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FREE RADICAL SCAVENGING CAPACITY AND ANTIOXIDANT ACTIVITY OF *Sesbania grandiflora* L. Pers. (WHITE VAIRY)

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ABSTRACT

Antioxidants act as a major defense against radical-medicated toxicity by protecting against the damage caused by free radicals. In addition, reactive oxygen species (ROS) have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer. The present study deals with in vitro antioxidant activity of acetone and ethanol extract of *Sesbania grandiflora* by using DPPH assay, total phenolic content, reducing power assay and Inhibition of lipid oxidation in linoleic acid emulsion. The percentage of scavenging activity at different concentrations was determined and the value of the extracts was compared with that of standard, ascorbic acid and tannic acid. The highest free radical scavenging effect and total phenolic content of the leaf ethanol extract was observed. The peroxidation inhibiting activity of the leaf acetone extracts recorded using the linoleic acid emulsion system, showed very good antioxidant activity.

Keywords: Free radical scavenging capacity, Antioxidant activity, Phenolic compounds

INTRODUCTION

Free radical reactions occur in the human body and food systems. Free radicals, in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology. An over-production of these reactive species can occur, due to oxidative stress brought about by the imbalance of the bodily antioxidant defense system and free radical formation. These reactive species can react with biomolecules, causing cellular injury and death. This may lead to the development of chronic diseases such as cancers and those that involve the cardio- and cerebrovascular systems. The consumption of fruits and vegetables containing antioxidants has been found to offer protection against these diseases. Dietary antioxidants can augment cellular defences and help to prevent oxidative damage to cellular components [1].

Besides playing an important role in physiological systems, antioxidants have been used in the food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats. This component in foods is readily oxidized by molecular oxygen and is a major cause of quality deterioration, nutritional losses, off-flavour development and discolouration. The addition of synthetic antioxidants, such as propyl gallate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone has been widely used industrially to control lipid oxidation in foods. However, the use of these synthetic antioxidants has been questioned due to their potential health risks and toxicity [2].

Sesbania grandiflora (L.) Pers. is a soft wooded tree belonging to the family Paplionaceae. Flowers are rich in nutrients and are used as vegetables in rural area. Bark is used in treating small pox and other eruptive fevers. The juice from the flower is used to treat head ache, head congestion, or stuffy nose. The powdered bark is also recommended for ulcers of the mouth and alimentary canal and infantile disorders of the stomach [3]. 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 on hydrolysis gave an acid. Besides saponins, the leaves contain an aliphatic alcohol [4]. The leaves are used as

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aperients, diuretic, 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 [3]. Leaves are chewed to disinfect the mouth and throat. Hence, the medicinally and nutritionally important flowers were used for the antioxidant activities in the present study.

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 procured from Merck, SRL / S.d.fine chem / Sigma, India.

Plant Material Preparation

Fresh leaves and young stem bark of *Sesbania grandiflora* L. Pers. were collected from Coimbatore, Tamil Nadu. The freshly collected plant materials were washed thoroughly in tap water, shade dried at room temperature (25ºC), powdered and used for solvent extraction. The plant samples were successively extracted with petroleum ether (for disposing lipid and pigments), 70% acetone and 50% ethanol using soxhlet apparatus. Each time before extracting with the next solvent, the material was dried in hot air oven at 40○C. The solvents were evaporated using a rotary vacuum-evaporator at 50ºC. The extract recovery in different solvents was expressed as percent of the plant sample dry matter. The freeze-dried extracts thus obtained were dissolved in the respective solvents at the concentration of 1mg/ml and used for assessment of antioxidant capacity through various chemical assays.

Determination of Total Carbohydrate and Total amino acids

Determination of Total Carbohydrate by Anthrone Method Aliquots of each extracts were taken and made up to 1 ml by adding distilled water. For each tube 4 ml of anthrone reagent was added. All the tubes were covered with glass marbles and heated for 8-10 min in a boiling water bath then the tubes were cooled for 5-7 min. The absorbency of green colour product was measured at 630 nm.

