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Pharmacognostical and Phytochemical Screening of Sesbania grandiflora

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Abstrat
A perusal of the literature reveals that the following uses have been reported from the various parts of the plant, they are laxative, diuretic, emetic, emmenagogue, febrifuge and tonic, agati is a folk remedy for bruises, catarrh, dysentery, eyes, fevers, headaches, smallpox, sores, sore throat, and stomatitis (Duke and Wain, 1981). Bark, leaves, gums and flowers are considered medicinal. The astringent bark was used in treating smallpox and other eruptive fevers. The juice from the flowers is used to treat headache, head congestion, or stuffy nose. As a snuff, the juice is supposed to clear the nasal sinuses. Leaves are poulticed onto bruises. Prescribe them for anemia, bronchitis, fever, pain, thirst, and tumors; the flowers, aperitif and refrigerant, for biliousness, bronchitis, gout, nyctalopia. Leaves, alexeteric, anthelmintic, for epilepsy, gout, itch, leprosy, nyctalopia, and ophthalmia. Yunani consider the tonic leaves useful in biliousness, fever, and nyctalopia. Leaf juice to cleanse the mouth and throat. Bark is applied to scabies. Leaves are chewed to disinfect the mouth and throat. Generally phyto constituents leaves contain saponin, oleanolic acid, galactose, Rhamnose, glucouronic acid seed contain saponin kaemferol-3,7, diglucoside, Leuco cyanidin and Cyanidin-3 glucoside. The literature survey revealed that no study has been performed on the pharmacognosy of the plant and phytochemical aspects and antimicrobial activity and anti-oxidant activity and Nutrient values of the leaves of Sesbania grandiflora (c) pers. Hence the present work is aimed at presenting a complete picture of the plant drug (leaves) from the pharmacognostical, preliminary phytochemical and pharmacological aspects.

Key words
Sesbania grandiflora, Anti-microbial activity of herbs, Anti-oxidant activity of herbs, Pharmacognostical and Phytochemical Screening

Introduction

The use of medicinal herbs is as old as 1500 BC in India. Underlying the medical culture of India both folk traditions as well as codified knowledge systems is a deep understanding of the medicinal value of the plants starting with the references in the Atharva veda, we have textual evidence of a tradition of use of medicinal plants that is more than three thousand years old. It is estimated that about 80,000 species of plants are utilized by the different systems of Indian medicine. The indigenous knowledge about plants and plant products is rather detailed and sophisticated and has evolved into a separate shastra (branch of learning) itself, called Dravya Guna Shastra. The codified traditions have about 25,000 plant drug formulations that have emerged from such studies. In addition to this over50,000 formulations are believed to be existing in the folk and tribal traditions. All these point to the deep passion for and exhaustive knowledge about medicinal plants that have existed in the land from time immemorial. The Vedas, epic poems contain rich material on the herbal lore of that time. These medicinal herbs become popular due to single active constituents. Keeping in mind the overall scenario, only selected medicinal herbs have been used in standardized from and market analysis clearly favours them.

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Vernacular Names:

Sanskrit	:	Agasti
Hindi	:	Bosna
Bengali	:	Bak
Tamil	:	Agati
Telugu	:	Agise
Malayalam	:	Akatti

Biological Source:

Leaves of Sesbaria Gradiflora (L) Pers.

Family	:	Fabaceae (Leguminosae)
Synonyms	:	Robinia Grandiflora L
		Agati Grandiflora (L) Desv.

GEOGRAPHICAL DISTRIBUTION :

Sesbania grandiflora is found in plains to 500 m. It is often planted as prop for piper betel..
it is distributed in tropical Africa, Asia, Indonesia and India.

SPECIES :

The Genus Sesbania scop. Includes 50 species which are distributed in warm and wet regions. Some of the species are cultivated and ornamental. In India, the following species have been reported.

S.bispinosa (S. esculenta)
S.gradiflora
S.sesban (S.aegyptiaea)
S.speciosa

CHEMICAL CONSTITUENTS :

Saponin, oleanolic acid, galactose, Rhamnose, glucouronic acid seed contain saponin kaemferol-3,7, diglucoside, Leuco cyanidin and Cyanidin-3 glucoside delphinidin glycosides & flavonols, seeds contain galactomannan and fattyacids bioflavonoids, isorhamnetin from the seeds of Sesbaria Gradiflora (L) Pers. Chromatographically pure saponin ester mixture.

NUTRIENT VALUES :

The leaf is reported to contain 73.1g H2O, 8.4 g protein, 1.4 g fat, 11.8g NFE, 2.2g fiber, 1,130 Ca, 80 mg.P, 3.9mg Fe, 9,000 IU vit.A, 0.21mg thiamine, 0.09 mg riboflavin, 1.2mg niacin, and 169 ascorbic acid. Leaves contain per 100g, 321 calories, 36.3g protein, 7.5 fat, 47.1 g carbohydrate, 9.2g fiber. 9.2g ash, 1684 mg Ca, 258 mg P, 21 mg Na, 2,005 mg K, 25,679 µg β-carotene, 242 mg ascorbic acid.

USES :

A perusal of the literature reveals that the following uses have been reported from the various parts of the plant, they are laxative, diuretic, emetic, emmenagogue, febrifuge and tonic, agati is a folk remedy for bruises, catarrh, dysentery, eyes, fevers, headaches, smallpox, sores, sorethroat, and stomatitis (Duke and Wain, 1981). Bark, leaves, gums and flowers are considered medicinal. The astringent bark was used in treating smallpox and other eruptive fevers.

- Studies on Erythrocyte membrane. In vitro haemolytic effect of sesbania grandiflora leaves [5]
- Sesbania mosaic, a new virus disease [7]
- Invitro haemolytic effect of the flowers of Ses bania grandiflora [9]
- S.grandifl;ora more crude protein [12]
- Nutritional value of Sesbania grandiflora leaves for monogastrics and ruminants [18]
- Anti inflammatory activity of S.grandiflora [21]
- Analgesic and anti pyretic activity of flowers of S.grandiflora [23]
- Anxiolytic & anti convulsive activity of S.gran diflora leaves in experimental animals [25]
- Beneficial effect on improved vision & preven tion of the disease [27]
- Determination of major carotenoids in a few Indian leafy vegetables by HPLC [28]
- Effect of S.grandiflora against cigarette smoke induced hyperlipidemia in rats [31]

3.2 MACROSCOPIC FEATURES

- The Plant grows up to 10m in height;the leav anchlets are pubescent.
- The leaves are unipinnate and even pinnate;
- Leaflets are 25-30 pairs, oblong, 1-3.5 cm long and 0.5 – 1 cm broad.
- Apex of the leaflets emarguiate, margins smooth.
- Rachis (petiole) 2 cm long; petiolule 2 mm long.
- Stipules lanceolate; 1 cm long;
- Inflorescence is 3-5 flowered raceme.
- Flowers 10 cm long, 5 cm broad.
- Calyx tube 5 lobed;
- Corolla Cream colured.
- Stamens (9)+1, Seeds Oblong, estrophiolate.

Fig. 1. Pinnately compound leaves seen in Sesbania Grandiflora (L) Pers.

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3.3 Pharmacognostical work:
Materials and Method for Studies

a) Collection of Specimen.

The plant specimens for the proposed study were collected from Sesbania grandiflora (L) Pers. Care was taken to select healthy plants and for normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Farmalin-5ml + Acetic acid – 5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual additional of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

b) Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Mictotome. The thickness of the sections was 10-12 µm. dewaxing of the sections was by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue as per the method published by O'Brien et al. (1964). Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast-green IKI (for Starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal section (section taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured.

c) Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books (Esau, 1964).

►

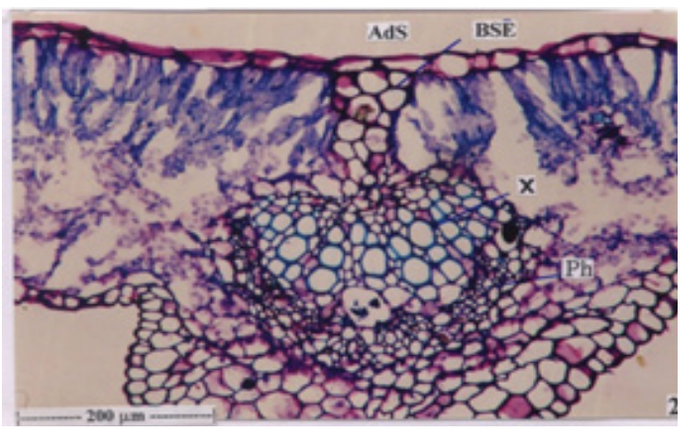
MICROSCOPIC FEATURES

Leaflets are dorsiventral with fairly disfuict adaxial and abaxial differentiation and prominent midrile (fig. 2.1.2)

Midrib: Midrib is planoconvex is sectional view with flat adaxial side and broadly conical abaxial sixe it is 450 µm thick in vertical plane 400 µm wide in horizontal plane. It has a district epidermal layer of small circular thick walled cells measuring 20 µm thick. The ground tissue of the abaxial part is homogeneous and parencymatous, the cells are polygonal and compact.

