Phytochemical Screening and *In-vitro* Evaluation of Antioxidant Activity and Antimicrobial Activity of the Leaves of *Sesbania sesban (L) Merr.*

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ABSTRACT

Introduction: Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions. *Sesbania sesban (L) Merr* is an ancient plant which is traditionally used as an antioxidant folklore plant. The present research deals with the phytochemical screening and in vitro evaluation of antioxidant activity of the leaves of *Sesbania sesban (L) Merr*. **Methods:** The ethanolic extract of the plant *Sesbania sesban (L) Merr* was subjected for the phytochemical screening. The preliminary screening reports the presence of Saponin, Tanin, Phenolic compound, Flavonoid in ethanolic extracts. DPPH scavenging activity or the Hydrogen donating capacity was quantified in presence of stable DPPH radical on the basis of Blois method. NO scavenging activity was performed in the presence of nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction according to the method of Marcocci. Ascorbic acid was used as standard for the both. **Results:** The scavenging was found to dose dependent. Thus extract has been established the as an antioxidant. The reducing capacity serves as significant indicator of antioxidant activity. The reducing power increased with the increasing concentration of sample. **Conclusion:** The folklore use of *Sesbania sesban (L)Merr* has been proved in present research work. Further studies along with isolation and molecular mechanism on extract of *Sesbania sesban (L) Merr* may lead to significant out come.

Key Words: *Sesbania sesban (L) Merr*, DPPH scavenging activity, NO scavenging activity, Ascorbic acid, Phytochemical screening

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INTRODUCTION

Oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others.[1-6]

Reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxynitrite radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases.[7,8] Antioxidant are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions.[9] Since ancient times, the medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities. The screening studies for antioxidant properties of medicinal and food plants have been performed increasingly for the last few decades in hope of finding an efficient remedy for several present-day diseases and means to delay aging symptoms.[10] Although several synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are commercially available, but are quite unsafe and their toxicity is a problem of concern. Hence strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Many other plant species have been investigated in the search for novel antioxidants,[11-14] but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive.

The present study deals the antioxidant activity of *Sesbania sesban (L) Merr* by in vitro studies.

PLANT PROFILE

Vernicular Names

Hindi: JAIT, JAYANTI
Bengali: JAYANTI
Marathi: SEVARI
Telugu: JALUGU

Description

It is a short-lived shrub or small tree up to 8 m tall. Its leaves are pinnately compound, 2-18 cm long with 6-27 pairs of linear oblong leaflets (26 × 5 mm). The raceme has 2-20 flowers which are yellow with purple or brown streaks on the corolla. Pods are sub cylindrical, straight or slightly curved up to 30 cm long and 5 mm wide containing 10-50 seeds. Five varieties of *S. sesban* are recognized botanically but their differences do not correlate strongly with their agricultural value.

Status

The origins of *S. sesban* are unclear but it is widely distributed and cultivated throughout tropical Africa and Asia.

Environmental Adaptation

S. sesban shows some cool tolerance. It grows well in the subtropics and is significant in extending the nitrogen fixing forage trees into cooler, higher elevation regions of the tropics up to 2,000 m.

MATERIALS AND METHODS

Collection and authentication of the plant

The whole plant of *Sesbania sesban* was collected from the field of Karipatti village, Salem, Tamilnadu. The plant was authenticated by Mr. A. Balasubramanian, Botanist, ABS Botanical Conservation, Research and Training Center, Karipatti village, Salem. (Dated 31.07.2008)

The plant material was dried in shade, coarsely powdered and passed through No.40 sieve and was used for the extraction.

Extraction

The powdered plant materials extracted with ethanol 95% v/v, (75-78 °C), until the extraction was completed. After completion of extraction, the solvent was removed by distillation. Dark brown colour residue was obtained. The residue was then stored in dessicator.

Preliminary Phytochemical Screening

All the extracts of *Sesbania sesban* were subjected to qualitative tests for the identification of various active constituents by different chemical tests.

The following active constituents have been identified from the tests Carbo hydrates, Glycosides, Proteins & Amino acids, Saponins, Tannins, Alkaloid, Phenolic compounds and Flavonoids.

The phytochemical constituents present in ethanolic extracts are presented in **Table 1.**

In vitro antioxidant properties

The ethanolic (50%) extract of seeds of *Sesbania sesban (L) Merr*. was used for the evaluation of antioxidant activity.