Estimation of Total Free Amino acids by Ninhydrin method

Aliquots of each extracts made up to 1 ml by distilled water. To this 1 ml of ninhydrin reagent was added. The tubes were placed in boiling water bath for 20 min. Then the tubes were cooled and 5 ml of diluents was added to each tube. Then the absorbance was measured at 570 nm [5].

Determination of total phenolic contents

The total phenolic content was determined according to the method described [6]. Aliquots of each extracts were taken in test tubes and made up to the volume of 1ml with distilled water. Then 0.5ml of folin-ciocalteu phenol reagent (1:1 with water) and 2.5ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as the tannic acid equivalents (TAE).

Reducing power assay

Aliquots of each extracts were taken in test tubes 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 [7]. The mixture was incubated 50°C for 20 min. Following this, 5 ml of trichloro acetic acid (10%) (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 ferric chloride solution (0.1%) and Absorbance was recorded at 700 nm against reagent blank. A higher absorbance of the reaction mixture indicates greater reducing power of the sample.

Free radical scavenging activity on DPPH•

The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH**,** according to the method of [8]. Sample extracts at various concentrations was taken and the volume was adjusted to 100µl with methanol. 5ml of a 0.1mM methanolic solution of DPPH was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27○C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

% DPPH radical scavenging activity = (Control OD – Sample OD / Control OD) X 100.

Inhibition of lipid oxidation in linoleic acid emulsion

Aliquots of each extracts were taken in test tube 0.5 ml of absolute ethanol were added with 0.5 ml of linoleic acid (2.5%) in 99.5% ethanol and 1 ml of 0.05 M phosphate buffer (pH7.0) 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 aliquot of the solution was 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 [9].

AA=100 [(Sample OD at 48hrs –Sample OD at 0h) / (Control OD at 48 hrs - Control OD at 0 h)] X100.

STATISTICAL ANALYSIS

Results were expressed as the means of three replicates ± the standard deviation of triplicate analysis.

RESULTS 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 leaf and flower of *Sesbania grandiflora* (white variety) are given in Table1. The percentage of amino acid of aqueous acetone extracts of flower found to be much lower (3.25%) than other three extracts namely aqueous acetone extracts of leaf (5.33%), aqueous ethanol extract of leaf (13.49%) and aqueous ethanol extract of flower (11.75%). The relatively high concentration of amino acid in leaf sample is accordance with other plant leaf extracts. On the other hand, aqueous acetone extracts of flower and leaf sample showed high concentration of total soluble sugars when compared with amino acids content of respective sample showed high concentration of total soluble sugars when compared with amino acid content of respective sample. Aqueous ethanol extract of flower and leaf sample showed low concentration of total /soluble sugars when compared with respective sample. Nonetheless, the amount of sugars present in the aqueous acetone extracts of flower (15.55%) found to be higher than that of aqueous acetone extracts of leaf(10.91%) and aqueous ethanol extracts of flower (10.62%) and leaf (4.81%).Similar results on high yield potential of different solvent extracts of various parts in Indian laburnum *Casia flstula* have also been reported [7]. The result indicate that the yield of extract is greater with more polar solvent and more over it is more effective in extraction of natural antioxidants [10, 11].

TOTAL PHENOLICS

The data on total phenolics of different solvent extracts of leaf and flower parts of *Sesbania grandiflora* (pink variety) shown in Figure1. Total phenolic content of acetone extract of flower (1.83%) is lower than the other three extracts namely ethanol extract of leaf (3.01), acetone extracts of leaf (3.06) and ethanol extract of flower (2.67%). 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 [12] and with in the levels reported [13] in certain fruits, grams, and vegetable. In general, leaf sample found to contain more phenolics than the flower sample. It is also interesting to note that the total phenolic content falls in the range of phenolics found in commonly consuming fruits, vegetables and grains. Hence, the consumption of both leaf and flowers as vegetable may root produce any harmful effects rather than beneficial impacts. [14] and [15] reported that the antioxidative properties of some vegetables and fruits are partly due to the low molecular weight phenolic compounds, particularly the flavanoids, which are known to be potent antioxidants. The major contributors of phenolic substances in *S.grandiflora* are simple phenolics acids. Apart from this the other bioactive compounds reported in this plant are saponins.