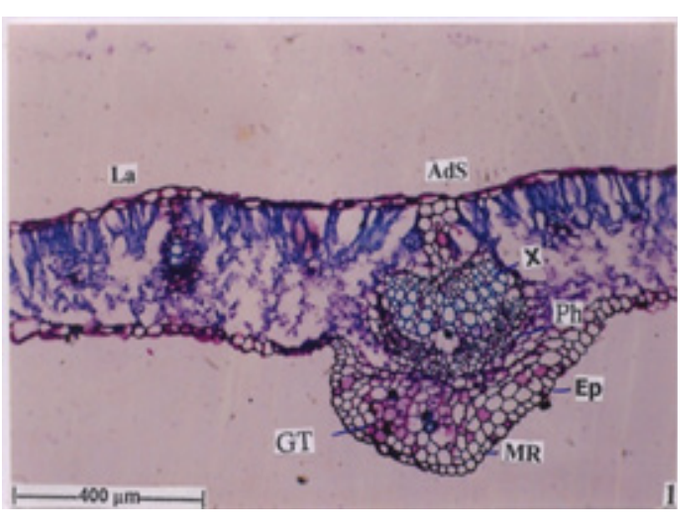
The vascular ground is single, prominent and broadly arc shaped. It consists of several radial parallel rows of xylem elements and thick bowl shaped bared of phloem. The xylem elements are thick walled wide angular metaxylem elements are nearly 30 µm wide. Phloem hand is 80 µm wide. On the adaxial side of the vascular bundle these is a vertical pillar of parenchymatous extension (fig. 2.2). The bundle sheath extension is 140 µm height and 80 µm in thickness.

Fig. 2.2 TS of midrib – enlarged.



Lamina (Fig. 2.1): The lamina is bifacial with smooth and even surfaces. It is 200 µm thick. The adaxial and abaxial epidermal layers are equally thick, comprising of circular to elliptical cells with prominent article. The mesophyll tissue is differentiated into a narrow zone of palisade cells and wide zone of spongy parenchyma. The palisade zone is 60 µm in height, the cells are cylindrical and loosely arranged the spongy mesophyll consists of 8-10 layers of small lobed cells farming reticulate and wide air chambers.

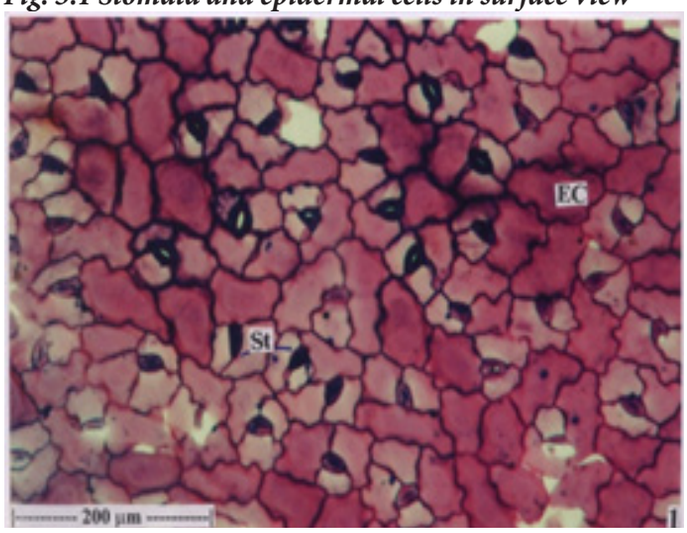
Fig. 2.1 TS of leaflet through midrib and lamina.

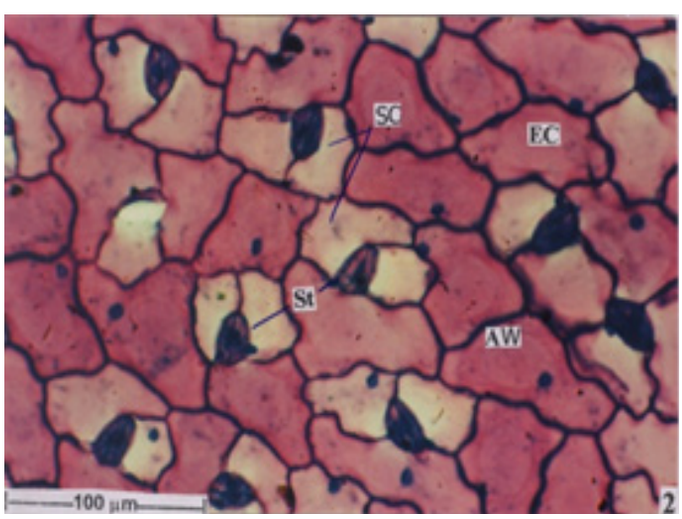


Epidermal cells and stomata (Fig. 3.1)

The adaxial and abaxial epidermal layers are stomatiferous. The stomata are similar on both sides both in morpholoty and frequency. The stomata are predominantly anisocytic one small and two large subsidiary cells. Some of the stomata are also paracylic having two weigshaped subsidiary cells (fig. 3.2). the guard cells are elliptic measuring 20x12 µm in size. Stomatal number is 140/mm2. The epidermal cells are fairly large, the anticlinical walls are slightly wavy and thick.

Fig. 3.1 Stomata and epidermal cells in surface view





Venation Pattern (fig. 4.1)

The Lateral view of fairly thick and the veinlets are thin and profusely branched (fig. 4.1). The vein islets are distinct; they are polygonal ranging from squarish to hexagonal in outline. Most of the larger vein islets have district vein terminations; the smaller islets lack distinct vein terminations. The venilets along the leaf margins are interconnected with each other forming what is known as intramarginal venations (fig. 4.2)

Fig. 4.1 Venation pattern under low magnification

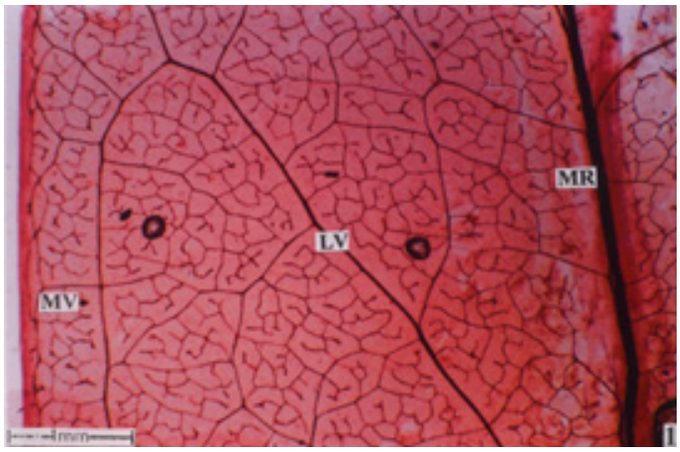
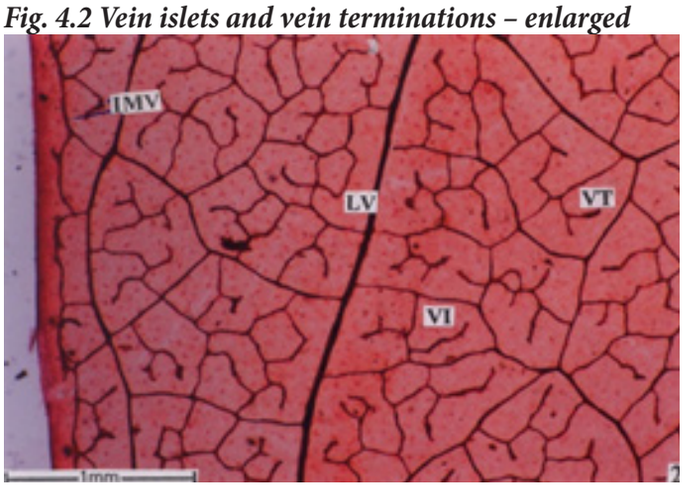


Fig. 4.2 Vein islets and vein terminations – enlarged



Petiolule : (Stack of the leaflet) :

Thepetiolule is circular in outline with irregular ridges and furrows (fig. 5.1). it has prominent epidermal layer comprising of thick walled cells with hemispeherical outer walls. The ground tissue is homogeneous and parenchymators, the cells are circular, darkly staining and compact (fig. 5.1). the vascular tissues occur in deeply curved outline of an arc. Xylem elements are arranged in parallel rows, each row comprising of 2 to 5 vessels. The Xylem elements are thick walled angular and lignified. Phloem is in a sheath along the outer part of the xylem are. The petiolule is 700 µm in diameter.

Fig. 5.1 TS of petiolule, showing gross sectional outline

Petiole : (fig. 5.2).

In cross sectional view, the petiole appears more or less in tetragonal outline with two adaxial thick, short winglike humps. It is 1.75 mm in vertical plane and 1.55 mm in horizontal plane. It has a distinct and continuous epidermal layer of small squarish cells (fig. 6.1). The outer ground tissue consists of circular to angular, then walled compact parenchyna cells in 5 to 8 layers. The central ground tissue has large, angular, polyhedral compact parenclyma cells. The vascular tissue occur in a wide closed cylinder in which there is a large median abaxial vascular bundle. Flanked on either side by small or bundle. These two larger lateral bundles and one wide, more prominent adaxial bundle all the bundles are collateral with patallel files of thin walled angular xylem elements. Phloem occurs in small arcs outside the xylem mass. A thin continuous sclerenchyma cylinder encircles all the vascular bundles (fig. 5.2, 6.2).

