Volume 1 Issue 2 2012

ISSN: 2320- 480X

The Journal of Phytopharmacology

(Pharmacognosy and Phytomedicine Research)

Preliminary Phytochemical Analysis of Leaf and Bark methanolic extract of Sesbania grandiflora

Mukul Bahera*[1], Roop Karki[1], Chandru Shekar[1]

1. A.S.P.M.'s K.T. Patil College of Pharmacy, Osmanabad, Maharashtra- 413501

[Email: mukeshbahera@rediffmail.com]

Abstract: Sesbania grandiflora L. (family- Leguminosae) is a small erect, fast-growing, and sparsely branched tree that reaches 10 m in height. The bark of this species is light gray, corky and deeply furrowed and the wood is soft and white. All Sesbania species have pinnately compound leaves where each leaf is divided into multiple leaflets. Agati leaves can be up to 30 cm long with 5-15 paired leaflets that are oblong to elliptic in shape and about 3 cm in length. The flowers of Sesbania grandiflora L. are large (7–9 cm long) and are borne on an unbranched, pendulous inflorescence. Two varieties of Sesbania grandiflora L. are recognized including variety grandiflora which has white flowers and variety coccinea which has rose pink or red flowers.

Keywords: *Sesbania grandiflora*, Extract, Percentage yield, Phytochemical

Introduction:

Sesbania grandiflora L. is an Indian medicinal plant which belongs to family Leguminosae. It is cultivated in south or west India in the ganga valley and in Bengal. The plant contains rich in tanins, flavonoides, coumarins, steroids and triterpens. The plant used in colic disorder, jaundice, poisoning condition, small-pox, eruptive fever, epilepsy etc.[1]

Taxonomic Classification:[2]

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Leguminosae
Genus	Sesbania
Species	Sesbania grandiflora

Chemical constituents[3-5]

The tanins, flavonoides, coumarins, steroids and triterpens were present on all organ tested, with more or less important contents according to the intensity of coloring obtained. The alkaloids are generally found in the form of traces. The saponosides were more often present in methanolic extracts than aqueous extract. The saponosides would be rather present in the form of triterpens and steroids that in the form of heterosides. Leucocyanidin and cyanidin are the active ingredients of *Sesbania grandiflora L.* seeds and oleanolic acid and its methyl ester & kaemferol-3-rutinoside are the major chemical constituents of flower. The bark contains tannins and gum. Saponin and Sesbanimide isolated from seeds.

Medicinal uses[6-7]

All parts of *Sesbania grandiflora L.* are utilized for medicine in Southeastern Asia and India including preparations derived from the roots, bark, gum, leaves, flowers, and fruit. In Folk Medicine it is resorted to be aperient, diuretic, emetic, emmenagogue, febrifuge, laxative, and tonic. Agati is a folk remedy for bruises, catarrh, dysentery, eyes, fevers, headaches, smallpox, sores, sore throat, and stomatitis. Different parts of this plant are used in Siddha system of Indian traditional medicine for the treatment of a wide spectrum of ailments including anemia, bronchitis, fever, headache, ophthalmia, nasal catarrh, inflammation, leprosy, gout and rheumatism. It also possesses anxiolytic

and anticonvulsive and hepatoprotective properties. In addition, *S. grandiflora* is mentioned as a potent antidote for tobacco and smoking-related diseases. However, the mechanisms underlying its beneficial effects against chronic smoking associated diseases are yet to be determined. The various parts of sesbania are used as medicine for many diseases and disorders.

Materials and method:

Collection of Plant material-

The leaves of *Sesbania grandiflora* were collected near Kanichikudi temple, Samalpatti to Oothngrai main road, Krishnagiri district, Tamil Nadu state in the month of October and was authenticated by Dr. K. Ravikumar, Assistant Director, Foundation of revitalisation of Local Health Traditions, Bangalore.

Preparation of plant extracts-

The freshly collected leaves and bark of *Sesbania grandiflora* were dried in shade under the control conditions and powdered. The powered leaves and bark of the plants (500g) were extracted in solvents with increasing polarity from Petroleum ether, Chloroform, Ethyl acetate, Methanol, and Hydroalcoholic medium for 24 hours with each solvent, by successive extraction method (Soxhlet apparatus) at a temperature of 30º to 35º C. The extracts were concentrated by evaporating the solvent on water bath until it got reduced to a semisolid mass. The concentrated extract was reduced to a semisolid mass by drying on water bath at 40±50˚C and packed into separate air tight containers. These extracts were subjected to phytochemical screening for the identification of the different phytoconstituents.

