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Phytochemical screening of *Sesbania grandiflora* L. bark

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Abstract
Sesbania grandiflora Linn. (Family: Fabaceae) is widespread distributed in West Bengal, Assam, Karnataka and North-Eastern parts. The present study intended with various phytochemical screening on *Sesbania grandiflora* bark extract**.** The present study was done with the help of soxhlet extraction**,** UV, andphytochemical tests. Preliminary phytochemical evaluation of the extracts revealed that presence of proteins, flavonoids, alkaloids, steroids, tannins and glycoside. As well total phenolic and total flavonoid content was also determined.

Keywords: phytochemical study, *Sesbania grandiflora* (L.) bark, total flavonoid content, total phenolic content etc.

1. Introduction
Sesbania grandiflora which belongs to family Fabaceae commonly known as 'sesbania', is widely used as Indian folk medicine. *S. grandiflora* has the common names of Agati, Corkwood Tree and West Indian Pea, The hummingbird tree (or scarlet wisteria). In India, it is known as vaka or basna. Traditionally *Sesbania gradiflora* is used alone or with other medicinal plants to treat a variety of ailments. It is a small tree believed to have originated either in India or Southeast Asia and grows primarily in hot and humid tropical areas in the world. A native to Asian countries such as India, Malasia, Indonesia and the Philippines where it is commonly seen growing on the dikes between rice paddies, along roadsides and in backyards vegetable gardens. The whole plant contains Grandifloral, arginine, cystine, histidine, isolucine, phenylalanine, tryptophan, valine, threonine, alanine, aspargine, aspartic acid and a saponin yielding oleanolic acid, galactose, rhamnose and glucuronic acid and it also contains flavonol glycoside, kaempferol. The root-bark of the red-flowered variety is useful in vitiated condition of *vata* and arthralgia. The bark is astringent, cooling, bitter, tonic, anthelmintic and febrifuge. The pounded bark is externally applied to cure scabies. The juice of the bark is good for dyspepsia, diarrhea and gastralgia. The leaves are acrid, bitter, sweet, cooling, aperient, tonic and diuretic and contain a non-poisonous saponin like substance. Leaves are chewed to disinfect mouth and throat and are useful in stomatalgia. The flowers are cooling, bitter, astringent, acrid and antipyretic. The juice of the flowers is applied to the eyes for nyctalopia and is used for intermittent fevers. The fruits are sweet, bitter, laxative and alexiteric and are useful in flatulent-colic, astringent, cooling, bitter, tonic, anthelmintic, febrifuge, cure scabies, dyspepsia, diarrhea and gastralgia, astringent, antipyretic [(Kirthikar and Basu,1998), (Chatterjee,1992), (Rastogi,1960)]. Based on the above medicinal properties of *Sesbania grandiflora*, in this study, we investigated the phytochemical screening of extracts of plant bark.

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

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

method. The extracts were concentrated to dryness by distilling the solvent at low temperature using rotary evaporator. The extract was stored in airtight container.

2.3. Preliminary Phytochemical Screening
[Khandelwal, 2002] [1]

A) Test for Carbohydrates
I) Molisch test
Two ml of extract solution was treated with few drops of 15 percent ethanolic α- napthol solution in a test tube and 2 ml of concentrated Sulphuric acid was added carefully along the side of tubes. The formation of reddish violet ring at the junction of two layers indicates the presence of carbohydrates.

B) Test for reducing sugars
I) Benedict's test
To 2 ml of Benedict's reagent, 1 ml of extract solution was added, warmed, and allowed to stand. Formation of red precipitate indicates presence of sugars.

II) Fehling's test
Five ml of extract solution was mixed with 5 ml Fehling's solution (equal mixture of Fehling's solution A and B) and boiled. Development of brick red precipitate indicates the presence of reducing sugars.

C) Test for monosaccharides
I) Barfoed's test
Mix equal volume of Barfoed's reagent and test solution. Heat for 1 – 2 min. in boiling water bath and cool. Red precipitate indicates presence of monosaccharides.