Fig. 5.2 TS of petiole, showing outline and vascular pattern

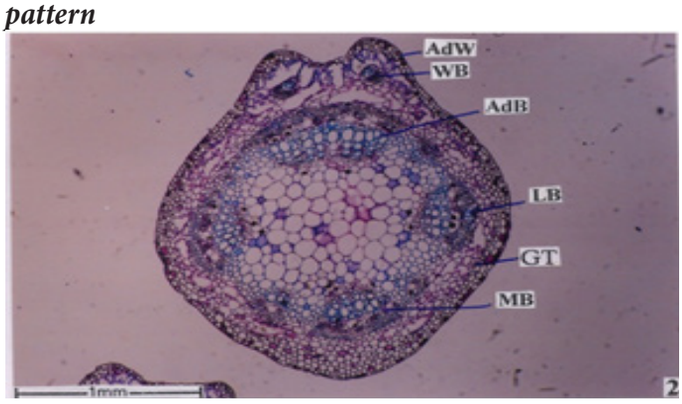
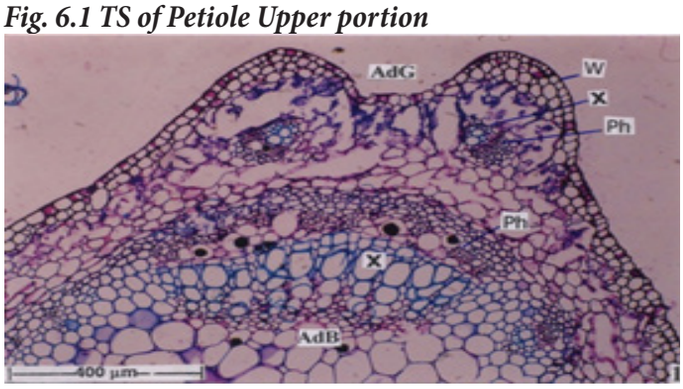


Fig. 6.1 TS of Petiole Upper portion



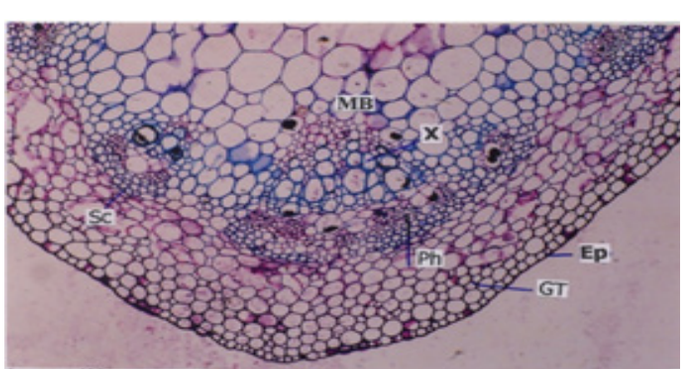


Fig. 6.2 TS of Petiole Lower portion

3.4 Powder Microscopic Observation (fig. 7.1)

The powder preparation of the leaf shows small fragments of the apidermal tissue. These pieces exhibit abundant stomata which are either anisocytic or paracytic type. The epidermal cells are highly lobed and amoeboid in shape; they have thick anticlinical walls, which are madulate no. of stomata per mm2 is about 138; the guard cells are Elliptic and measure 20 µm long and 15 µm wide. Epidermal trichomes are absent cuticular striations are not evident.

Fig. 7.1 Powder Mycroscopy Fragments of epidermis as seen in the powder

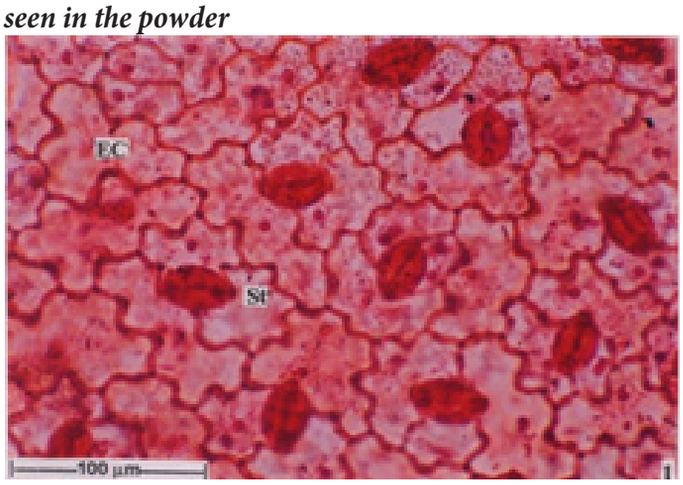
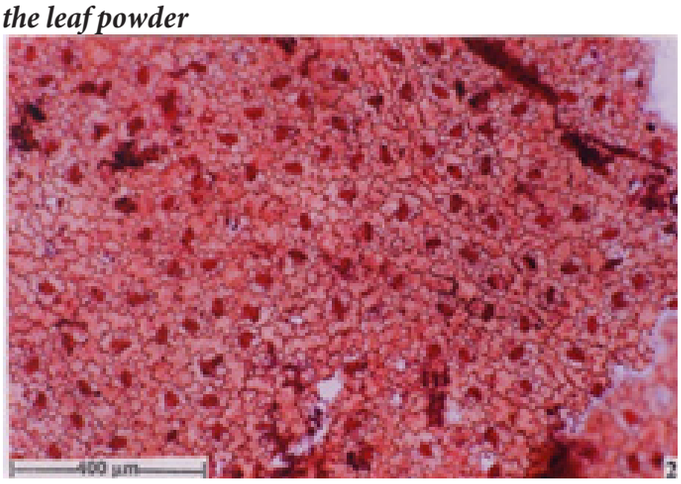


Fig. 7.2 Powder Mycroscopy Stomatal distribution in the leaf powder



Physiochemical Standards

METHODOLOGY OF PLANT ANALYSIS

Collection and processing :

The fresh leaves of Sesbania grandiflora (L) Pers viscose was collected and cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender and sieved to get uniform particles by using sieve No 60. Then the final uniform powder was used for the extraction of active constituents of the plant.

Physiochemical Standards

1) Ash Value :

The ash content of the crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may include inorganic matter added for the purpose of adulteration. Ash value varies with narrow limits in case of the individual drug but varies considerably in case of different drugs.

(a) Determination of Total Ash:

About 3 g of powdered drug was accurately weighed in a silica crucible, which was previously ignited and weighed. The powdered drug was spread as a fine layer on the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450° C until free from carbon. The crucible was cooled and weighed. The procedure was repeated to the constant weight. The percentage of total ash was calculated with reference to the air-dried drug. The ash value of the 10 leaf samples was calculated and tabulated in the table 3.5.1

(b) Determination of water-soluble ash:

The ash obtained in the determination of total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a tared silica crucible and ignited for 15 minutes at a temperature not exceeding 450° C. The procedure was repeated to get the constant weight. The weight of the insoluble matter was subtracted from the weight of total ash. The difference in weight was considered as the water -soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug and tabulated in the table 3.5.1

(c) Determination of Acid insoluble ash:

The ash obtained as described in the determination of total ash was boiled with 25 ml of hydrochloric acid for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water; the insoluble ash was transferred into pre-weighed silica crucible. The procedure was repeated to get constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug and tabulated in the table 3.5.1

2) Solubility Value

Solubility values of crude drugs are useful for their evaluation especially when the constituents of a drug cannot be readily estimated by any other means. Fur-

ther, these values indicate the nature of the constituents present in a crude drug.

i) Ethanol soluble extractive method:

4g of the each air-dried coarse powder of 10 leaf samples were macerated with 100 ml 90% of ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing to stand for 18 hours. It was then filtered rapidly taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow extractive with reference to the air-dried drug was calculated and tabulated in the table 3.5.1.

ii) Water soluble extractive method :

4g of the each air-dried coarse power of 10 leaf samples were macerated with 100 ml of Chloroform water (95ml of water + 5ml of Chloroform) in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowed to stand for 18 hours. It was then filtered rapidly taking precautions against loss of the solvent. 25ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish dried at 1050C and weighed. The percentage of water-soluble extractive with reference to the air-dried drug was calculated and tabulated in the table 3.5.1.

3) Extractive Value:

Purification of Solvents:

The solvents obtained commercially (LR – Grade Extra pure) were purified by distillation methods prior to use for extraction and for phytochemical investigation. Required quantity of grounds powder was weighed and then it was extracted with various solvents of varying polarity [Eg. N-hexane, Water, Ethyl acetate, Ethanol] by using Cold maceration technique or by using continuous hot percolation method (Soxhlet method).

PREPARATION OF EXTRACTS :

Hot maceration method using Soxhlet apparatus :

Freshly collected plant material (leaves) was dried in shade, and then coarsely powdered in a blender. The coarse power (1 kg) was extracted successively with (a) N-Hexane b) water c) Ethanol d) Ethyl acetate, each 2500 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Watman No. 41 filter paper and evaporated on a water bath and finally dried in vacuum. The residues obtained were used for screening the phytochemical and antibacterial and antioxidant properties.

The final residue thus obtained was then subjected to preliminary phytochemical tests for identification of the individual components (active principles) that are present in the plant under study. The extractive values for various solvents were tabulated in the table 3.5.1.

PHYSICO – CHEMICAL CHARACTERISTICS OF SESBANIA GRANDIFLORA (L.) PERS

Table 3.5.1

S. No.	Parameter	Lead Value (Percentage w/w)
1.	Total Ash	18.631%
2.	Acid Insoluble Ash	9.28%
3.	Water Soluble Ash	11.296%
4.	Sulphated Ash	7.36%
5.	Loss on Drying	9.23%
6.	SOLUBILITY	
	a) Ethanol	6.728%
	b) Water	4.761%
7.	Extractive Value	
	a) N-hexane	6.8 gms.
	b) Water	7.2 gms.
	c) Ethanol	5.9 gms.
	d) Ethyl Acetate	6.3 gms.