The percentage extractive yield was calculated by formula as mentioned below:

$$\% \text{ Extractive yield (w/w)} = \frac{\text{weight of dried extract}}{\text{weight of dried fruit}} \times 100$$

Determination of Extractive value:- The extractive values of dried leaf and bark powder of *Sesbania grandiflora* were determined with different solvents i.e. Petroleum ether, Chloroform, Ethyl acetate, Methanol, and Hydroalcoholic medium.

Preliminary physical analysis of dried leaf and bark (mixture) extract:- The property of selective reactivity of phytochemical present in an extract forms the basis of chemical tests for identification of different constituents.

Preliminary analyses of *Sesbania grandiflora* leaf and bark extract was performed initially to identify various chemical compounds present and to assess physicochemical properties.

The performed preliminary analyses included:

a) Macroscopic evaluation of leaf & bark (mixture) extract:- Macroscopic evaluation of leaf and bark extract was performed with respect to colour, odour, taste, touch etc.

b) Analysis of solubility parameters:- Solubility of prepared leaf and bark extract of *Sesbania grandiflora* was determined in various solvents i.e. Petroleum ether, Chloroform, Ethyl acetate, Methanol, and Hydroalcoholic medium.

Preliminary phytochemical screening:- The various extracts of *Sesbania grandiflora* i.e. Petroleum ether, Chloroform, Ethyl acetate, Methanol, and Hydroalcoholic medium were subjected to qualitative chemical analyses to detect the presence of various phytoconstituents.[8, 9]

Test for Carbohydrate:

A small quantity of the extracts was dissolved separately in 5 ml distilled water and filtered. The filtrates were subjected to the following tests to detect the presence of carbohydrates.

Molish′s test:- Extract filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube separately and 2 ml of concentrated sulphuric acid was added carefully along the sides of the test tubes. Formation of violet ring at the junction may indicate the presence of carbohydrates.

Test for reducing sugar:

Fehiling′s test:- Extract filtrates were treated in equal volumes with 1ml Fehling A and 1ml Fehling B solutions, boiled for one minute separately. The mixtures were boiled for 5-10 minutes on water bath. Reddish brown colour was obtained due to formation of cuprous oxide which indicated the presence of reducing sugar.

Benedict′s test:- Extract filtrates were treated with equal volumes of Benedict′s

reagent in test tubes separately. The mixtures were boiled for 5-10 minutes on water bath. Solution appeared green, yellow or red depending on amount of reducing sugar present in each filtrate.

Test for Glycosides:

Test for cardiac glycosides:

Keller kelliani test (test for deoxysugar):- Leaf and bark mixture extract were treated with chloroform and evaporate it to dryness. Separately 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added and transferred to a small test tube added with carefully 0.5 ml of concentrated Sulphuric acid by the side of the test tube, blue colour appears in the acetic acid layer indicating the presence of glycosides.

Test for Anthraquinone Glycosides:

Borntrager's test:- Leaf and bark mixture extract were boiled with 1 ml of dilute Sulphuric acid in a test tube separately for 5 min, filtered while hot, pipette out the supernatant or filtrate, cooled and shaken with an equal volumes of dichloromethane. The lower levels of dichloromethan separated and shaken with half its volume with dilute ammonia. A rose pink to red color appeared in the ammonical layer, indicating the presence of glycosides.

Test for Saponin Glycosides:

Froth test:- Leaf and bark extracts were treated with water in a semi-micro tube separately shaken well. The froth appeared thus indicating the presence of glycosides.

Tests for Amino acid and Protein:

Biuret test (General test):- Leaf and bark extract were treated with 1 ml 10% sodium hydroxide solution separately and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation of purplish violet colour may indicate the presence of proteins.

Million′s test (for proteins):- 3 ml test solutions were mixed with 5 ml Million's reagent separately. White precipitate was formed which on heating turned to brick red. It may indicated the presence of amino acids.

Tests for Sterols and Triterpenoids:

Libermann-Burchard test:- Leaf and bark extract were treated with few drops of acetic anhydride separately. Boiled and cooled, concentrated sulphuric acid was added from the side of the test tubes. A brown ring at the junction of two layer and the upper layer turning green which indicated the presence of sterols while formation of deep red colour indicated the presence of triterpenoids.

