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RESEARCH ARTICLE

A STUDY ON IN VITRO ANTIOXIDANT ACTIVITY OF AQUEOUS SEED EXTRACT OF *SESBANIA SESBAN* (L) MERR.

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ABSTRACT

The present study was done to investigate the *in vitro* antioxidant activity of aqueous extract of *Sesbania sesban* seeds. The assays such as DPPH, Chelation, ferrous ion, ABTS, Superoxide radical, hydroxyl radical assay, FRAP assay and total antioxidant activity were done to assess the antioxidant potential of the seed extract. The extract was tested at a concentration range of 100 – 500 µg/ml for all the assays and the values were compared with a standard. The results obtained showed that the radical scavenging activity was in a dose dependent manner and found to increase with increase in concentration of the extract. The IC50 value was calculated for the assays and tabulated for inference. Different assays revealed different levels of radical scavenging potential of the extract and exhibited as a better antioxidant source for therapeutic applications.

Keywords: *Sesbania sesban*, antioxidant, aqueous extract, radical scavenging, dose dependent.

1. INTRODUCTION

The traditional medicine all over the world is nowaday revealed by an extensive activity of researches on different plant species and their therapeutic principles. Plants contain phytochemicals with various bioactivities including antioxidant, anti-inflammatory and anticancer activities. Currently, about 25% of the active component was identified from plants that are used as prescribed medicines [1].

Free radicals and reactive oxygen species (ROS) such as superoxide, hydroxyl and peroxyl radicals are normal by-products of aerobic metabolism produced *in vivo* during oxidation. These ROS are generated in the mitochondria and microsome organelles under normal physiological conditions. They can also be produced externally by exposure to radiation, toxic chemicals, cigarette smoking and alcohol consumption, and by eating oxidized polyunsaturated fats. Overproduction of ROS can result in oxidative damage to various biomolecules including lipids, proteins, DNA and cell membranes. They also lead to the development of a variety of diseases such as coronary heart diseases, cancer, diabetes, hypertension and neurodegeneration. While compounds capable of scavenging free radicals possess great potential in ameliorating these diseases, most of the ROS are scavenged by endogenous defense enzymes such as catalase, superoxide dismutase and peroxidase-glutathione system. However, the activities of these endogenous defense systems may not be sufficient to mop up the free radicals [2].

Antioxidants can protect the human body from free radicals and reactive oxygen species (ROS) effects. Antioxidant agents are well known to retard the progress of many chronic diseases as well as lipid peroxidation [3].

Currently, there is a great interest in the study of antioxidant substances mainly due to the findings concerning the effects of free radicals' in the organism. Phenolic plant compounds have attracted considerable attention for being the main sources of antioxidant activity, in spite of not being the only ones. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential. The antioxidant activities of phenolics play an important role in the adsorption or neutralization

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of free radicals. Several synthetic antioxidants are commercially accessible but have been reported to be toxic. Plants have been reported to exhibit antioxidant activity due to the presence of antioxidant compounds such as phenolics, proanthocyanidins and flavonoids [4].

Commonly used synthetic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate (PG) and tertbutylhydroxyltoluene (TBHQ). Though important, they are known to constitute potential health risks and toxic effects. Their applications are, therefore, strongly restricted. Hence, the need to search, develop and utilize more effective antioxidant from natural origin. Inspite of our dependence on modern medicine and the tremendous advances in synthetic drugs, a large number of the world populations (80% of people) cannot afford the products of the western pharmaceutical industry and have to rely upon the use of traditional medicines, which are mainly derived from plant material [5].

Fabaceae, which is the third largest family among the angiosperms after Orchidaceae (orchid family) and Asteraceae (aster family), consists of more than 700 genera and about 20,000 species of trees, shrubs, vines, and herbs and is worldwide in distribution. *Sesbania sesban* (L) Merr belongs to fabaceae family has a long history of use in India, grows in a wide range of soils from loose sands to heavy clays. Root and bark used as bitter tonic used in debility nervous disorders and act as a CNS stimulant. Root of plant used as dysuria, retention of urine, hepatoprotective activity. Leaves are used as anthelmintic activity [6].

