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RESEARCH ARTICLE

In Vitro Evaluation of Antioxidant and Hepatoprotective Potential of Sesbania grandiflora Linn. Leaf Extract

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Abstract: Sesbania grandiflora Linn., commonly known as Agathi, is a traditional Indian medicinal plant belonging to the family Fabaceae. It is widely cultivated in southern and western parts of India, particularly in the Ganga valley and Bengal regions. Traditionally, S. grandiflora has been used in the management of colic disorders, jaundice, smallpox, fever, leprosy, night blindness, and gout. Although various parts of this plant have demonstrated hepatoprotective properties, the in vitro hepatoprotective activity of the ethanolic leaf extract has not been previously explored. The present study aimed to evaluate the phytochemical constituents, in vitro antioxidant, and hepatoprotective potential of S. grandiflora leaf extract. The leaves were collected from Tambaram, Tamil Nadu, and authenticated. Hepatotoxicity was induced using acetaminophen in Chang liver cell lines, and the protective effect of the extract was assessed through the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. Phytochemical screening revealed the presence of flavonoids, saponins, tannins, and steroids. The in vitro antioxidant activity was confirmed by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay. The results indicated that the ethanolic leaf extract of S. grandiflora exhibited a dose-dependent hepatoprotective effect in Chang liver cells. Overall, the findings suggest that Sesbania grandiflora leaves possess significant antioxidant and hepatoprotective potential, supporting their traditional use in managing hepatic disorders.

Keywords: Antioxidant, Agathi, sesbania, acetaminophene, Hepatoprotectiv

INTRODUCTION

Nature has provided an excellent storehouse of remedies to cure all the ailments of mankind. Plants have been used for healing and treatment of disease from time immemorial. Even when the modes of medicine have changed from time to time, plants continue to be the main stay of medicine. India is considered to be the emporium of herbal drug for the prevention and treatment of disease[1].The plant *Sesbania grandiflora* (L.) Poir (Fabaceae) is a short lived and fast growing tree, native of India and Malaysia. In India it is present in Punjab, Dehli, Bengal, Assam and Tamil nadu. *Sesbania grandiflora*, commonly known as "Agathi," is used in Indian traditional medicine for the treatment of several diseases. Different parts of *Sesbania grandifolia* Linn was used as folk medicine for various purposes. The roots were used in rheumatism, inflammation and painful swelling, bark as astringent, in snake bites, small pox and also as antipyretic and anthelmintic agent[2]. Leaves were used as tonic, antipyretic, leprosy, night blindness and gout[3]. It is useful in ophthalmia and in poultice for bruise. Flowers were used in eyes to relive dimness of vision and to improve appetite. Fruits were used as laxative, cures fever, pain, anemia, and also to improve taste and thirst[4].

Various research have been done in the different parts of the *Sesbania grandiflora* and has shown anti inflammatory, anti-arthritic[5,] antiulcer[6,7] ,analgesic, antidiarrheal, antibacterial ,antifungal activity[8], antihelmintic[9], hypolipedmic[10,11], diuretic, CNS depressant and laxative[12] actions. But there are very few reports on the Hepatoprotective activity on *Sesbania grandiflora.* Despite the large number of compounds available in the market there is still a need to identify potent leads with chemotherapeutic significance.

MATERIAL AND METHODS

Plant collection and authentication:

Sesbania grandiflora leaves were collected around Tambaram, India during April. The plant material was identified and authenticated by Prof. P Jayaraman, Plant Anatomy Research Centre, Chennai, Tamil nadu.(Reg.No: PARC/2010/577). The leaves were washed with double distilled water to remove dirt and then shade dried. The dried leaves were powdered.

Extraction :

The powdered materials of *Sesbania grandiflora* was extracted with 70% ethanol by Soxhlation. The extracts were filtered and concentrated using rotary flask evaporator and dried over hot water bath.

Cell lines and growth media

Chang liver cells (normal human liver cells) were purchased and cultured in Dulbecco's modified eagles medium (DMEM) supplemented with fetal bovine serum (10%), penicillin G (100 IU/ml) and streptomycin (100 µg/ml). Cells were maintained at a temperature of 37°C in a 5% CO2 atmosphere with 95% humidity.