D) Test for Proteins
I) Biuret test
The extract was treated with 1 ml of 10 percent sodium hydroxide solution and heated. A drop of 0.7 percent copper sulphate solution was added to the above mixture. The formation of purple violet color indicates the presence of proteins.

II) Million's test
The extract was treated with 2 ml of Million's reagent. Formation of white precipitate indicates the presence of proteins and amino acids.

III) Xanthprotein test
Mix 3 ml T.S. with 1 ml conc. sulphuric acid. White precipitate is formed. Precipitate turns Yellow. Add NH_4OH, precipitate turns orange.

E) Test for amino acids
Ninhydrin test
The extract was treated with Ninhydrin reagent at pH range of 4-8 and boiled. Formation of purple color indicates the presence of amino acids.

F) Test for Steroids
I) Salkowski test
One ml of concentrated Sulphuric acid was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish brown color exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.

II) Liebermann – Burchard reaction
Mix 2 ml of extracts with chloroform. Add 1 – 2 ml acetic anhydride and two drops of conc. sulphuric acid from the side of the test tube. First red, then blue and finally green color appears.

III) Liebermann's reaction
Mix 3 ml. of extract with 3 ml. acetic anhydride. Heat and cool. Add few drops of conc. sulphuric acid. Blue color appears.

G) Test for Cardiac Glycosides
I) Test for deoxysugars (Keller - Killiani test)
To 2 ml of extract, glacial acetic acid, one drop 5% ferric chloride and conc. sulphuric acid was added. Presence of cardiac glycosides is indicated by formation of reddish brown color at junction of the two liquid layers and upper layer appeared bluish green.

II) Legal's test (Test for cardenoloids)
To the extract add 1 ml pyridine and 1 ml sodium nitroprusside. Pink to red color appears.

H) Test for Anthraquinone Glycosides.
I) Borntrager's test
To 3 ml extract, add dil. sulphuric acid. Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic layer. Add ammonia. Ammonical layer turns pink or red.

II) Modified Borntrager's test
To 5 ml extract add 5 ml, 5% $feCl_3$ and 5 ml dil. hydrochloric acid. Heat for 5 minutes in boiling water bath. Cool and add benzene or any organic solvent. Shake well. Separate organic layer, add equal volume dilute ammonia. Ammonal layer shows pinkish red color.

I) Test for Saponin glycoside
Foam test
Shake the drug extract or the dry powder vigorously with water. Persistent foam observed.

J) Test for Alkaloids
To the extract, add dilute hydrochloric acid. Shake well and filter. With filtrate perform following tests.

I) Dragendorff's Test
0.1 ml dilutes hydrochloric acid and 0.1ml Dragendorff's reagent was added in 0.2ml of extract in test tube. Formation of orange brown precipitate indicates the presence of alkaloids.

II) Mayer's test
Two ml of extract was taken in test tube .0.2ml of dilute hydrochloric acid and 0.1ml of Mayer's reagent was added. Formation of yellowish buff precipitate indicates the presence of alkaloids.

III) Wagner's test
Two ml of extract was treated with 0.2ml of dilute hydrochloric acid and 0.1ml of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

IV) Hager's test
Two ml of extract was taken in test tube and was allowed to react with 0.2ml of dilute hydrochloric acid and 0.1ml of Hager's reagent. Formation of yellowish precipitate indicates the presence of alkaloids.

K) Test for Tannins and Phenolic compounds
I) Ferric Chloride test
Five ml of extract solution was allowed to react with 1 ml of 5% ferric chloride solution. Greenish black coloration indicates the presence of tannins.

II) Lead acetate test
Five ml of extract solution was allowed to react with 1 ml of 10 percent aqueous lead acetate solution. Development of yellow colored precipitate indicates the presence of tannins.

III) Dilute Iodine test
Five ml of extract solution was allowed to react with 1 ml of dilute iodine solution. Development of transient red color indicates the presence of tannins.

IV) Dilute nitric acid test
Two ml of extract solution was allowed to react with few drops of dilute nitric acid solution. Formation of reddish to yellow color indicates the presence of tannins.

V) Dilute potassium permanganate solution test
To 2-3 ml of extract, add few drops of dilute potassium permanganate solution. Decolorization of the solution indicates presence of tannins.