REDUCING POWER ASSAY

Figure 2-5 shows the reducing power of the extracts using potassium ferric cyanide 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 9 and [6]. 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 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 Prussian blue at 700nm [16]. Among the two different extracts, the aqueous acetone extracts of flower showed higher reducing power. The absorbance value and concentration of extracts show linear relationship. Therefore the increasing OD value indicates increasing trend of reducing power. Polyphenolics in the *S. grandiflora* flowers and 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 is in the following order: 70% acetone extracts of flower > 50% ethanol extract of flower. However when compared with the standard, tannic acid, all the extracts have 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 hydroxyl radical and super oxide anion. DPPH has the advantage of being unaffected by such as metal ion by certain side reactions, such as metal ion chelating and enzyme inhibition brought about by various additives. A freshly prepared DPPH solution exhibits a deep purple colour generally fades / disappears when a present in medium. Thus antioxidant molecule can quench DPPH free radicals (by providing 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) resulting in a decreasing absorbance at a 517nm [17]. Hence the more rapidly the absorbance decreases implies more potent antioxidant activity of extract in terms of hydrogen atom donating capacity / electron transfer capability.

The free radical scavenging activity values of the crude 50% ethanol and 70% acetone extracts of leaf and flower were examined and compared against one another. Figure 6-9 show that the dose response curve for the free radical scavenging activity of flower 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 0.54-1.63 dosage of acetone extracts of leaf expressed high percentage of free radical scavenging activity compared with the acetone extracts (22.55%-63.09%).

Radical scavengers may protect tissues from free radicals, there by preventing disease such as cancer [18] even though it is unclear whether active constituents in plant extracts, such as those from *S. grandiflora* flower, 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 flower of *S. grandiflora* in linolic acid emulsion system were shown in Figure 10. The peroxidation inhibiting activity of the 70% acetone extracts of flower and leaves and 50% ethanol extract of flowers are comparable to each other and the values are relatively higher than the values of 50% ethanol extract of leaves of *S. grandiflora*. 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 an opposite 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.

Table 1. Recovery per cent of extracts, total soluble carbohydrates and total free amino acids of different solvent extracts of leaf and bark of *A. nilotica* g/100g

Parameter	Leaf		Bark	
	50% Ethanol	70% Acetone	50% Ethanol	70% Acetone
Recovery per cent of extract	2.8	38.5	2.3	43.5
Total soluble carbohydrates	2.41± 0.20	7.85 ± 1.98	6.11 ± 1.30	9.51 ± 1.27
Total free amino acids	7.09 ±0.39	3.88 ± 0.63	8.07 ± 0.21	4.34 ± 0.68

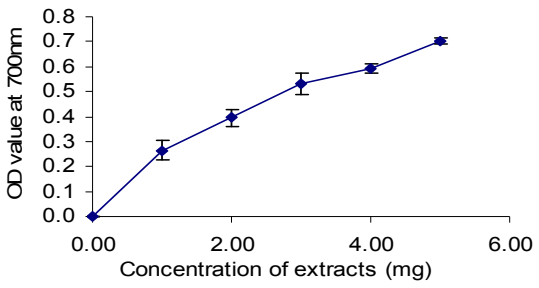

Figure 2: Reducing power of 50% ethanol extracts of *Sesbania grandiflora* leaf (white variety)

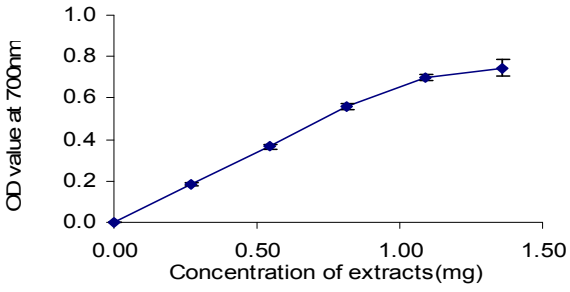

Figure 3: Reducing power of 70%acetone extracts of *Sesbania grandiflora* leaf (white variety)

