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ORIGINAL ARTICLE

In vitro Anti Oxidant Activity of Bark of *Sesbania grandiflora* L.

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ABSTRACT
Sesbania grandiflora L. is a well known medicinal plant. The aim of the present study is to evaluate the antioxidant activity of bark extracts of Sesbania grandiflora L. The antioxidant screening was done by using the methods like DPPH, Reducing power assay and Hydrogen peroxide scavenging capacity. Aqueous and methanolic extracts were used for this investigation. The results showed strong antioxidant activity of methanolic extracts as compared with that of the aqueous extract. Further studies are empowered for the isolation and characterization of these natural antioxidants.
Keywords: *Sesbania grandiflora, Antioxidant activity, DPPH Assay, Reducing power assay, Ascorbic acid*

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INTRODUCTION

The term antioxidant can be defined as any substance that delays or inhibits oxidative damage to a target molecule [1]. Antioxidants are compounds which have ability to bring either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. Antioxidants are substances used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals. Antioxidants are involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals. Reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxy nitrite radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases [2].

Sesbania grandiflora which belongs to family Fabaceae commonly known as 'sesbania', is widely used as Indian folk medicine. *S. grandiflora* has the common names of Agati, Corkwood Tree and West Indian Pea, The hummingbird tree (or scarlet wisteria). In India, it is known as vaka or basna. Traditionally *Sesbania gradiflora* is used alone or with other medicinal plants to treat a variety of ailments. It is a small tree believed to have originated either in India or Southeast Asia and grows primarily in hot and humid tropical areas in the world. A native to Asian countries such as India, Malasia, Indonesia and the Philippines where it is commonly seen growing on the dikes between rice paddies, along roadsides and in backyards vegetable gardens. The whole plant contains Grandifloral, arginine, cystine, histidine, isolucine, phenylalanine, tryptophan, valine, threonine, alanine, aspargine, aspartic acid and a saponin yielding oleanolic acid, galactose, rhamnose and glucuronic acid and it also contains flavonol glycoside, kaempferol. The root-bark of the red-flowered variety is useful in vitiated condition of *vata* and arthralgia. The bark is astringent, cooling, bitter, tonic, anthelmintic and febrifuge. The pounded bark is externally applied to cure scabies. The juice of the bark is good for dyspepsia, diarrhea and gastralgia. The leaves are acrid, bitter, sweet, cooling, aperient, tonic and diuretic and contain a non-poisonous saponin like substance. Leaves are chewed to disinfect mouth and throat and are useful in stomatalgia. The flowers are cooling, bitter, astringent, acrid and antipyretic. The juice of the flowers is applied to the eyes for nyctalopia and is used for intermittent fevers. The fruits are sweet, bitter, laxative and alexiteric and are useful in flatulent-colic, astringent, cooling, bitter, tonic, anthelmintic, febrifuge, cure scabies, dyspepsia, diarrhea and gastralgia, astringent, antipyretic

[3,4,5]. Based on the above medicinal properties of *Sesbania grandiflora*, in this study, we investigated the antioxidant activity of methanolic and aqueous extracts of plant bark.

MATERIALS AND METHODS

Plant Material

The plant material of *Sesbania grandiflora*. (*Fabaceae*) bark was collected from local area of Dhule district, Maharashtra, India. The plant material was clean and dried. Also was identified and authenticated from Department of Botany, S.S.V.P.S's L. K. Dr. P. R. Ghogarey Science College, Dhule (M.S.) by Voucher Specimen No.110.

Preparation of the Extract

Dried bark material were mechanically reduced to a coarse powder and then sieved and stored in an air tight container at room temperature. The extraction method was based on the presence of active constituents in the drug, using various solvents ranging from non-polar to polar. Dried powder was extracted sequentially with methanol and distilled water by using soxhlation method. The extracts were concentrated to dryness by distilling the solvent at low temperature using rotary evaporator. The extract was stored in airtight container.