L) Test for Flavonoids
I) Lead acetate test
Few drops of 10 per cent lead acetate were added to the extract. Development of yellow precipitate confirms the presence of flavonoids.

II) Ferric chloride test
Few drops of alcoholic ferric chloride were added to the extract. Development of yellowish orange precipitate confirms the presence of flavonoids.

III) Sodium Hydroxide test
Few drops of 0.1N Sodium Hydroxide were added to the extract. Development of white precipitate confirms the presence of flavonoids.

2.4 Quantitative Estimation of Phytoconstituents
2.4.1 Total Phenolic Content
[(Shukla *et al*, 2007**)** [2], (Chaiyan et.al, 2009) [3], (Lydia *et al*, 2012) [4]

Chemicals: Folin ciocalteu reagent, gallic acid, sodium carbonate, distilled water,

Preparation of reagents
1. **Folin reagents:** Dilute 15ml foline reagent in 15ml distilled water.
2. **Sodium carbonate:** (2%w/v) Dissolve 2gm of Na_2CO_3 in 100ml distilled water.
3. **Gallic acid solution:** (mg/ml) Dissolve 50mg of gallic acid in 50ml distilled water (1000 µg/ml) from this solution different concentration are prepared.
4. **Preparation of Extract Solution:** (mg/ml) The solution of extract of plant *Sesbania grandiflora* were prepared as, the methanolic extract were prepared as 10 mg in 10 ml methanol and aqueous extract were prepared as 10 mg in 10 ml distilled water.
5. **Instrument:** UV 1800 Shimadzu, Japan.

Dillution for Gallic acid: 50gm Gallic acid + 50ml distilled water. (1000 µg/ml).

Procedure
Total soluble phenolic in the extracts were determined with Folin–Ciocalteau reagent according to the method using gallic acid as a standard phenolic compound, 1.0 ml of extract solution containing 1.0 g extract in a volumetric flask was diluted with 46 ml of distilled water. 1.0 ml of Folin–Ciocalteau reagent was added and mixed thoroughly. Three minutes later 3.0 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 3 hr. with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm. The concentration of total phenols was expressed as mg/g of dry extract. The concentration of total phenolic compounds in the extract was determined as µg of gallic acid equivalent using an equation obtained from the standard gallic acid graph.

2.4.2 Total Flavonoid Content
[(Jia et.al, 1999) [5], (Tiwari and Patel, 2012) [6]]

Chemicals: Quercetin, methanol, aluminum chloride, $NaNO_2$, NaOH, Distilled water.

Preparation of solution
1) Quercetin solution:
Add 30mg of Quercetin in 30ml of methanol. From this solution different concentration are preapared.

2) 10% Aq.Aluminium chloride ($Alcl_3$)
Dilute 10gm Aluminum chloride with 100ml of distilled water.

3) Preparation of Extract Solution: (mg/ml) The solution of extract of plant *Sesbania grandiflora* were prepared as, the methanolic extract were prepared as 10 mg in 10 ml methanol and aqueous extract were prepared as 10 mg in 10 ml distilled water.

4) Instrument
UV 1800 Shimadzu, Japan.

Dilution of Quercetin
First dilution 30 mg of quercetin in 30ml methanol (1000 µg/ml)

Procedure
A known volume of extract was placed in a 10 ml volumetric flask. Distilled water was added to make 5 ml, and 0.3 ml Sodium nitroxide ($NaNO_2$), (1:20) were added. 3 ml Aluminum chloride ($AlCl_3$) (1:10) were added 5 min later. After 6 min, 2 ml 1 mol litre^{-1} Sodium hydroxide (NaOH) was added and the total was made up to 10 ml with distilled water. The solution was mixed well again and the absorbance was measured against a blank at 510 nm with a UV-Visible spectrophotometer. Quercetin was used as the standard for a

calibration curve. The flavonoid content was calculated using the linear equation based on the calibration curve.

