and 1 ml of 0.1% ferric chloride solution and mixed. Absorbance of the reaction mixture was read spectrophotometrically at 700 nm against water blank. The OD value of the reagent blank (without sample) was considered as 0 concentration of the sample. A higher absorbance of the reaction mixture indicates greater reducing power of the sample.

Radical Scavenging Activity Using DPPH Method: Different concentrations of 0.91-4.29 mg extracts were taken in different test tubes. The volume was adjusted to 100 µl by adding methanol. 5 ml of a 0.1 mM methanolic solution of DPPH was added to these and shaken vigorously. The tubes were allowed to stand at 27°C for 20 min. The control was prepared as above without any extract and methanol was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

% radical scavenging activity = (Control OD - Sample OD / Control OD) X 100

Inhibition of Lipid Oxidation in Linoleic Acid Emulsion: 500 µg of a weighed sample extract (0.25% w/v) in 0.5 ml of absolute ethanol were added with 0.5 ml of 2.5% linoleic acid in 99.5% ethanol (absolute ethanol). 1 ml of 0.05 M phosphate buffer (p^H 7.0) and 0.5 ml of distilled water was placed in a screw capped tube and then in dark oven at 40°C. A control without sample extracts was used. Every (12 hrs) 0.1 ml aliquotes of this solution were taken and 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate were added. After exactly 3 min 0.1 ml of 2 x 10^{-2}M ferrous chloride in 3.5% hydrochloric acid was added. The reading was taken at 500 nm until the absorbance of the control reached maximum [15].

$$AA = 100 - \frac{(\text{Sample OD at 48 hrs - Sample OD at 0 h})}{(\text{Control OD at 48 hrs - Control OD at 0h})} \times 100$$

RESULT AND DISCUSSION

Total Free Aminoacids and Soluble Sugars: Total free amino acid content and total soluble sugars of 50% ethanol and 70% acetone extracts and recovery percent of extracts of leaves of *Sesbania grandiflora* (pink variety) were given in Table 1. The relatively high concentration of amino acid in leaves sample is accordance with other plant leaf extracts. On the other hand, aqueous acetone extracts of leaves showed high concentration of total soluble sugars when compared with amino acids content of respective sample. Aqueous ethanol extract of leaves sample showed low concentration of total /soluble sugars when compared with respective sample. Similar results on high yield potential of different solvent extracts of various parts in Indian laburnum *Casia fistula* have also been reported [15]. The result indicates that the yield of extract is greater with more polar solvent and more over it is much effective in extraction of natural antioxidants [17, 18].

Total Phenolics: The data on total phenolics of different solvent extracts of leaves of *Sesbania grandiflora* (pink variety) was shown in Figure1. Total phenolic content of ethanol extract of leaves (3.01), acetone extracts of leaves (3.06). In general the total phenolic compound in *S.grandiflora* reported in this study was lower than that of phenolic content of various accessions of *centella asiatica* leaf samples [19]. And with in the levels reported [20]. In certain fruits and vegetables. The results also however, lower than that of the total phenolic content in DU- Zhong [21]. It is also interesting to note that the total phenolic content falls with in the range of phenolics found in commonly consuming fruits, vegetables and grains [22]. Reported that the antioxidative properties of some vegetables and fruits are partly due to the low molecular weight of phenolic compounds, particularly the flavanoids, which are known to be potent antioxidants. The major contributors of phenolic substances in *S.grandiflora* are simple phenolic acids. Apart from this the other bioactive compounds reported in this plant was saponins (Fig. 1).

Table 1: Total soluble carbohydrates and total free amino acids of leaves extracts if Sesbania grandiflora(pink varity) g $100g^{-1}$

Parameter	50% Ethanol extract	70% Acetone extract
Total soluble carbohydrates	3.50±0.80	7.64±0.38
Total free amino acids	19.56±0.13	5.96±0.03

Reducing Power Assay: Figure 2 Show the reducing power of the extracts using potassium ferricyanide reduction method. It has been reported that the reducing power was associated with the antioxidant activity and its relationship of phenolic constituents have been well established in several plant sources including vegetables [23]. The yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of each extract. The presence of ductant (Antioxidants) in the herbal extracts causes the reduction of Fe^{3+}/ Ferric cyanide complex to ferrous form. Therefore Fe^{2+} complex can be monitored by measuring the formation of Perl's prcussian blue at 700nm [24]. Among the two different extracts, the aqueous acetone extracts of leaves show higher reducing power. The absorbance value and concentration of extracts show linear relationship. Therefore the increasing OD value indicates increasing trend of reducing power. Polyphenols in the *S.grandiflora* leaf extracts appear to function as good electron and hydrogen atom donors and therefore should be capable of converting free radicals to more stable products. In the present study the reducing power was in the following order: 70% acetone extract of leaves > 50% ethanol extract of leaves. However when compared with the standard, tannic acid, all the extracts showed lower reducing power activity.