3)Fluorescence Analysis

Fluorescence analysis of the powder / solution of 10 leaf samples were observed in day/visible light and UV light (Long wavelength – 365 nm and Short wave length – 265 nm).

Method :

The drug powder was treated with neutral solvents like benzene, chloroform, ethyl acetate and 50% aqueous-ethanol and acids like 1M Hydrochloric acid and alkaline solutions like sodium hydroxide solution. They were subjected to fluorescence analysis in daylight and in UV-light. The results were tabulated in the table 3.5.2.

PHYTOCHEMICAL SCREENING OF SESBANIA GRANDIFLORA (L.) PERS

4.1 INTRODUCTION:

Plants posses a wide variety of chemical compounds and are classified as primary metabolites and secondary metabolites. Primary metabolites are substances widely distributed in nature and most of them are occurring in one form or the other virtually in all organisms and perform the basic cell metabolism; example includes starch, cellulose, carbohydrates etc. Secondary metabolites perform no apparent function in plants, primary metabolism but often have an ecological role; example includes alkaloids, steroids, terpenoids, flavonoids, quinoids, iridoids, coumarins, tannins, phenols and their glycoside. Secondary metabolites contrast to primary metabolites tends to be synthesized in specialized cell type and distinct developmental stages making their extraction purification difficult. As a result, secondary metabolites after isolation, structure elucidation and screened for activity are used commercially as biologically active compounds known as phytopharmaceuticals.

Chemical evaluation comprises of different chemical tests and chemical assays. The isolation, purification and identification of active constituents are chemical method of evaluation. Preliminary phytochemical screening is a part of chemical evaluation. The qualitative chemical tests are useful in detection of adulteration.

The systematic investigations of plant material for its phytochemical behavior involve four different stages.

a) The procurement of raw material and quality control.

b) Extraction, purification and characterization of the constituents of pharmaceutical interest in process quality control.

c) Investigation of biosynthetic pathways to particular compounds.

d) Quantitative evaluation.

4.2 Preliminary Phytochemical Analysis

The chemical tests for various phytoconstituents in the ethanolic extract and water extract were carried out as described below.

(a) Test for alkaloids

i) DragendorfPs Test - In a test tube containing 1ml of extract, few drops of Dragendorff s reagent was added and the colour developed was noticed. Appearance of orange colour indicates the presence of alkaloids.

ii) Wagner's Test - To the powder, two ml of Wagner's reagent was added, the formation of a reddish brown precipitate indicated the presence of alkaloids.

iii) Mayer's Test - To the powder, two ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

iv) Hager's Test - To the powder, 3 ml of Hager's reagent was added, the formation of yellow precipitate confirmed the presence of alkaloids.

S. No.	Treatment	Day Light	UV Light (254 NM)	UV Light (365 NM)
1.	Drug Powder	Pale Green	Green	Brown
2.	Powder + 1N NaOH (aq)	Pale Yellow	Pale Green	No visible Colour
3.	Powder + 1N NaOH (al)	Pale Yellow	Pale Green	No visible Colour
4.	Powder + 1N Hcl	Pale Green	Pale Green	No visible Colour
5.	Powder + 50% H2SO4	Brown	Pale Green	Pale Green
6.	Powder + 50% HNO3	Orange	Pale Yellow	Pale Green
7.	Powder + Picric acid	Yellow	Pale green	Pale Green
8.	Powder + Acetic Acid	Pale Green	Pale Green	No visible colour
9.	Powder + Fed3	Pale Brown	Pale Yellow	Pale Yellow
10.	Power + HNO3 + NH3	Orange PPT	Pale Yellow	Pale Green

(b) Tests for Terpenoids (Noller's Test):
To 1 ml of extract, tin (one bit) and thionyl chloride (1 ml) were added. Appearance of pink colour indicates the presence of Terpenoids.

(c) Test for steroids:
i)) Liebermann - Burchard's Test- The powder was dissolved in two ml of chloroform in a dry test tube. Ten drops of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution became red, then blue and finally bluish green, indicated the presence of steroids.
ii) Salkowski Test- The powder was dissolved in chloroform and equal volume of concentrated sulphuric acid. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer represented the steroid components in the test samples.

(d) Test for Coumarin - To 1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarin is indicated by the formation of yellow colour.

(e) Test for Tannin - To few mg of powder, ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of tannins.
i) To few mg of powder, potassium dichromate solution was added, formation of a precipitate showed the presence of tannins and phenolics.
ii) The sample powder was mixed with basic lead acetate solution. Formation of white precipitate indicated the presence of tannins.

(f) Test for saponin - To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Formation of honeycomb like froth indicates the presence of saponins.

(g) Test for Flavanoids (Shinadow's Test) :
i) Shinoda's Test : To few mg of the powder, magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. Formation of red colour showed the presence of flavonoids.
ii) Zinc-Hydrochloric Acid Reduction Test: To few mg of the powder Zinc dust and concentrated Hydrochloric acid was added. Formation of magenta colour showed the presence of flavonoids.
iii) Ferric Chloride Test: To few mg of the powder, a small quantity of ethanolic solution and few drops of neutral ferric chloride were added. Blackish red colour indicated the presence of flavonoids.
iv) Alkaline Reagent Test: To few mg of the powder, a few drops of dilute sodium hydroxide were added. An intense yellow colour, which became colourless on addition of a few drops of dilute acid, indicated the presence of flavonoids.

(h) Tests for Quinones- To 1 ml of the extract 1 ml of concentrated sulphuric acid were added. Formation of red colour shows the presence of Quinones.
i) Test for Anthraquinones (Borntrager's Test) - The powder / extract was macerated with ether and after filtration, aqueous ammonia or caustic soda was added. Pink red or violet colour in the aqueous layer after shaking indicates the presence of anthraquinones.

i) Test for phenols- To 1 ml of the extract, 2 ml of distilled water was added followed by few drops of 10 % aqueous ferric chloride. Appearance of blue or green colour indicates the presence of phenols,

(j) Test for protein (Biuret test):
i) Biuret Test: To few mg of powder, one ml of forty percent sodium hydroxide solution and two drops of one percent copper sulphate solution were added. Formation of violet colour indicated the presence of proteins.
ii) Xanthoprotein Test: To few mg of powder, one ml of concentrated nitric acid was added. As a white precipitate was formed, it is boiled and cooled. Then, 20% of sodium hydroxide or ammonia was added. Orange colour indicated the presence of aromatic amino acids.
iii) Tannic Acid Test: To the powder, 10% tannic acid was added. Formation of white precipitate indicated the presence of proteins.

k) Test for carbohydrates (Sugar):
i) Molisch's Test: To the powder, one ml of alpha-naphthol solution, and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.
ii) Fehling's Test: To the powder, equal quantities of fehling's solution A and B were added and on heating, formation of a brick red precipitate indicated the presence of carbohydrates.
iii) Benedict's Test: To five ml of Benedict's reagent, few mg of powder was added and boiled for two minutes and cooled. Formation of a red precipitate showed the presence of carbohydrates.

l) Test for Glycosides – The extract was mixed with a little anthrone on a watch glass. One drop of concentrated sulphuric acid was added and made into a paste, warmed gently over water bath. The presence

of glycosides was identified by dark green coloration.

m) Test for Gum – The extract mixed with water leads to the thickening of the substance, indicates the presence of gum.

n) Test for starch – The extract mixed with 1% iodine potassium Iodide (IKI) solution. Formation of blue colour indicates the presence of starch.

o) Test for fixed oil (Spot test) – A small quantity of powder extract was pressed between the filter papers. Formation of grease spot indicates the presence of fixed oils and fats.

p) Test for catachin – A test solution of drug and Ehrlich's reagent followed by few drops of concentrated hydrochloric acid gives pink colour indicating the presence of catachin.

q) Volatile Oil
a) Substance is treated with Sudan red III (in alcohol) gives red coloration, indicates the presence of volatile oil.
b) Spot Test : Extract leaves no greasy spot on filter paper – presence of volatile oil.
The results had been tabulated in the table 4.1

PRELIMINARY PHYTOCHEMICAL ANALYSIS
Table 4.1

Sl. No.	Particulars	Water Extract	Alchohol Extract
1.	Alkaloids	+	+
2.	Terpenoids	-	-
3.	Steroids	-	-
4.	Coumarin	+	-
5.	Tannin	+	+
6.	Saponin	+	-
7.	Flavonaoids	+	-
8.	Quinones	-	-
9.	Phenols	+	+
10.	Protein	-	-
11.	Carbohydrate	+	+
12.	Glycosides	-	-
13.	Gum	-	-
14.	Fixed Oil	-	-

Thin layer chromatography :
When a mixture of compounds is spotted on a TLC plate; the compound which is readily soluble and not readily adsorbed, moves up along with the solvent. Those which are insoluble or sparingly souluble are more strongly adsorbed, moves up less readily, leading to the separation of the compound.

Preparation of TLC plate :
Eighty grams of silica gel G was weighed and shaken to a homogenous suspension with 85 ml distilled water for 90 seconds. This suspension was poured in TLC applicator, which was adjusted to 0.25 mm thickness. 24 carrier plates were laid together in a row on a template for air drying until the transparency of the layer disappears. The plates were dried in a hot air oven at 1100C for 30 minutes. The plates were then stored in a dry atmosphere and used whenever required.