Qualitative Phytochemical Analysis:
The individual extract was subjected to the qualitative phytochemical screening for the presence of some chemical constituents. Phytochemical test were carried out adopting standards procedure.

Test for Tannins: 4ml extract was treated with 4 ml $FeCl_3$ formation of green colour indicates that presence of condensed tannin.

Test for Saponin: 5 ml extract was mixed with 20 ml of distilled water then agitated in graduated cylinder, formation of foam within 15 minutes indicates saponin.
Test for Alkaloids: 3 ml of concentrated extract was taken into a test tube and 1 ml HCl was added to the mixture was heated gently for 20 min cooled and filter, the filtrate was used for following test.

a) Wagner test: 1ml of the extract was treated with Wagner's reagent; formation of brown reddish precipitate indicates presence of alkaloids.
b) Dragen droff's test: 2 drops of Dragen droff's reagent were added to 1ml of the extract. The development of a creamy precipitate was indicative of the presence of alkaloids.
c) Hager's test: 1ml of the extract was treated with Hager's reagent, presence of alkaloids confirmed by the yellow colored precipitate.
Test for Flavonoid:
a) Alkaline reagent test: Extract was treated with 10 % NaOH solution, formation of intense yellow colour indicates presence of Flavonoid.
b) Mg turning test: Extract were treated with Mg turning and add conc. HCl to this solution add 5ml of 95 % ethanol, formation of crimson red colour indicates
Test for Anthraquinone: 5ml of Extract was hydrolyzed with dilute H_2SO_4, then add 1ml of benzene and 1ml of NH3, formation of Rose Pink coloration suggest anthraquinone
Test for Steroid: 1ml extract was dissolved in 10 ml of chloroform & equal volume of concentrated H_2SO_4 acid was added from the side of test tube .The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence .This indicates the presence of steroid.

Antioxidant assay by DPPH method
The DPPH radical scavenging assay was first described by Blois (1958) and this was modified later by many researchers. The free radical scavenging activities of ethanolic extracts and the standard L-ascorbic acid (vitamin C) were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH). DPPH, being a stable free radical it is frequently used to determine radical scavenging activity of natural compounds. DPPH(radical form) absorbs at 517 nm, but upon reduction with an antioxidant, its absorption decreases. It is due to the formation of its non-radical form, DPPH–H.

DPPH **Antioxidant** DPPH–H

The reaction mixture contained 2 mL of 0.1 mol/L DPPH in ethanol and 2 mL of the serial dilution of extract at the concentration of 50, 75, 100, 125, 150 and 200. The mixture was incubated at 25 °C for 15 min, and the absorbance was determined at 517 nm. Distilled water was used as the control and L-Ascorbic acid was used as standard antioxidant. The scavenging activity of DPPH radicals in samples was calculated according to the following equation:
Percentage antioxidant activity = (absorbance at blank) – (absorbance at test) × 100
(absorbance at blank)

***In-vitro* cytotoxicity assay:**
The Cytotoxicity of ethanolic extract of *sesbania grandifolia* leaves on Chang Liver cells was determined by the MTT assay (*Mosmann* 1983). It is based on the concept that dead cells and their products do not reduce tetrazolium. The number of viable cells is proportion to the extent of formazan formation.

Procedure
- The monolayer cell culture was trypsinized and cell count was adjusted to $1x10^5$ cells/ml.
- To each well of the 96-well tissue plate , 0.1 ml diluted suspension was added and incubated for 24 hr.
- The supernatant was removed and the monolayer was washed once. 100µl of different concentration of extract was added to the well plate and incubated at 37°C for 72 hours in 5% CO_2. The plant extract in the wells were discarded.
- Add 50 µL MTT solution (5mg/ml) were added to each well and Incubated at 37°C for 3 hours.
- The supernatant was removed. Add 50µL propanol and the plates were gently shaken(to dissolve the formazan salt)
- The absorbance was measured at 540 nm.