Therefore, this study has been designed to evaluate the *invitro* antioxidant activity of the aqueous seed extract of *Sesbania sesban.*

2. MATERIALS AND METHODS

2.1. Plant material and Preparation of extract

The plant was authenticated with Botanical Survey of India, Southern Regional Centre, Coimbatore. Seeds of *Sesbania sesban* were shade dried and healthy seeds were selected and grinded well to a coarse powder. Aqueous extract was prepared with 10g of the powdered sample in 300 ml of water. The extract that was obtained was condensed in an oven and was preserved in an air tight container and stored at 4^0C for further use.

2.2. In vitro antioxidant activity of aqueous extract of seeds of *Sesbania sesban*

2.2.1. Superoxide Radical Scavenging Assay [7]

Superoxide radical O_2^- scavenging capacity of aqueous extract was examined by a pyrogallol autooxidation system. The reaction mixture contained 70µl 10mM pyrogallol, 4.5ml 50mM Tris Hcl (pH 8.2) and 0.5ml various concentrations of samples. The absorbance at 325nm was recorded immediately at 30 seconds and then recorded once every minute. The scavenging rate was obtained according to the formula: O_2^-, scavenging rate (%) = $[1-(A_1-A_2)/A_0]$ X 100, where A_0 was the absorbance of the control (without extract), A_1 was the absorbance in the presence of the extract; A_2 was the absorbance of without pyrogallol.

2.2.2. Hydroxyl Radical Scavenging Assay [8]

Two sets were prepared for the hydroxyl radical scavenging effect, one with extract and sodium salicylate and the other with extract but without sodium salicylate. One set of the tubes containing the mixture 0.5ml of $FeSO_4$, 0.35 ml of H_2O_2, 0.5 ml of the extract and 0.15 ml of sodium salicylate and the other set of tubes containing all the components except sodium salicylate were incubated at 37^0C for 1 hr. The absorbance was read at 562nm.

2.2.3. DPPH Radical Scavenging Activity [9]

Various concentrations of the extract (1.0 ml) were mixed with 1.0ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.135 mM. The mixture were shaken vigorously and left to stand for 30 min in dark, and the absorbance was measured at 517 nm. BHA was used as control. The percentage of DPPH decolorization of the sample was calculated according to the equation:

% decolorization = $[1-(ABS_{sample}/ABS_{control}]$ x 100

2.2.4. Reducing Power Assay [10]

The reaction mixture contained 2.5 ml of various concentrations of methanol extract of the sample, 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 0.2 M sodium phosphate buffer. The control contained all the reagents except the sample. The mixture was incubated at 50°C for 20 min and were terminated by the addition of 2.5 ml of 10% (w/v) of trichloroacetic acid, followed by

centrifugation at 3000 rpm for 10 min. 5.0 ml of the supernatant upper layer was mixed with 5.0 ml of deionized water and 1.0 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm against blanks that contained distilled water and phosphate buffer. Increased absorbance indicates increased reducing power of the sample.

2.2.5. Ferrous Ion Chelating Assay [11]

The reaction mixture contained 1.0 ml of various concentrations of the extract, 0.1 ml of 2 mM $FeCl_2$ and 3.7 ml methanol. The control contained all the reaction reagents except sample. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine. After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. A lower absorbance of the reaction mixture indicated a higher Fe^{2+} chelating ability. The capacity to chelate the ferrous ion was calculated by

% chelation = [1-(ABS $_{sample}$/ABS $_{control}$] x 100.

2.2.6. ABTS Radical Scavenging Activity [12]

Samples were diluted to produce 0.2 to 1.0 mg/ml. The reaction was initiated by the addition of 1.0 ml of diluted ABTS to10 µl of different concentration of the sample or 10 µl of methanol as control. The absorbance was read at 734 nm and the percentage inhibition was calculated. The inhibition was calculated according to the equation

$I = A_o - A_1/A_o \times 100$, where A_o is the absorbance of control reaction, A_1 is the absorbance of test compound.