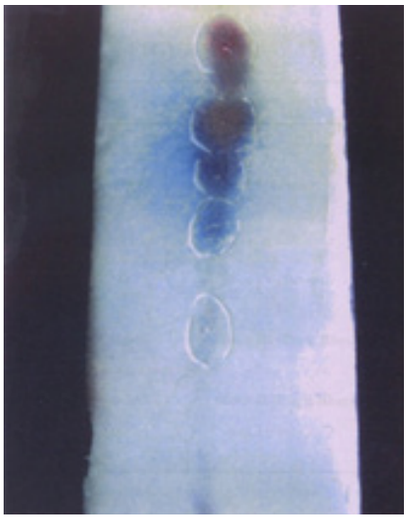
Application of the extract :
The extract was dissolved in solvent taken in a capillary tube and it was spotted on a TLC plate, 2 cm above its bottom. The starting points were equally sized as far as possible and had a diameter ranging from 2-5 mm.

Method :
The extract was subjected to thin layer chromatography for separation using the solvent system. The TLC plates were made with silica gel G and activated. The spots were spotted by means of a micropipette and dried. It was then developed in the following solvent system – Toluene : Ethyl acetate (7:3).
The different spots developed in the solvent system were identified by using sulphuric acid as the spray reagent. The different spots developed are shown in figure 4.2. The Rf values were correspondingly calculated and shown in the Table 4.3

TLC elution pattern of ethanolic extract of Sesbania grandiflora(L.)pers.
Adsorbant: hand made silica gel
Solvent system:
Toluine: Ethyl acetate (7:3)
Spray reagent:
VanillinSulphuric acid

Explanation:
• Ethanol extract (A) separated in to 5 spots indicating the presence of mixture of compounds such as alkaloids, flavanoids, saponoins, reducing sugars etc.
• Rf value of each constituent was found to be

A1 - 0.55
A2 - 0.63
A3 - 0.71
A4 - 0.76
A5 - 0.79



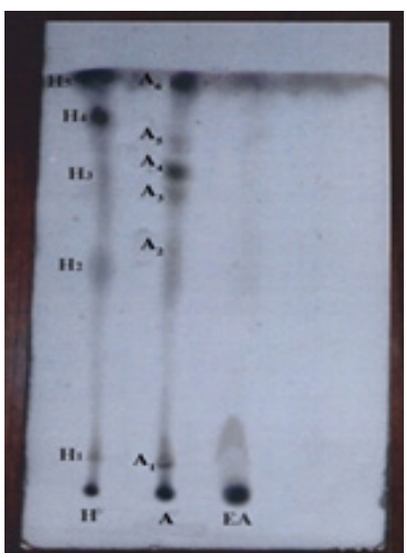


Fig. 4.2 TLC Profile of Ethanolic Extract of Sesbaria Gradiflora (L) Pers. Using vanillin sulphuric acid as spray reagent.

Fig. 4.3 TLC Elution Pattern – Various extracts of Sesbaria Gradiflora (L) Pers.

TLC Elution Pattern Various extract of Sesbaria Gradiflora (L) Pers.

Solvent System : 10% Ethyl acetate in Benzene

Spray reagent : 1:1 conc. Sulphuric acid with water

Explanation:

• N-Hexane extract (H) separated into 5 distinct spots, showing the presence of falvanoids, reducing sugars, Saponins.

• Ethanol extract (A) Separated into 6 sports indicating the presence of mixture of compounds such as proteins, reducing sugars and alkaloids.

• Ethyl acetate extract (EA) eluted but no such distinct spots were observed.

Rf value of each compound was found to be :

H1	-	0.1	A1	-	0.08
H2	-	0.52	A2	-	0.55
H3	-	0.63	A3	-	0.66
H4	-	0.88	A4	-	0.72
H5	-	0.98	A5	-	0.77
			A6	-	0.97

ANTI BACTERIAL ACTIVITY

5.1 INTRODUCTION

In the traditional system of medicine, leave of Sesbaria Gradiflora (L) Pers. used in laxative, Tonic, diuretic, dysentery, cough hence we selected microbes that affects the respiratory system to study the Anti bactyerial properties of Sesbaria Gradiflora (L) Pers..

1. Bacterial Strains :
2. Escherichia coli
3. Bacillus substilis
4. Staphylo coccus aureus
5. Pseudomonas aeruginosa
6. Proteus

The Anti Bacterial studies were carried out on Nutrient Agar Media and Blood Agar Media for (Proteus), Authentic Bacterial cultures were obtained from Sri Krishnadevaraya University, Anantapur and JNT University, Anantapur.

5.2.1 Anti Bacterial Activity

Nutrient Agar of Himedia laboratories used as the media for Escherchia coli, Bacillus substilis, Staphylo coccus aureus, Pseudomonas aeruginosa.

Composition of Nutrient Agar Media (formula per litre)

Yeast extract	5 g
Meat extract	10 g
Peptone	5 g
Sodium chloride	5 g
Agar	20 g

* Blood agar media was used for Proteus.

Formula for Blood Agar Media :

Nutrient Agar	2.8%
Blood	10%

Requirements :

7. Petriplates (sterile)
8. Cotton swabs (sterile)

9. Nutrient Agar media (sterile)
10. Blood agar media (sterile)
11. Borers – 8 mm diameter (sterile)
12. Finn pipettes
13. Needles (sterile)
14. Glass wares – pipettes, test tubes, glass rods etc.

All the glasswares were sterilized in the hot air oven at 1600C for one hour and the media was autoclaved at 15 lb pressure for 20 minutes at 1210C.

Procedure :
The anti-bacterial studies were carried out aseptically under-in-vitro conditions by "cup plate method".
The authentic bacterial cultures were inoculated in Nutrient broth overnight and used. Strains of Proteus were cultured in blood agar media and used.
The sterile nutrient agar media at 400C-500C was transferred aseptically to sterile Petri plates and allowed to solidify. The bacterial cultures were then inoculated by swabbing technique. Bores of 8mm diameter were made on the Bacteria seeded agar.
The various fractions of the plant extracts are dissolved in DMSO, so as to contain 1000, 2500, 5000 µg/ml of the drug. 100 microlitre of each drug solution were added to the respective cups along with the standard Ciprofloxacin (5µg/ml) for each organism, along with the solvent control DMSO.
The Bacteria seeded agar plates were aseptically transferred to incubator and incubated at 370C for 24 and 48 hour. The diameter of the zone of inhibition was measured after 24 and 48 hours of incubation and compared with standard antibiotic, Ciprofloxacin.
The extract which shown a zone of inhibition above 12mm were considered for minimum inhibitory concentration by Double Dilution Method20.
The observations were tabulated in Table-6.

INV1TRO ANTIOXIDANT STUDIES OF SESBANIA GRANDIFLORA (L.) PERS

6.1 INTRODUCTION

Chemical compounds and reactions capable of generating potential toxic oxygen species/free radicals are referred to as pro-oxidants. On the other hand, compounds and reactions disposing off these species, scavenging them, suppressing their formation or opposing their action are called as Anti-oxidants. In a normal cell, there is an appropriate pro-oxidant : antioxidant balance. However, this balance can be shifted towards the pro-oxidant when production of oxygen species is increased or when levels of anti-oxidants are diminished. This state is called oxidative stress and can result in serious cell damage if the stress is massive or prolonged. Oxidative stress is implicated in the etiopathogenesis of a variety of human diseases.

Table - 6: Anti-bacterial activity of varying concentration of Sesbaria Gradiflora extracts

Concentration (µg/ml)	**Escherichia coli**				**Bacillus Substillis**				**Staphylococcus aureus**				**Pseudomonas aerugenosa**				**Proteus**			
	H	**W**	**A**	**EA**	**H**	**W**	**A**	**EA**	**H**	**W**	**A**	**EA**	**H**	**W**	**A**	**EA**	**H**	**W**	**A**	**EA**
1000	2	1	2	1	2	2	4	2	-	-	2	2	3	1	4	4	3	3	3	4
2500	3	5	3	2	3	3	3	3	4	3	3	3	4	4	4	3	5	2	6	5
5000	4	5	4	3	5	5	2	3	3	3	4	4	5	4	6	6	8	10	8	8
Standard (Ciprofloxacin)	9	9	9	9	8	8	10	10	9	9	8	8	7	7	8	8	12	12	12	12
Solvent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Zone of inhibition in mm
N-Hexane extract, W- Water extract, A – Ethylalcohol EA – Ethyal acetate
- No inhibition observed, -- Not done

During the last two decades, there has been a growing interest in studies that concern with the prevention of uncontrolled oxidative process leading; to, various diseases in living system. Several studies have shown the role of oxidative stress in the causation and progression of different diseases including atherosclerosis, carcinogenesis, neurodegenerative diseases, chronic inflammatory diseases, radiation damage, ag-

ing and various other pathobiological effects.

Free radical is a molecule with an impaired electron. Normally it steals an electron from weakly bonded structure. The molecule, which loses an electron, also becomes a free radical giving rise to a perpetuating chain system. The free radicals are species with very short half life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids. These species may be either oxygen derived (Superoxide, hydroxyl, hydroperoxyl, peroxyl, alkoxyl, hydrogen peroxide, and ozone) or nitrogen derived (Nitric oxide, peroxynitrite, nitrogendioxide, and dinitrogen trioxide).

Free radicals often attack DNA, protein molecules, enzymes and cells leading to alteration in genetic material and proliferation (formation of tumor masses). A majority of carcinogenic agents are regarded as powerful generators of free radicals, which initiate chain reaction and damage the cell and its components.