Dpph Radical Scavenging Activity: The radical scavenging activities of the various extracts were tested using metabolic solution of the stable free radical DPPH. Unlike laboratory generated free radicals such as the

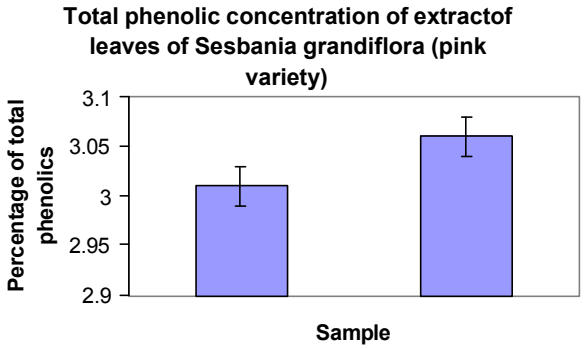

Fig. 1: Total phenolic concentration of extract of leaves of *Sesbania grandiflora* (L.) Pers

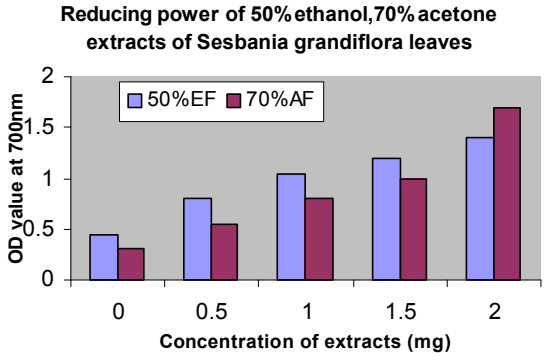

Fig. 2: Reducing power of 50% ethanol, 70% acetone extracts of *Sesbania grandiflora* leaves

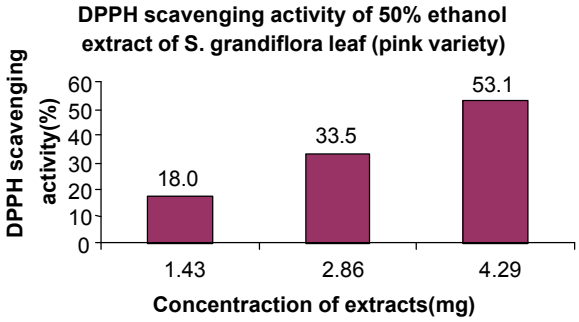

Fig. 3: DPPH Scavenging activity of 50% ethanol extract of *Sesbania grandiflora* leaf (pink variety)

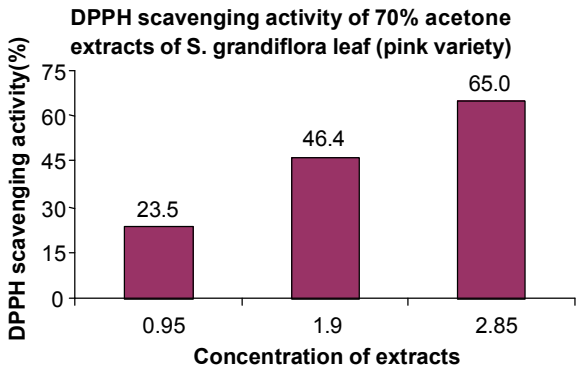

Fig. 4: DPPH Scavenging activity of 70% acetone extracts of *Sesbania grandiflora* leaf (pink variety)

hydroxyl radical and super oxide anion. DPPH has the advantage of being unaffected by such as metal ion due to certain side reactions, such as metal ion chelation and enzyme inhibition brought about by various additives. A freshly prepared DPPH solution exhibits a deep purple colour generally fades / disappears when they are present in medium. Thus antioxidant molecule can quench DPPH free radicals (by providing by hydrogen atom or by electron transfer, conceivably via a free radical attack on the DPPH molecule) and convert them to a colourless product (2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine) alogous of hydrazine) resulting in a decreasing absorbance at a 517nm [25]. Hence more rapidly the absorbance decreases implies more potent antioxidant activity of extract in terms of hydrogen atom donating capacity / electron transfer capability (Fig. 3 and 4).

The free radical scavenging activity of the crude 50% ethanol and 70% acetone extracts of leaves were examined and compared against one another. Figure 7-10 showed that the dose response curve for the free radicals scavenging activity of plant extracts. The results were expressed as a percentage of the ratio of the decrease in absorbance of DPPH solution in the absence of phenolics at 517nm. In the range of 1.43-4.29 dosage of ethanol extracts of leaf expressed low percentage of free radical scavenging activity (18.03%-53.12%) than the rest of the plant extracts. The three extracts showed about equivalent free radical scavenging activity with dose dependent manner.

Radical scavengers may protect tissues from free radicals, there by preventing disease such as cancer [26]. Even through it unclear whether active constituents in plant extracts, such as those from *S.grandiflora* leaf are active against free radicals after being absorbed and metabolized cells in the body. Radical scavenging assay were gained acceptance for their capacity to rapidly screen materials of interest.

Antioxidant Activity in Linoleic Acid Emulsion: The results of peroxidation inhibiting activity of aqueous ethanol and aqueous acetone extracts of leaves of *s.grandiflora* in linolic acid emulsion system are shown in (Table 4). The peroxidation inhibiting activity of the 70% acetone extract of leaves are comparable to each other and

the values are relatively higher than the values of 50% ethanol extracts. However, the peroxidation inhibiting activity of tannic acid registered highest value than all other extracts.

The polar paradox occurs in emulsion then nonpolar compounds have strong antioxidant activity in an emulsion due to the concentration of antioxidant at the lipid: air thereby ensuring strong protection of the emulsion against oxidation. Conversely polar compounds exhibit weak antioxidants activity in emulsion due to the dilution of these compounds in the aqueous phase. Moreover the opposite in antioxidant activity profile is observed in bulk lipid or oil system. In the present study the antioxidant activity against linoleic acid emulsion system might be due to the presence of low molecular phenolic acids, carotenoids and vitamin C.

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