In vitro Antioxidant activity

Reducing power determination [6,7, 8,9]

Reagents-

Phosphate Buffer (50mM)

Weight accurately 17.34 gm of potassium di-hydrogen phosphate and 43.85 gm of di-sodium hydrogen phosphate both are previously dried at 110 to 130^{0}C for 2 hours in volumetric flask and dissolve in sufficient distilled water to produce 1000 ml.

potassium ferricyanide (1% w/v)

Weight accurately potassium ferricyanide in 100 ml volumetric flaks and dissolve in sufficient amount of distilled water to produce 100 ml.

Trichloroacetic acid (10% v/v)

Take accurate volume of trichloroacetic acid in 100 ml volumetric flask and add sufficient amount of distilled water to produce 100 ml.

Ferric chloride solution (0.1% w/v)

Weight accurately ferric chloride in 100 ml volumetric flaks and dissolve in sufficient amount of distilled water to produce 100 ml.

Preparation of Standard Solution:-

Standard solution was prepared by dissolving 100 mg ascorbic acid in 100 ml distilled water.

Preparation of Extract Solution

The solution of extract of plant *Sesbania grandiflora* were prepared by, the methanolic extract were prepared as 50 mg in 50 ml methanol and aqueous extract were prepared as 50 mg in 50 ml distilled water.

Procedure

The reducing power of methanolic and aqueous extract was determined.Different concentration of methanolic and aqueous extract (25, 50, 75,….200µl/ml) was prepared and mix 1.0 ml of each sample with 2.5 ml of phosphate buffer solution (50mM, pH 7.0) and 2.5 ml of 1% potassium ferric cyanide separately, incubate at 50^{0}C for 20 minutes. Then add 2.5ml of trichloroacetic acid (10%) to the mixture, centrifuge at 3000 rpm for 10 min. finally mix 1.25 ml from supernatant with 1.25 ml of distilled water and 0.25 ml of $FeCl_3$ solution (0.1 % w/v). immediately measure the absorbance at 700 nm. Carry out the same assay in triplicate. Prepare the different concentration of sample results of this method are based on the fact of increased absorbance value of different concentrations indicates higher reducing power.

Hydrogen Peroxide Scavenging Capacity [10]

Reagents

Phosphate Buffer (50mM)

Weight accurately 17.34 gm of potassium di-hydrogen phosphate and 43.85 gm of di-sodium hydrogen phosphate both are previously dried at 110 to 130^{0}C for 2 hours in volumetric flask and dissolve in sufficient distilled water to produce 1000 ml.

Hydrogen peroxide (H_2O_2) (40Mm)

Dissolve 0.046 ml of Hydrogen Peroxide solution in 100 ml of Phosphate Buffer (50mM).

Preparation of Standard Solution

Standard solution was prepared by dissolving 100 mg ascorbic acid in 100 ml distilled water.

Preparation of Extract Solution

The solution of extract of plant *Sesbania grandiflora* were prepared by, the methanolic extract were prepared as 50 mg in 50 ml methanol and aqueous extract were prepared as 50 mg in 50 ml distilled water.

Procedure

The ability of the *Sesbania grandiflora* extracts to scavenge hydrogen peroxide was determined according to the method of keser et.al. 2012. A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Extracts (10 to100 µg/ml) in particular solvents were added to a hydrogen peroxide solution (0.6 ml, 40 mm). Absorbance of hydrogen peroxide at 230nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of *Sesbania grandiflora* extracts and standard compound were calculated by using the formula.

% Scavenged [H_2O_2] = [(Ac –As)/Ac] x100

Where, Ac is the absorbance of the control and As is absorbance of standards**.**

DPPH radical scavenging assay [10]

The antioxidant activity of the methanolic and aqueous extracts of the plant *Sesbania grandiflora* was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH carried out by using the method of Molyneux. About 1 ml of 100 µM DPPH solution in methanol, equal volume of the extract in methanol of different concentrations of the extract in methanol was added and incubated in dark for 30 min and 1ml of methanol served as control. The change in colour was observed in terms of absorbance using a spectrophotometer at 517 nm. The different concentrations of ascorbic acid were used as reference compound. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

$$\text{Percentage Inhibition} = \frac{\text{Absorbance Control - Absorbance test}}{\text{Absorbance Control}} \times 100$$