Salkowski's test:- Leaf and bark extract were treated in chloroform separately with few drops of concentrated sulphuric acid, shaken well and allowed to stand for some time, red colour appeared in the lower layer indicated the presence of sterols while formation of yellow coloured lower layer indicated the presence of triterpenoids.

Tests for tannins and phenolic compounds:

Ferric chloride test:- Small amount of leaf and bark extract were shaken with water separately and warmed. Then about 2 ml of 5% ferric chloride solution was added and observed for the formation of green or blue colour which may indicate the presence of phenols.

Gelatin test:- 1% gelatin solution containing 10% sodium chloride was added to each leaf and bark extract. Formation of precipitate indicated the presence of tannins and phenolic compounds.

Iodine test:- Leaf and bark extract were treated with diluted iodine solution separately. Appearance of transient red colour indicated the presence of tannins and phenolic compounds.

Nitric acid test:- Leaf and bark extract were treated with dilute nitric acid separately. Formation of reddish to yellowish colour indicated the presence of tannins and phenolic compounds.

Test for alkaloids:

About 500 mg of the leaf and bark extract were stirred with about 5 ml of dilute hydrochloric acid separately and filtered. Each filtrate was tested with the following reagents:

Dragendroff's test:- Few drops of Dragendroff's reagent (solution of potassium bismuth iodide) were added to each filtrate and observed for the formation of orange yellow precipitate which may indicate the presence of alkaloids.

Mayer's test:- Few drops of Mayer's reagent (Potassium mercuric iodide solution)

were added to each filtrate and observed for the formation of white or cream colour precipitate which may indicate the presence of alkaloids.

Hager's test:- Few drops of Hager's reagent (saturated aqueous solution of picric acid) were added to each filtrate and observed for the formation of yellow precipitate which may indicate the presence of alkaloids.

Wagner's test:- Few drops of Wagner's reagent (solution of iodine in potassium iodide) were added to each filtrate and observed for the formation of reddish brown precipitate which may indicate the presence of alkaloids.

Tests for flavonoids:

Shinoda test (Magnesium Hydrochloride reduction test):- To leaf and bark extracts, 5ml. 95% ethanol was added separately. Each mixture was treated with 0.5g magnesium turnings and few drops of conc. HCL. Pink colour, if produced, may confirm the presence of flavonoids.

Alkaline reagent test: - Small quantity of each extract sample was taken and added with lead acetate solution. After few minutes appearance of yellow colour precipitates which indicated the presence of flavonoids.

Result:

The powdered leaves and bark of *Sesbania grandiflora* extracted with different solvent. The resultant extract was dried in air until constant weight of the plant extract was obtained. The plant extract was then observed for the physical characteristics are mentioned in Table no. 1

Table no. 1: Physical characteristics of *S. grandiflora* plant extracts

Parameters	Physical properties of *S. grandiflora*
Colour	Blackish green
Odor	Characteristic
Taste	Bitter
Consistency	Solid
Sense of touch	Bit sticky

Percentage yield-

Table 2:- Quantitative determination of extractive value of leaves extract of *S. grandiflora.*

S. No.	Extracts	% yield	Colour	Consistency
1.	Petroleum ether	5.6	Blackish green	Waxy
2.	Chloroform	14.4	Blackish green	Sticky solid
3.	Methanolic	16.8	Blackish green	Sticky solid
4.	Hydroethanolic	15.2	Blackish green	Solid

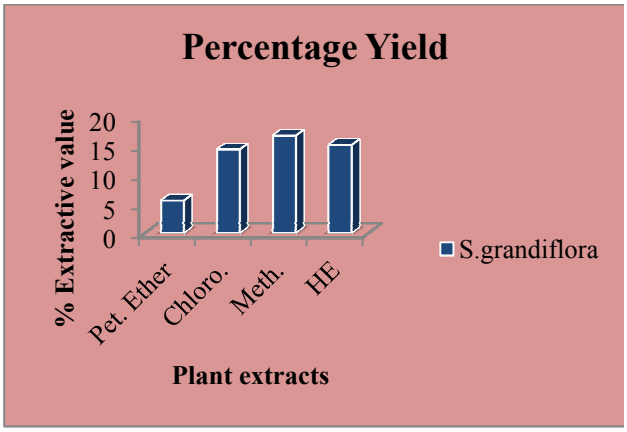

Fig no. 1: % yield of leaves and bark extracts of *S. grandiflora* and in different solvent system Pet.Ether (petroleum ether), Chloro (Chloroform), Met. (Methanol), HE (hydroethanol)

Assessment of solubility parameters:-

The solubility analysis of leaves and bark extract of *S. grandiflora* was performed by using different solvents of variable polarity. The whole plant extract showed good solubility in variable solvents. The obtained results were as shown in Table no. 3.