When the normal level of antioxidant defense mechanism is not sufficient to the eradication of free radicals induced injury, administration of antioxidants has a protective role to play. These antioxidants are produced either endogenously or received from the exogenous sources and include enzymes like superoxide dismutase , catalase , glutathion peroxidase and glutathion reductase, minerals like manganese , zinc and copper , vitamins like vitamin A, C and E . Other compounds with antioxidant activity include glutathione, flavonoids, bilirubin and uric acid etc.

Several anti oxidants of plant origin are experimentally proved and used as effective protective agents against oxidative stress. They play an important role in major health problems such as cancer, cardio vascular diseases, cardio vascular diseases, rheumatoid arthritis, cataracts, Alzheimer's disease and degenerative diseases associated with aging.

Hence an attempt has been made to evaluate the antioxidant property by in vitro methods described as following.

6.2 Determination Of Percentage Inhibition Of Lipid Peroxidation

Principle:

The lipids in the cell membranes are highly susceptible to peroxidative damage and are broken down into number of small units to form Malondialdehyde. This reacts with Thio Barbituric acid (TEA) to form Thiobarbituric acid reacting substances (TEARS) which has a pink colour with absorption maxima at 532 nm.

Reagents Required:

• 0.15M Potassium chloride (Ranbaxy Fine Chemicals Ltd. New Delhi

• 10% Liver Homogenate

• 25 nm Ferrous Sulphate (Ranbaxy. Fine Chemicals Ltd. New Delhi)

• lOμm Ascorbic acid (Fischer Inorganics & Aromatics Ltd. Madras)

• lOμM Potassium dihydrogen phosphate (Fischer Inorganics & Aromatics Ltd, Madras)

• Extracts (1.5, 3, 7, 15, 30, 62, 125, 250, 500 and 1000 micro gram/ml concentrations) in ethanol, N-hexane and ethyl acetate.

• 15% TriChloro Acetic Acid (TCA) (Merck Ltd., Mumbai)

• 0.375% ThioBarbituric acid (TBA) (Himedia Laboratories Pvt. Ltd., Mumbai).

Procedure:

Liver homogenate was prepared from the male albino Wister rats. The liver was quickly excised after decapitation and exsanguinations and washed several times with ice cold saline solution (with 0.15M potassium chloride pH 7.4), then homogenized in the same saline solution. A 10% liver homogenate was prepared and the test system contained homogenate with a protein concentration of 500 micro gram/ml. Lipid peroxidation was initiated by the addition of 25 μM ferrous sulphate, lOOμM ascorbate and lOμM potassium dihydrogen phosphate. The homogenates were incubated at 37°C for 1 hour with different concentrations of 1.5, 3,7,15, 30, 62,125, 250, 500 and 1000 μg/ml of Sesbania Grandiflora (L.) Pers. extract. 1ml of 15% TCA and 0.375% s.% Thiobarbituric acid were added. The tubes were placed in boiling water bath for 30 minutes. Then centrifuged and the supernatant is measured at 532nm. The percentage Lipid peroxidation inhibition was calculated from the following formula

6.3 Nitric Oxide Scavenging Activity

Principle:

This method is based on the inhibition of nitric oxide radical generated from sodium nitroprusside in buffered saline and measured by Griess reagent. The absorbance of the chromophore is evaluated at 546nm.

Reagents Required:

• Sodium nitro prusside (lOmM) [S.d.fine-chem Ltd., Boisar]

• Phosphate buffered saline (PBS)

• Griess Reagent:

• 1% Sulfanilamide (Sigma chemicals, U.S.A)
• 2% O -Phosphoric acid (Sigma Chemicals, U.S.A)
• 0.1% Naphthyl ethylene diamine dihydrochloride (Sigma chemicals, U.S.A)

Procedure:
Aqueous solution of Sodium nitroprusside spontaneously generates nitric oxide (NO) at physiological pH, which interacts with oxygen to produce nitrate ions and which was measured colorimetrically. 3ml (DPPH) by free radical scavenger.

Reagents Required:
• l,l-diphenyl-2-picryl hydrazide (DPPH) (Sigma Chemicals, U.S.A)
• Dimethyl Sulphoxide (DMSO) (Sigma chemicals, U.S.A)
• Ethanol of reaction mixture containing sodium nitroprusside (lOmM) in phosphate buffered saline (PBS) and various concentrations of the extracts of Sesbania Grandiflora (L.) Pers. was incubated at i.-c 37°C for 4 hours. Controls with out test compound were kept in an identical manner. After incubation 0.5ml of Griess reagent (1% Sulfanilamide. 2% O-Phosphoric acid and 0.1% Naphthyl ethylene diamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and those of test compounds.

TABLE 6.1 - INVITRO LIPID PEROXIDE INHIBITORY ASSAY

Concentration (mcg/ml)	Percentage Inhibition		
	Total Ethanolic Extract	N-Hexane Extract	Ethyl acetate Extract
1.5	39.74	13.24	9.93
3	40.49	13.49	10.12
7	46.18	14.43	11.84
15	46.52	14.53	12.24
30	48.69	16.23	13.15
62	49.69	16.78	13.80
125	49.44	18.31	17.65
250	50.11	20.04	12.52
500	52.04	21.68	13.69
1000	52.46	22.80	14.98
IC50 (mg/ml)	150.5		

6.4-DPPH Method of determination of Antioxidant Activity
Principle:
This is one of the widely used methods for screening of antioxidant activity of plant drugs. DPPH assay method is based on the reduction of absorbance of methanol solution of l,l-dipheny l-2-picryl hydrazide

Procedure :
DPPH scavenging activity was measured by the spectrophotometric method. A stock solution of 25 mg of DPPH (200μM) was prepared in 100 ml of ethanol, 0.05 ml of test compounds dissolved in ethanol were added at different concentrations (1.5, 3, 7, 15, 30, 62, 125, 500 and 1000 μg/ml). An equal amount of ethanol was added to the control, the reaction was allowed to be completed in the dark for about 20 minutes. After 20 minutes the decrease in absorbance of test mixtures was read at 517 nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical.

6.5 Determination Of Total Antioxidant Activity
Principle:
It is a spectroscopic method for the quantitative determination of antioxidant capacity through the formation of Phospho-molybdenum complex. The assay is based on the reduction of Molybdenum (Mo) (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. This method is routinely applied to evaluate the total antioxidant capacity of plant extracts.

Reagents Required:
• Vitamin K (Merck limited, Mumbai)
• Sulphuric acid (0.6M)
• Sodium phosphate (28mM)
• Ammonium molybdate (4mM) [E.merck (India) Ltd, Mumbai]

Procedure:
The total antioxidant activity was evaluated by Prieto et al 999 method. An aliquot of 0.1 ml of sample solution/Vitamin E equivalent to 500micro gram was combined with 1ml of the reagent solution (0.6M sulphuric acid, 28mM Sodium phosphate and 4mM-ammonium molybdate). In case of blank, 0.1 ml of methanol was used in place of sample. The tubes were capped and incubated in a boiling water bath at 95°C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank in PerkinEImer UV-Visible spectrophotometer (Milton

Roy, NY, USA). The antioxidant activity was expressed as equivalents of Vitamin E (mg/g).nol was added to the control, the reaction was allowed to be completed in the dark for about 20 minutes. After 20 minutes the decrease in absorbance of test mixtures was read at 517 nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical.

6.5 Determination Of Total Antioxidant Activity

Principle:

It is a spectroscopic method for the quantitative determination of antioxidant capacity through the formation of Phospho-molybdenum complex. The assay is based on the reduction of Molybdenum (Mo) (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. This method is routinely applied to evaluate the total antioxidant capacity of plant extracts.

Reagents Required:

- Vitamin K (Merck limited, Mumbai)
- Sulphuric acid (0.6M)
- Sodium phosphate (28mM)
- Ammonium molybdate (4mM) [E.merck (India) Ltd, Mumbai]

Procedure:

The total antioxidant activity was evaluated by Prieto et al 999 method. An aliquot of 0.1 ml of sample solution/Vitamin E equivalent to 500micro gram was combined with 1ml of the reagent solution (0.6M sulphuric acid, 28mM Sodium phosphate and 4mM-ammonium molybdate). In case of blank, 0.1 ml of methanol was used in place of sample. The tubes were capped and incubated in a boiling water bath at 95°C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank in PerkinEImer UV-Visible spectrophotometer (Milton Roy, NY, USA). The antioxidant activity was expressed as equivalents of Vitamin E (mg/g).