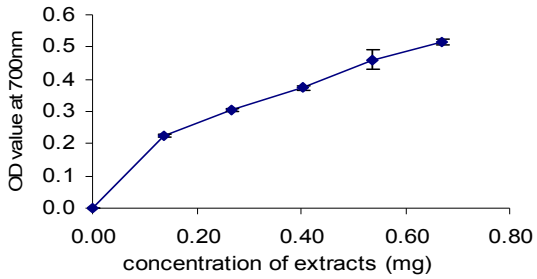

Figure 4: Reducing power of 50% ethanol extracts of *Sesbania grandiflora* flower (white variety)

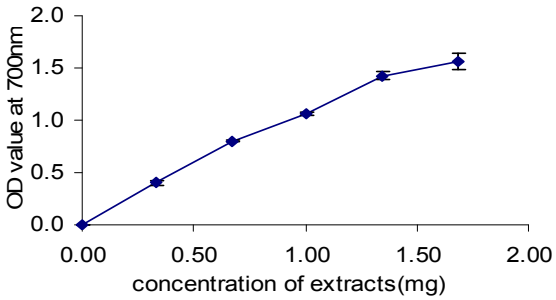

Figure 5: Reducing power of 70% acetone extracts of *Sesbania grandiflora* flower (white variety)

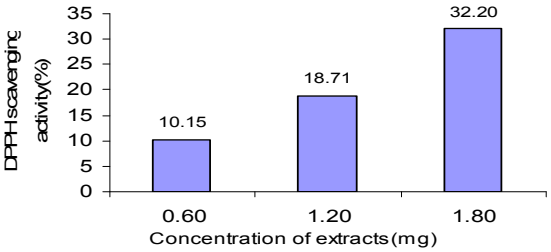

Figure 6: DPPH scavenging activity of 50%ethanol extracts of *Sesbania grandiflora* leaf (White variety)

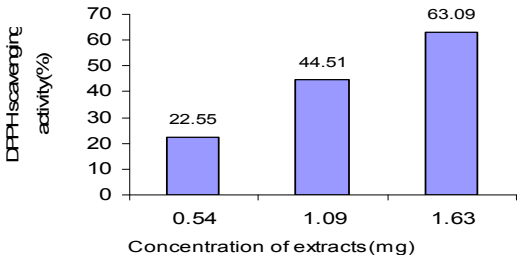

Figure 7 : DPPH scavenging activity of 70% acetone extracts of *S.grandiflora* leaf (White variety)

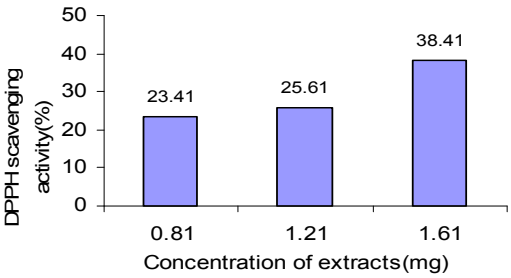

Figure 8: DPPH scavenging activity of 50% ethanol extracts of *S.grandiflora* flower (white variety)

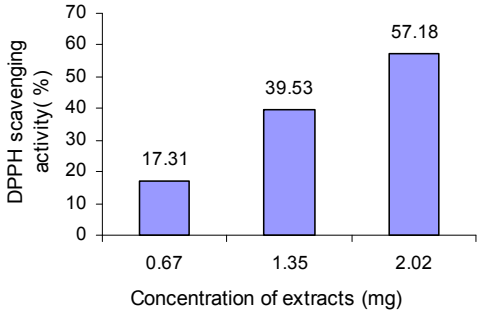

Figure 9: DPPH scavenging activity of 70% acetone extracts of *Sesbania grandiflora* flower (white variety)

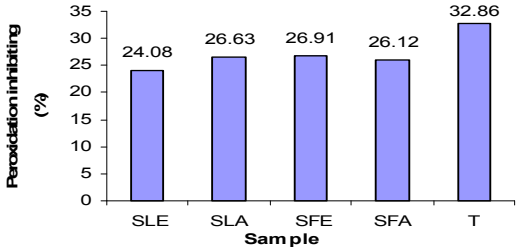

Figure 10. Peroxidation inhibiting activity of *S. grandiflora* white variety extracts in linoleic acid emulsion system

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