DPPH radical scavenging activity

DPPH scavenging activity or the Hydrogen donating capacity was quantified in presence of stable DPPH radical on the basis of Blois method.[15] Briefly, to a methanolic solution of DPPH (100 μM, 2.95 ml), 0.05 ml of test compounds dissolved in methanol was added at different concentration (20-100 μg/ml). Reaction mixture was shaken and absorbance was measured at 517nm at regular intervals of 30 seconds for 5 minutes, and the reading was taken till 20 min. Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract.

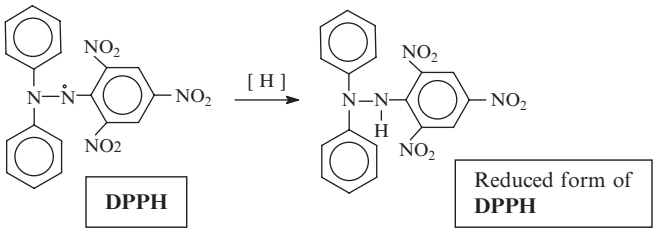
Table 1. Preliminary phytochemical screening of *Sesbania sesban (L)Merr.*

	Ethanolic extract of *Sesbania sesban (L) Merr.*
Carbo hydrates	+
Glycosides	+
Fixed Oils & Fats	–
Proteins & Amino acids	+
Saponins	+
Tannins	+
Phyto sterols	–
Alkaloids	+
Phenolic compounds	+
Flavonoids	+
Gums & Mucilage	–

+ = Presence , – = Absence

Table 2. Antioxidant activity of different extract of dried seeds of *Sesbania sesban (L) Merr.*

Concentration µg/ml	DPPH Scavenging% Ethanolic Extract	NO Scavenging% Ethanolic Extract
20	16.71	14.26
40	28.77	25.32
60	37.48	36.14
80	58.18	56.89
100	76.25	72.18
Ascorbic Acid (100 µM)	92.41	93.48



Reduction of 1,1, Diphenyl -2-Picryl hydrazyl – (DPPH) free radical

The capability to scavenge the DPPH radical was calculated using the following equation

$$\% \text{ DPPH Scavenging} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

The % scavenging activity has shown in **Table no. 2**

Nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction according to the method of Marcocci. The chemical source NO was sodium nitroprusside (5 mM) in 0.5 M phosphate buffer, PH 7.4, spontaneously generates nitric oxide in aqueous solution**.** Nitric oxide interacts with oxygen to produce stable products, leading to the production of nitrites.[16]

About 1 ml sodium nitroprusside (5 mM) in 0.5 M phosphate buffer was mixed with 3.0 ml of different concentrations (20 – 100 µg/ml) of the drugs dissolved in the suitable solvent systems and incubated at 25 °C for 150 min.

Ascorbic acid was used as standard.

The capability to scavenge the NO radical was calculated using the following equation:

$$\% \text{ NO Scavenging} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

DPPH is stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, then losing colour stoichometrically with the number of electrons consumed, which is measured spectrophotometricallty at 517 nm. As shown in **Table 2**, Sesbania sesban(L) Merr. extract strongly scavenged DPPH radical. The Change in colour of $DPPH^-$ is directly proportional to the amount of antioxidant present in the reaction mixture (antioxidant react with stable free radical i.e DPPH) and the 100 µg/ml Ethanolic extract was found the active free radical scavenging activity increase from 16.71 % (20 µg/ml) to 76.25 % (100 µg/ml). The Change in colour of Sodium Nitropruside is directly proportional to the amount of antioxidant present in the reaction mixture (antioxidant react with stable free radical i.e NO) and the 100 µg/ml ethanolic extract was found (**Table 2)** the active free radical scavenging activity increase from 14.26 % (20 µg/ml) to 72.18 % (100 µg/ml). The scavenging was found to dose dependent. Thus extract has been established as an antioxidant.

CONCLUSION

The folklore use of *Sesbania sesban (L)Merr* has been proved in present research work. The reducing capacity serves as significant indicator of antioxidant activity. The reducing power increased with the increasing concentration of sample. The antioxidant activity of the plant is attributed to the presence of saponins and flavonoids, for which the modern research to isolate the compounds, should be governed.

Further phytochemical and pharmacological studies along with isolation and molecular mechanism on extract of *Sesbania sesban (L) Merr* may lead to significant out come.

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