Table 6.2 - INVITRO NITRIC OXIDE SCAVENGING ACTIVITY

Concentration (mcg/ml)	**Percentage Inhibition**		
	Total Ethanolic Extract	***N-Hexane Extract***	***Ethyl acetate Extract***
1.5	0.25	--	0.13
3	11.09	3.69	0.76
7	13.59	4.68	3.66
15	13.55	4.83	4.22
30	20.06	7.71	5.49
62	32.84	12.63	6.65
125	62.57	25.02	16.91
250	74.56	29.82	23.30
500	65.39	30.24	21.79
1000	44.38	35.63	16.87
IC50 (mg/ml)	177.30		

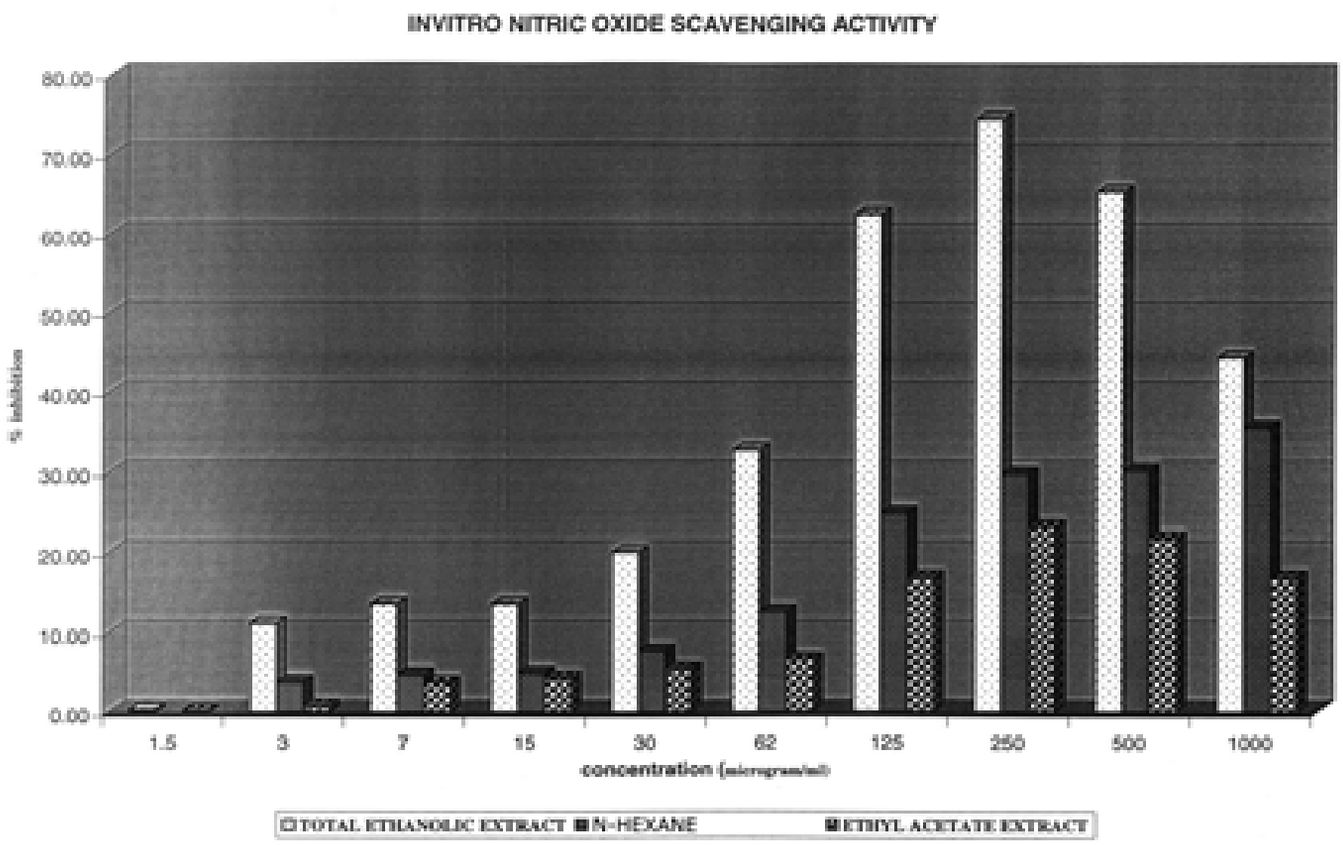
Table 6.3 - DPPH RADIAL SCAVENGING ASSAY

Concentration (mcg/ml)	***Percentage Inhibition***		
	Total Ethanolic Extract	***N-Hexane Extract***	***Ethyl acetate Extract***
1.5	0.32	0.13	--
3	0.66	0.382	--
7	8.36	2.32	--
15	11.24	3.56	--
30	24.77	9.28	--
62	47.58	15.59	--
125	78.27	27.95	--
250	80.79	35.12	--
500	80.26	39.35	--
1000	81.94	43.56	--
IC50 (mg/ml)	68.69	--	--

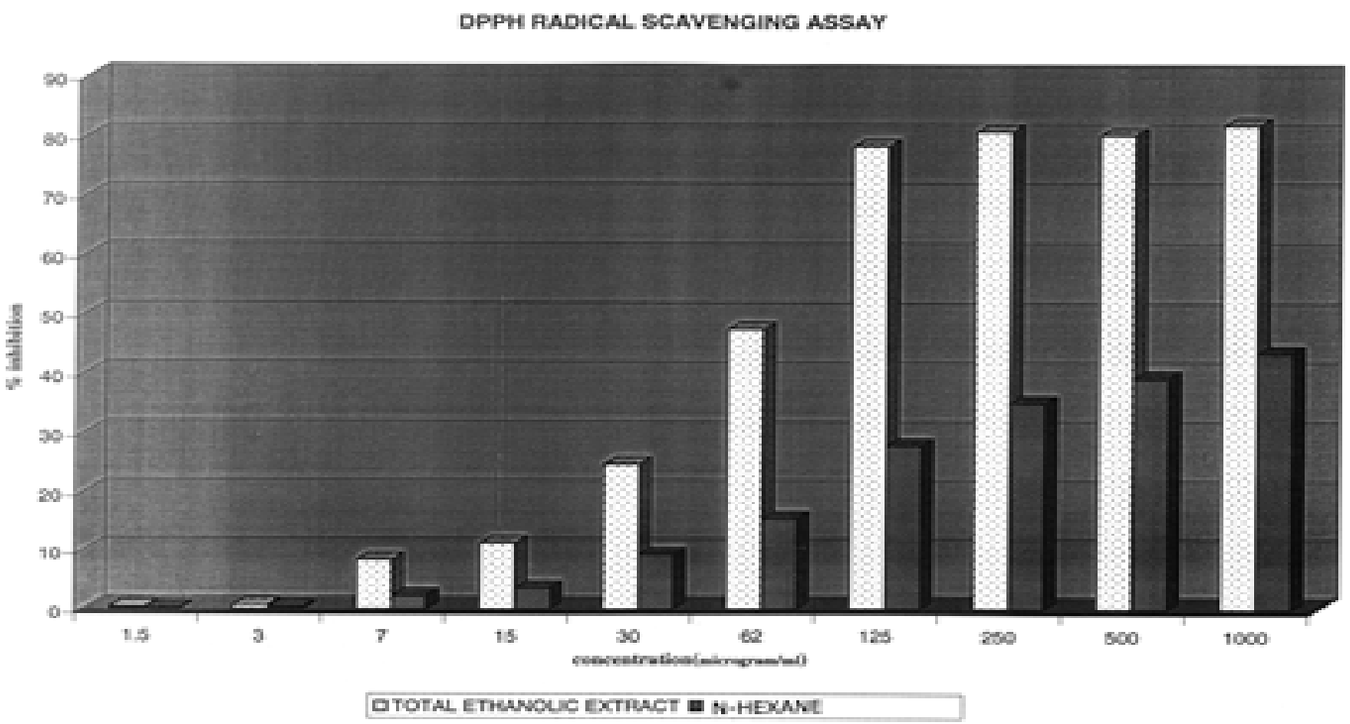
TOTAL ANTIOXIDANT ASSAY - TABLE 6.4

Concentration (mcg/ml)	Percentage Inhibition		
	Total Ethanolic Extract	*N-Hexane Extract*	*Ethyl acetate Extract*
5000	2.240	--	--
1000	2.632	--	--

Invitro Nitric oxide Scavenging Activity



DPPH Radical Scavenging Assay



Results

Results of the Plant Material – Leaf Powder

Powder Characters :

Colour	Pale green
Appearance	Coarse powder
Odour	No Characteristic Smell
Taste	No Characteristic Taste

Powder Analysis :

Treatment	**Observation**
Powder triturated with water	Non Sticky
Powder shaken with water	Foam like froth
Powder treated with 5% aqueous NaOH	Pale Yellow
Powder treated with 60% Aquous sulphuric acid	Brown colour
Powder pressed between filter paper For 24 hrs.	No Oil stain

PHYSICO – CHEMICAL CHARACTERISTICS OF SESBANIA GRANDIFLORA (L.) PERS Table 3.5.1

S. No.	**Parameter**	Lead Value (Percentage w/w)
1.	Total Ash	18.631%
2.	Acid Insoluble Ash	9.28%
3.	Water Soluble Ash	11.296%
4.	Sulphated Ash	7.36%
5.	Loss on Drying	9.23%
6.	SOLUBILITY	
	a) Ethanol	6.728%
	b) Water	4.761%
7.	Extractive Value	
	a) N-hexane	6.8 gms.
	b) Water	7.2 gms.
	c) Ethanol	5.9 gms.
	d) Ethyl Acetate	6.3 gms.