Table 3: Solubility analysis of *S. grandiflora* and *A. niotica* leaves and bark extract respectively

Solvent	Solubility of *S. grandiflora* extract
Water	Completely soluble
Methanol	Completely soluble
Ethanol	Completely soluble
Propanol	Slightly soluble
Benzene	Completely soluble
Chloroform	Slightly soluble
Diethyl	Completely soluble

ether	

The plant extract showed complete solubility in all solvents used in study.

Phytochemical Test Result;

Identification for various phytochemical constituents was performed with leaves and bark of Methanolic extract of *S.grandiflora* by performing relevant phytochemical tests. The results were as depicted in Table no. 4.

Table no. 4: Phytochemical evaluation *Sesbania grandiflora* and *Acacia nilotica*

Phytochemical tests	**Results for *S.grandiflora***
Test For Flavonoids	
Lead acetate test	**Positive**
Ferric chloride test	**Positive**
Shinoda Test	**Positive**
Alkaline test	**Positive**
Test For Carbohydrates	
Molisch test	**Negative**
Test for pentoses	**Negative**
Barfoed's Test	**Negative**
Fehling's test	**Negative**
Test For Saponins	
Forth test	**Positive**
Test for Triterpenoids and Steroids	
Salkowaski test	**Negative**
Libermann-Burchard Test	**Negative**
Test For Protein and Amino Acids	
Biuret test	**Negative**
Million's Test	**Negative**
Xanthoprotein Test	**Negative**
Test for proteins containing	**Negative**

sulphur	
Test for Tannins and Phenols	
Ferric Chloride Test	**Positive**
Lead acetate Test	**Positive**
Test for Glycosides	
General test	**Positive**
Test for Cardiac Glycisides	
Legal's Test (test for cardenoloids)	**Positive**
Keller killiani's Test (for deoxysugars)	**Positive**
Liebermann's Test (for bufadenoloids)	**Positive**
Test for Anthraquinone Glycosides	
Borntrager's Test	**Negative**
Modified Borntrager's Test	**Positive**
Test For Alkaloids	
Mayer's Reagent	**Positive**
Dragendroff's Reagent	**Positive**
Hagers test	**Positive**
Wagner's test	**Positive**

Conclusion: Phytochemical screening of Petroleum ether, Chloroform, Ethyl acetate, Methanol, and Hydroalcoholic medium extracts revealed the presence of presence of alkaloids, glycoside, flavonoids, saponins, tannins and phenols by positive reaction with the respective test reagent.

Acknowledgement: The authors are grateful to the Director, college of Pharmacy for providing facility for the research work.

Reference:

1. Kirtikar basu, "Indian Medicinal Plants", 2nd edition, *Bishen singh Mahendra pal Singh publishers*, 1991
2. Wagh V.D., et al, "Phytochemical, pharmacological and phytopharmaceutics aspects of *Sesbania grandiflora* (Hadga) : A review", *Journal of Pharmacy Research*, 2009, Vol 2, issue 5, pp 889-892
3. Outtara M.B., et al, "Antibacterial Potential and Antioxidant Activity of Polyphenols of Sesbania grandiflora", *Current Research Journal of Biological Sciences"*, 2011, Vol 3, Issue 4, 351-356
4. Nadkarni A.K., Nadkarni K.M., "Indian materia medica", *popular book depot originally by University of Michigan*, 3rd edition, 1955
5. Gutteridge R.C., "Forage tree legumes in tropical agriculture", *Tropical grassland society of Australia Inc*, 1998.
6. Alli L.A., Salwau.A., et al,. Antiplasmodial activity aqueous root extract of acacia nilotica. *AJBR* 2011; Vol 5: pp 214-219.
7. Mbatchou V.C., et al, "Antibacterial activities of seed pod extracts of acacia nilotica wild to artemia salina larvae. *Journal of applied biosciences* 2011; Vol 40: pp 2738-2745.
8. Kokate C.K.,Purohit A.P, Gokhale S.B., "Pharmacognosy" 17th edition , Nirali Prakashan, 2009 pp. 99, 231, 185, 271, 445, Pune.
9. Evans WC, Trease, "Text Book of Pharmacognosy." ELBS, 3rd ed, (1994), pp. 177- 179 and, 247, London.