RESULTS

Reducing power determination

Reducing capability of an antioxidant substance can be assessed using its ability to convert Fe^{3+} to Fe^{2+}. Intensity of Perl's Prussian blue color caused by this reduction is measured at 700 nm. Higher absorbance indicates higher reducing power. The reducing power of the compound can be contributed to its antioxidant potency. The reducing power assay of the plant extracts of plant *Sesbania grandiflora* were tested in this study illustrated in Table No. 1 The findings revealed that the values of reducing power of the plant extracts of *Sesbania grandiflora* were functions of their concentrations. In this study, results showed that all plant extracts had significant levels of Reducing Power activity in a dose dependent manner. At concentration 200ug/ml, methanolic, and aqueous extract had reducing power values of 0.120, 0.118 as compare to standard Ascorbic acid 0.142. At this concentration, methanolic extract showed a remarkable reducing power that was significantly greater than those of the aqueous extract as compared to standard ascorbic acid.

Table 1 Absorbance of reducing power determination of sample and standard

Concentration (ug/ml)	Absorbance (nm) (Mean ± SEM) (n=3)		
	Ascorbic acid Standard	Methanolic extract	Aqueous extract
25	0.057±0.0098	0.060 ± 0.0003	0.048 ± 0.0003
50	0.079± 0.0003	0.068 ± 0.0003	0.062 ± 0.0003
75	0.097± 0.0003	0.092 ± 0.0008	0.068 ± 0.0008
100	0.102± 0.0003	0.100 ± 0.0008	0.078 ± 0.0008
125	0.117± 0.0003	0.102 ± 0.0008	0.093 ± 0.0012
150	0.131± 0.0003	0.104 ± 9.8130	0.094 ± 0.0006
175	0.136 ± 0.0003	0.111 ± 0.0003	0.107 ± 0.0006
200	0.142 ± 0.0003	0.120± 0.0012	0.118 ± 0.0003

Hydrogen peroxide scavenging activity

Scavenging of H_2O_2 by extracts may be attributed to their polyphenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The ability of plant extracts to effectively scavenge hydrogen peroxide. The plant extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner (Table No. 2). Although hydrogen peroxide itself is not very reactive, but sometimes it can cause

cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removal of H_2O_2 is very important living systems. The Hydrogen peroxide -derived IC_{50} values of plant extracts are also illustrated in Table 2. The methanolic and aqueous extract of plant inhibited Nitric oxide upto 80.02% and 50.70% at concentration 200ug/ml.. Amongst the plant extracts of *Sesbania grandiflora* methanolic extract was found to be the most potent Hydrogen peroxide scavengers, as they could inhibit Hydrogen peroxide free radicals up to 80.02 % at 200 ug/ml concentration compared to the aqueous extract whereas standard Ascorbic acid were shows 93.84% of Hydrogen peroxide scavenging activity.

Table 2 Absorbance of Hydrogen peroxide scavenging capacity of sample and standard

Concentration (ug/ml)	**Absorbance (nm) (Mean ± SEM) (n=3)**		
	Ascorbic acid Standard	**Aqueous extract**	**Methanolic extract**
25	0.820 ± 0.001	0.938 ± 0.0006	0.944 ± 0.0006
50	0.798 ± 0.0003	0.811 ± 0.0013	0.919 ± 0.0013
75	0.696 ± 0.001	0.735 ± 0.0013	0.880 ± 0.0013
100	0.604 ± 0.0006	0.648 ± 0.0006	0.852 ± 0.0006
125	0.567 ± 0.0003	0.593 ± 0.0006	0.806 ± 0.0016
150	0.428 ± 0.001	0.514 ± 0.0006	0.797 ± 0.001
175	0.333 ± 0.0003	0.479 ± 0.001	0.767 ± 0.0013
200	0.209± 0.001	0.400 ± 0.0006	0.750 ± 0.0013
IC_{50}	93.84	50.70	80.02