The effect of the samples on the proliferation of Chang Liver cells was expressed as the % cell viability, using the following formula:
% cell viability = Mean OD of TEST group / Mean OD of control group × 100

***In-vitro* hepatoprotective activity against Acetaminophen induced toxicity in Chang liver cells by MTT assay**
Chang liver cells (1x105cells/well) were maintained in culture media containing 125 µg/ml of acetaminophen, in the presence of Ethanol extract of *Sesbania grandifolia leaves* at the concentrations of 100, 50, 25, 10 µg/ml for 24hrs. Later, supernatants from each well were removed and the % of cell viability was calculated.
- The monolayer cell culture was trypsinized and cell count was adjusted to $1x10^5$ cells/ml.

- To each well of the 96-well plate , 0.1 ml diluted suspension was added and incubated for 24 hr.
- The supernatant was removed and the monolayer was washed once. 100µl of different concentration of extract was added to the well plate and incubated 24 hours.

- After 24 hours pretreatment with the extract, 100 µl of Acetaminophen ( ) was added to well and incubated at 37°C for 72 hours in 5% CO_2..The supernatant was discarded.
- Add 50 µL MTT solution (5mg/ml) were added to each well and Incubated at 37°C for 3 hours.
- The supernatant was removed. Add 50µL propanol and the plates were gently shaken(to dissolve the formazan salt)
- The absorbance was measured at 540 nm.

% Growth inhibiton = 100 - Mean OD of individual Test group X 100

Mean OD of control group

Statistical analysis

Data are expressed as mean±SEM. Mean difference between groups were analysed by student T test. p value <0.001

RESULTS AND OBSERVATIONS:

The preliminary phytochemical screening of *sesbania grandifolia* leaf has shown the presence of saponins, flavonoids, tannins, alkaloids and steroids.The % inhibition of DPPH free radical by different concentration of the standard ascorbic acid and methanol extract of the plants were depicted in table 1.The IC50 values of Ascorbic acid and ethanolic extract of *Sesbania grandifolia* leaf was found to be **131.58 µg/ml.**

Table1: DPPH assay

Conc.in **(µg/ml)**	% of antioxidant activity
50	26.62±0.21
75	34.43±0.36
100	42.62±0.29
125	48.08±0.12
150	55.37±0.22
200	64.32±0.16

***Table 2: In vitro* Acetaminophen induced hepatotoxicity in Chang liver cells**

S.No	**Treatment**	**Concentration (µg/ml)**	**% Cell Viability**
1	**Control**	-	-
2	**Acetaminophen**	**125**	**39.92**
3	**Acetaminophen + Sesbania ethanolic leaf extract**	**100**	**71.3**
		50	**65.8**
		25	**60.4**

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DISCUSSION

Hepatic system of an organism is involved in metabolic activities of it. In this process it may exposed to various challenges and hence, hepatic system is not only evolved to perform its function but also to protect itself to various challenges like exposure to antibiotics, chemicals etc. Liver is such an organ that its physiological role and its self protective mechanism are well developed and orchestrated. Inspite of such balanced internal milieu, hepatic aberration, damage and necrosis commonly occurring due to over exposure to hepatotoxic causes to such an extent that it over powers the mechanism. However there are several herbs and herbal formulation which are found to be claimed for treating hepatic disorders.

In the present study the extract was subjected phytochemical screening, it was observed that the extract has shown a marked presence of flavonoids, tannins and steroids. Hepatoprotective activity was associated with antioxidant activity, since it is free radical mediated damage. The probable mechanism by which Sesbania grandiflora (Linn) exerted its protective action might be due to the stimulation of hepatic regeneration through an improved synthesis of proteins, or with interference with the liberation of microsomal activation to toxicants.

Flavonoids and tannins were reported to possesses various of pharmacological activity including hepatoprotective activity. In the present investigation, preliminary phytochemical study on Sesbania grandiflora (Linn) gave positive tests for flavonoids and tannins. This could be the reason for the dose dependent hepatoprotective property of the leaf extract.

CONCLUSION

The present investigation revealed that the ethanolic extract of leaves of Sesbania grandiflora Linn exerted dose dependent protection against hepatotoxicity. The extract has also shown good in-vitro cytoprotective activity. Our results indicated that the potent hepatoprotective activity of the extract may be due to its free radical scavenging properties which may be due to presence of flavonoids in the plant. Further investigation has to be done to identify the active constituent responsible for the activity and to evaluate the in vivo hepatoprotective property using animal models. Also the underlying mechanism of action contributing to the hepatoprotective activity has to be enumerated and validated.

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