2.2.7. Ferric reducing antioxidant power (FRAP) assay [13]

A solution of 20 mM $FeCl_3 \cdot 6H_2O$, 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ in 16 ml $C_2H_4O_2$, pH 3.6) and 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl) was prepared. At the time of establishing the assay, 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$ was mixed to prepare the FRAP solution. Plant extract (150 µl) was mixed with 2850 µl of FRAP solution and incubated at room temperature in the dark for 30 min. Absorbance of the intense blue-coloured product (ferrous tripyridyltriazine complex) was measured at 593 nm. The observed absorbance of the sample was calculated by putting the values on a linear standard curve plotted between 200 µM to 1000 µM $FeSO_4$.

2.2.8. Total Antioxidant Capacity Assay [14]

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH. The reagent solution consists of 0.6 M H_2SO_4, 28.0 mM sodium phosphate and 4.0 mM ammonium molybdate. An aliquot of 0.1 ml of sample was combined with 1 ml of reagent solution. The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. The blank solution contained 1 ml of reagent solution and the solvent used for the sample, and it was incubated under the same conditions as the rest of the samples.

3. RESULTS AND DISCUSSION

The *in vitro* antioxidant activity of aqueous seed extract of *Sesbania sesban* was done using different assays and the results are tabulated and discussed as follows.

Table 1. Percentage inhibition activity of aqueous extract of seeds of *Sesbania sesban*

Sample concentration (µg/ml)	DPPH	Chelation	ABTS	Superoxide radical	Hydroxyl radical
100	21.70 ± 0.92	13.27 ± 0.85	12.39 ± 1.02	28.16 ± 0.69	16.60 ± 0.52
200	39.06 ± 1.25	22.24 ± 1.43	35.68 ± 1.50	39.43± 1.72	29.31± 1.37
300	56.43 ± 1.54	58.44± 2.70	55.66 ± 2.84	54.92± 1.50	43.19± 1.94
400	70.09 ± 1.86	66.67 ± 3.52	71.18 ± 3.98	67.60± 2.01	76.32 ± 2.25
500	79.09 ± 2.48	81.72 ± 4.87	81.71± 4.37	78.87± 0.74	83.88± 4.68

Value expressed as mean ± SD (n = 3)

In the DPPH test, the stable, nitrogen centered, coloured, DPPH free radical is reduced either by hydrogen donor or antioxidant to a non-radical DPPH-H and the decrease in colour of DPPH radical is monitored over a time period. The seed extract exhibited stronger radical scavenging ability and its 50% inhibition reached at 265 ± 12.5 µg/ml which indicates its good antioxidant potential. The percentage inhibition ranged from 21.70 ± 0.92 to 79.09 ± 2.48 % corresponding to 100-500 µg/ml of the extract.

Metal chelating activity is significant since it reduces the concentration of the catalyzing transition metal in lipid peroxidation [15]. It has been reported that the chelating agents, which forms σ bonds with the metal are effective as secondary antioxidants, because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [16]. The EC_{50} value was found to be 275 ± 12.75 µg/ml.

ABTS is a compound frequently used in phytomedicine research to measure the antioxidant properties of plants for better elucidation of their biological properties. The radical is generated through the oxidation of ABTS to an intensely coloured nitrogen centered cation by reacting with potassium persulfate for 12-14 h. The IC_{50} values of the plant extracts were also determined for $ABTS^{+}$ which ranges at 275 ± 20.5 µg/ml.

Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals that are generated. The scavenging activity of this radical by the plant extract compared favourably with the standard reagents such as gallic acid suggesting that the plant is also a potent scavenger of superoxide radical [4]. The extract was found to have the property to scavenge the superoxide radical at various concentrations ranging from 100-500 µg/ml. The activity increased with increase in concentration of the extract and reached its EC_{50} value of 275 ± 17.06 µg/ml.