FLUORESCENCE ANALYSIS OF SESBANIA GRANDIFLORA (L.) PERS Table 3.5.2

S. No.	**Treatment**	**Day Light**	**UV Light (254 NM)**	**UV Light (365 NM)**
1.	Drug Powder	Pale Green	Green	Brown
2.	Powder + 1N NaOH (aq)	Pale Yellow	Pale Green	No visible Colour
3.	Powder + 1N NaOH (al)	Pale Yellow	Pale Green	No visible Colour
4.	Powder + 1N Hcl	Pale Green	Pale Green	No visible Colour
5.	Powder + 50% H2SO4	Brown	Pale Green	Pale Green
6.	Powder + 50% HNO3	Orange	Pale Yellow	Pale Green
7.	Powder + Picric acid	Yellow	Pale green	Pale Green
8.	Powder + Acetic Acid	Pale Green	Pale Green	No visible colour
9.	Powder + Fed3	Pale Brown	Pale Yellow	Pale Yellow
10.	Power + HNO3 + NH3	Orange PPT	Pale Yellow	Pale Green

PRELIMINARY PHYTOCHEMICAL ANALYSIS Table 4.1

Sl. No.	**Particulars**	**Water Extract**	**Alchohol Extract**
1.	Alkaloids	+	+
2.	Terpenoids	-	-
3.	Steroids	-	-
4.	Coumarin	+	-
5.	Tannin	+	+
6.	Saponin	+	-
7.	Flavonaoids	+	-
8.	Quinones	-	-
9.	Phenols	+	+
10.	Protein	-	-
11.	Carbohydrate	+	+
12.	Glycosides	-	-
13.	Gum	-	-
14.	Fixed Oil	-	-

4.3 Results And Discussion

The extracts were prepared by using previously distilled water, ethanol, Ethyl acetate, N-Hexane by hot maceration method using soxlet apparatus. The qualitative organic analysis has revealed that the water extract consists of phenols, Alkaloids, Coumarin, Tannin, Flavonoids, Carbohydrate and ethanolic extract consists of Flavanoids, Tannins, Saponin, Glycosides, Proteins, Reducing sugar, etc. These less quantity with Ethylacetate conformed with the TCL further trails is required.

5.3 Results & Discussions

The water fraction shows pronounced activity against Proteus moderate activity against E.Colli, Bacillus, slight activity against Pseudomonas aerugenosa and Staphylococcus aureus.

The Alcohol extract shows pronounced activity against Proteus, Moderate activity against Pseudomonas aerugenosa , Staphylococcus aureus. Slight activity against E.Colli, Bacillus substillis.

N-Hexane extract shows pronounced activity against Proteus moderate activity against Pseudomonas aerugenosa, Bacillus substillis.

Slight activity against Staphylococcus, E.Colli.

Ethylacetate extract shows moderate activity against Pseudomonas aerugenosa, Proteus, slight activity against Staphylococcus aureus, E.Colli and Bacillus.

• The total ethanolic extract showed good antioxidant activity in all in vitro free radical scavenging models when compared to N-Hexane and ethyl acetate extracts of Sesbania Grandiflora (L.) Pers. leaves.

• In lipid peroxide inhibitory assay, the total ethanolic extract of of Sesbania Grandiflora (L.) Pers. showed only 39.74% inhibition at 1.5 meg/ml concentration; whereas a increase in inhibition of lipid peroxidation was observed at higher concentration (52.46% inhibition at 1000 meg/ml). Similarly the response in percentage inhibition was observed in N-Hexane fraction, ethyl acetate fraction. But the effect was found to be lesser in the N-Hexane and the ethyl acetate extract than the ethanolic extract. The effect is represented as Total ethanolic extract> N-Hexane extract> Ethyl acetate extract. The 1C 50 value for the total ethanolic extract was found to be 150.5 meg/ml. The percentage inhibition of all the extracts was found to show dose dependent increase in invitro lipid peroxide inhibitory assay.

• In invitro nitric oxide scavenging assay, the percentage inhibition was 74.56%, 35.63% and 23.3% in total ethanolic extract, N-Hexane extract and ethyl acetate extract at 250mcg/ml, lOOOmcg/ml and 250mcg/ml respectively. The total ethanolic extract and ethyl acetate showed a biphasic response where as the N-Hexane extract showed a dose dependent increase. The 1C 50 value for the total ethanolic extract was found to be 177.3 meg/ml.

• In DPPH radical scavenging assay, the total ethanolic extract showed 81.94% inhibition at 1000 mcg/ml and the N-Hexane extract showed 43.56% inhibition at 1000 mcg/ml. Both the extracts showed a dose dependent increase in activity. The IC 50 value for the total ethanolic extract was found to be 68.69 mcg/ml.

• A does dependent increase in total antioxidant property was shown by total ethanolic extract of Sesbania Grandiflora (L.) Pers. The values were expressed as grams equivalent to 1 gram of vitamin E (mg/g)

6.7 DISCUSSION

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radicals can bring about thousands of reactions ant thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals. Anti oxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation etc.

Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like brain and liver. In this study, invitro lipid peroxidation was induced to rat liver by using ferrous sulphate and ascorbic acid. Lipid peroxidation occurs either through ferryl-preferryl complex or through OH' radical by Fenton's reaction. Whatever may be the process of lipid peroxidation, leaf extracts of Sesbania Grandiflora (L.) Pers. (LESG) (total ethanolic extract, N-Hexane extract and ethyl acetate extract) shows dose dependent prevention towards generation of lipid peroxides.

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc and involved in the regulation of various physiological processes. Excess concentration of NO is associated with nitric oxide and thus inhibits the generation of the anions.

DPPH is a relatively stable free radical and the assay determines the ability of LESG to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electrons to the paired ones. Antioxidants can act by converting the unpaired electrons to paired ones. The dose dependent inhibition of DPPH indicates that LESG causes reduction of DPPH radical.

A substance may act as an antioxidant due to its ability to reduce ROS by donating hydrogen atom. The reducing property of LESG implies that it is capable of donating hydrogen atom in a dose dependent

manner. The high content of phenolic compounds are known to have direct anti oxidant property due to the presence of hydroxyl groups, which can function as hydrogen donor.

Preliminary phytochemical analysis indicates tannins and flavonoids in LESG. Polyphenols, particularly flavonoids and tannins are well known natural antioxidants. Thus, the antioxidant potential of leaf extracts of Sesbania Grandiflora (L.) Pers. may be due to the presence of polyphenolic compounds, which needs further analysis.

Units of Measurement: Measurements of length, height, weight, and volume should be reported in metric units (metre, kilogram, or litre) or their decimal multiples. Temperatures should be given in degrees Celsius. Blood pressures should be given in millimetres of mercury. All haematological and clinical chemistry measurements should be reported in the metric system in terms of the International System of Units (SI). The symbol L for litre is recommended in order to avoid the risk of confusion between the letter l and the number 1.

Discussion on Results

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The plant Sesbania Grandiflora (L.) Pers. Belongs to the family Fabaceae. The leaves are used in laxative, febrifuge, emetic, emmenagogue, tonic, catarrh, dysentery, eyes, headaches, smallpox, sores, sorethroat, poulticed onto bruises, Rheumatic swellings (23,24). Anatomical and physiochemical studies on the leaf has been carried out which is described in chapter 3.3 & 3.4. Preliminary phytochemical analysis revealed the presence of phenols, flavonoids, triterpenoids, Alkaloids, Coumarin, Saponin, Carbohydrate, Kaempherol, Quercetin, rhamnetin, tannins, gums and glycosides in both water and alcohol extracts wand saponins in alcohol extract.

Invitro Antioxidant and free radial scavenging activity studies on leaf extract of Sesbania Grandiflora (L.) Pers.revealed the presence of antioxidant activity. The total alcoholic extract had good antioxidant property when compared to N-Hexane and ethyl acetate extracts. The antioxidant activity is due to the presence of phenols, and flavonoids in the leaf extract, which are known to have antioxidant property.

The results obtained suggested that the use of this leaf extract widely in the treatment of many diseases may be due to ins antioxidant and free radical scavenging ability. Thus a varied therapeutic activity of the plant (leaf) extract may be in part due to its antioxidant activity.

Legend for Figures

Fig. 1 Pinnately compound leaves seen in adaxial and abaxial views (right).

Fig. 2 Microscopic features of the leaflet

1) TS of leaflet through midrib and lamina.
2) TS of midrib – enlarged.

(Ads – Adaxial side; BSE – Bundle sheath extension; Ep-Epidermis; GT – Ground tissue; La Lamina; MR Midrib ; Ph – Phloem ; X – Xylem)

Fig. 3. Paradermal sectional view of the epidermis of the leaflet.

1) Stomata and epidermal cells in surface view.
2) Same as above enlarged.

(AW – Anticlinical walls; EC – Epidermal Cells ; SC – Subsidiary Cells; St – Stomata)

Fig. 4. Lamina–Cleared to show theVenation pattern in surface view.

1) Venation pattern under low magnifica tion.
2) Vein islets and vein terminations – en larged.

(IMV – Intramarginal Veins ; LV – Lateral veins ; MV – Marginal vein ; MR _ Midrib ; VI – Vein islet ; VT – Vein termination.)

Fig. 5 Anatomy of the Petiolule and petiole

1) TS of petiolule, showing gross sectional outline.
2) TS of petiole, showing outline and vascu lar pattern.

(ADB – Adaxial side; Adw – Adaxial wing; Ep – Epiderms ; GT-Ground tissue ; LB – Lateral bundle ; MB – Median bundle ; Ph Phloem; WB-Wing bundle ; X-Xylem).

Fig.6.TS of petiole showing structural details of the vascular cylinder.

1) Upper portion 2) Lower por tion

(ADB – Adaxial bundle ; AdG – Adaxial Groove ; Ep – epidermis ; GT – Ground tissue ; MB – Median bundle ; Ph – Phloem ; Se – Scleren Chyma Cap ; W-Wing ; X – Xylem).

Fig. 7 Powder Mycroscopy

Fragments of epidermis as seen in the powder

1) Stomata and epidermal cells.
2) Stomatal distribution in the leaf powder.

• (EC – Epidermal cells ; St – Stomata)

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