DPPH free radical scavenging assay

The DPPH assay is purely based on the assumption that an antioxidant serves as a hydrogen donor and thus reduces the DPPH free radicals (the color turns from purple to yellow). This assay is known as a basic and quick tool to carry out evaluation of antioxidant activity of plant extracts. The antioxidant potency of a compound is relative to loss of DPPH free radicals that can be quantified through a decrease in the maximum absorption of DPPH at 517 nm. In this study, results showed that all plant extracts had significant levels of radical scavenging activity in a dose dependent manner (Table No.3). The DPPH-derived IC_{50} values of plant extracts are also illustrated in Table No.3 The methanolic and aqueous extract of plant inhibited DPPH upto 87.13%, and 72.35% at concentration 200ug/ml. Amongst the plant extracts of *Sesbania grandiflora* methanolic extract *was* found to be the most potent DPPH scavengers, as they could inhibit DPPH free radicals up to 87.13% at 200ug/ml concentration compared to the aqueous extract where as standard Ascorbic acid were shows 94.29% of DPPH scavenging activity.

Table 3 DPPH free radical scavenging activity of extracts of plant *Sesbania grandiflora*

Concentration (ug/ml)	**Absorbance (Mean ± SEM) (n=3)**		
	Standard Ascorbic acid	**Methanolic extract**	**Aqueous Extract**
25	15.68±0.09	10.04±0.19	8.82±0.18
50	28.4±0.18	17.54±0.12	13.2±0.10
75	41.36±0.03	32.27±0.07	24.17±0.14
100	53.98±0.16	45.09±0.15	41.96±0.26
125	64.74±0.15	56.79±0.22	49.76±0.06
150	72.54±0.33	63.55±0.26	55.52±0.14
175	84.97±0.03	74.41±0.13	61.41±0.10
200	94.29±0.51	87.13±0.09	72.35±0.08
IC50	101.21	115.74	147.52

DISCUSSION AND CONCLUSION

To determine the best possible action of the drugs, it is highly essential to understand the phytochemical constituents of the plant. Antioxidants and its mechanism can be explained by redox reactions, wherever one reaction species diminished at the expense of the oxidation of the other, where reductants can inactivate the oxidants. Reducing power activities of *Sesbania grandiflora* extracts were significantly different and dose dependent; as the concentration of extracts increases, the reducing power also increases and exhibits the highest correlation with bioactive compounds [8, 4,2].

It was observed that the both methanolic and aqueous extract of plant *Sesbania grandiflora* demonstrated dose dependant increase in the reducing power. To find the active species which is capable of donating hydrogen and subsequently its leads to the reducing power activity was determined. The high reducing power is indicative of the hydrogen donating ability of the active species present in extract. In

the present study the reducing power of the methanolic extract of plant *Sesbania grandiflora* were found to be excellent and increase in direct proportional to the increasing concentration of extract as compared with aqueous extract and standard ascorbic acid. The reducing power of methanolic extract, aqueous extract and standard ascorbic acid at concentration 200µg/ml was found to be 0.142, 0.120, and o.118 respectively. [Table no.1]. The hydrogen peroxide scavenging capacity of methanolic extract of plant *Sesbania grandiflora* was found to be (80.02 %) at concentration 200 µg/ml. Similarly, for the aqueous extract were found to be (50.70 %) at concentration 200 µg/ml with reference to standard ascorbic acid 93.84 % at concentration 200 µg/ml [9-13].

From this study, it was found that methanolic extract of plant *Sesbania grandiflora* have potent antioxidant activity as compared to be aqueous extract of plant *Sesbania grandiflora* with reference to standard ascorbic acid. The results were shown in table no.1 and 2.

DPPH is a stable free radical which is a sensitive way to determine the antioxidant property of plant extracts. The DPPH method as antioxidant activity was evidently introduced nearly 50 years ago by Blois and is used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant potency. The parameter IC_{50} is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color). In this method also methanolic extract shows higher potency as compared to the aqueous extract [6-9].

Higher plants are act as a source of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times. Present study shows that plant may contain a polyphenolic compound which exhibits strong antioxidant activity compared to that of the reference compound. The results would help to determine the potency of the extract and plant as a potential source of natural antioxidants.

CONFLICTS OF INTEREST

None

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