The hydroxyl radical (OH) is said to be detrimental and initiates auto-oxidation, polymerization and fragmentation of biological molecules. The identification of compounds that have excellent hydroxyl scavenging activity would be significant for some diseases caused by oxidative stress. It has been demonstrated that plants contain many natural antioxidants compounds which have been identified as hydroxyl radical scavengers. Therefore, OH scavenging effects of *S. sesban* aqueous extract were assessed in the present study. The result shows that the scavenging activity of seed extract are significant. The percentage inhibition was at rise with increase in concentration of the extract. The 50% percentage inhibition was at 340 ± 15.2 µg/ml.

Table 2. EC_{50} (µg/ml) values of aqueous extracts of *Sesbania sesban*

Antioxidant assay	EC_{50} (µg/ml)
DPPH	265 ± 12.50
Chelation	275 ± 12.75
ABTS	275 ± 20.50
Superoxide radical	276 ± 17.06
Hydroxyl radical	340 ± 15.20
Reducing Power	300 ± 15.15
FRAP	250 ± 14.01

EC_{50} value represents scavenging efficiency at 50% concentration of the extract on DPPH, ABTS radical, OH radical, chelation of ferrous ions, superoxide radical, hydroxy radical, and by reducing power and FRAP assay
Value expressed as mean ± SD (n = 3).

Table 3. Total antioxidant activity of the aqueous extracts of *Sesbania sesban* by phosphomolybdenum assay

	mg/g (GAE)	mg/g (AAE)
Total antioxidant activity	5.84 ± 0.05	16.90 ± 0.13

Total Antioxidant Capacity (TAC) assay by phosphomolybdenum method that based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate/Mo (V) complex at acidic pH, usually detects antioxidants such as some phenolics, ascorbic acid, α-tocopherol and carotenoids [14]. The total antioxidant activity of the aqueous seed extract of *Sesbania sesban* was determined and was found to be 5.84 ± 0.05 mg/g of gallic acid equivalent (GAE) and 16.90 ± 0.13 mg/g of ascorbic acid equivalent (AAE). The values

represent a significant antioxidant potential of the extract.

Table 4. Scavenging capacity of the aqueous extracts of *Sesbania sesban*

Sample concentration (µg/ml)	Reducing Power(A_{700nm})	FRAP(A_{540nm})
100	0.153± 0.008	0.282± 0.01
200	0.241± 0.01	0.415± 0.02
300	0.512± 0.03	0.627± 0.02
400	0.685± 0.03	0.795± 0.04
500	0.889± 0.04	0.971± 0.05

Value expressed as mean ± SD (n = 3).

The table.4 shows the scavenging capacity of the aqueous extracts of *Sesbania sesban* assessed by Reducing Power and FRAP assay. The reducing capacity of the extract, another significant indicator of antioxidant activity was also found to be appreciable. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Increasing absorbance indicates an increase in reductive ability. The 50% inhibition was found at 300 ± 15.15 µg/ml. The extract was found to be effective at all concentrations. The results show that there was increase in reducing power of the plant extract as the extract concentration increases [4].

In FRAP assay, reduction of the ferric-tripyridyltriazine to the ferrous complex forms an intense blue colour which can be measured at a wavelength of 593 nm. The intensity of the colour is related to the amount of antioxidant reductants in the samples [17]. Since FRAP assay is easily reproducible and linearly related to molar concentration of the antioxidant present, thus it can be reported that extract of *S. sesban* may act as a free radical scavenger, capable of transforming reactive free radical species into stable non radical products. The antioxidant potentials of the extract of seeds of *S. sesban* were estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II) at 593 nm and its antioxidant activity increased proportionally with the polyphenol content [18]. The 50% inhibition was found at 250 ± 14.01µg/ml and the scavenging activity was a dose dependent manner.

4. CONCLUSION

The present study demonstrates the *invitro* antioxidant activity of aqueous seed extract of *Sesbania sesban.* Various assays supported the free radical scavenging potential and antioxidant potential of the extract at various concentrations. As per the literature available and the results of the present study, the *Sesbania sesban* seed extract can serve as a potent antioxidant source. A validation of the purified compounds from the extract need to be done for making it to be used for pharmaceutical and therapeutic applications.

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