3. Results

3.1 Preliminary Phytochemical Screening

Preliminary Phytochemical Screening of Various Extracts of bark of *Sesbania grandiflora.*

Table 1: Observation Table for Chemical Test for Extracts of *Sesbania grandiflora.*

Sr. No	Chemical test	Petroleum Ether Ext.	Chloro. Extract	Methanol Extract	Aqueous Extract
1.	**Test for Carbohydrates** a) Molisch Test b) Fehilings Test c) Benedicts Test a) Barfoed's Test	 - - - -	 - - - -	 - - - -	 - - - -
2.	**Test for Proteins** a) Biuret Test b) Millions Test c) Xanthoprotien Test	 - - -	 + + +	 - - -	 - - -
3.	**Test for Amino Acids** a) Ninhydrin Test	 -	 -	 -	 -
4.	**Test for Steroids** a) Salkowski Test b) Liebermann – c) Burchard reaction d) Liebermann's reaction	 + + +	 + + +	 - - -	 - - -
5.	**Test for Glycosides** a) Deoxysugares b) (Killer-KillaniTest) c) Legal's Test d) Brontrager's Test e) Modified Brontrager's f) Test	 - - - -	 - - - -	 + + - -	 + + - -
6.	**Test for Alkaloids** a) Drogendroff's Test b) Mayers Test c) Hagers Test d) Wagners Test	 - - + -	 + + + -	 + + + +	 + + + +
7.	**Test for Flavonoids** a) Lead Acetate b) Sodium Hydroxide c) Ferric Chloride Test	 - - -	 + - -	 ++ + ++	 + + +
8.	**Test for Tannins** a) 5% Ferric Chloride Test b) Lead Acetate Test c) Dilute Iodine Test. d) Dilute Nitric acid Test. e) Potassium Permanganate Solution.	 - - - - -	 + - + - -	 ++ ++ ++ ++ ++	 + ++ + + - ++
9.	Test for Triterpenoids a) Libermann Burchardad's reaction	 ++	 ++	 -	 -
10.	**Test for saponins** a) Foam test	 -	 -	 +	 +

(-) Absent, (+) Less color intensity, (++) More color intensity

3.2 Quantitative Estimation of Phytoconstituents

3.2.1 Total Phenolic Content

Table 2: Absorbance of standard Gallic acid at different concentration

Sr. no.	Concentration	Absorbance
1	10	0.035
2	20	0.073
3	30	0.098
4	40	0.123
5	50	0.188
6	60	0.188
7	70	0.213
8	80	0.244
9	90	0.268
10	100	0.298

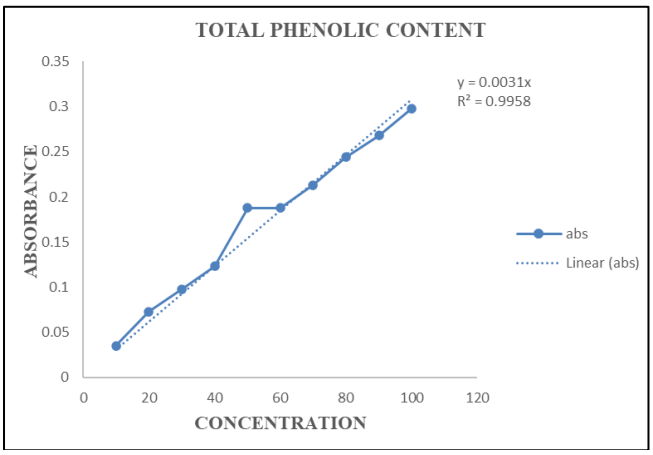

Fig 1: Concentration response curve for standard gallic acid at different concentration.

Table 3: Result of total phenolic content of *Sesbania grandiflora* bark extract

Sr. No.	Sample	Absorbance	Concentrationug/ml
1	Methanolic extract	0.170	54.83
		0.180	58.06
2	Aqueous extract	0.105	33.87
		0.168	54.19

3.2.2 Total Flavonoid content

Table 4: Absorbance of standard qurecetin at different concentration.

Sr.no	Concentration	Absorbance
1	10	0.397
2	20	0.408
3	30	0.424
4	40	0.448
5	50	0.448
6	60	0.456
7	70	0.463
8	80	0.509
9	90	0.609
10	100	0.774

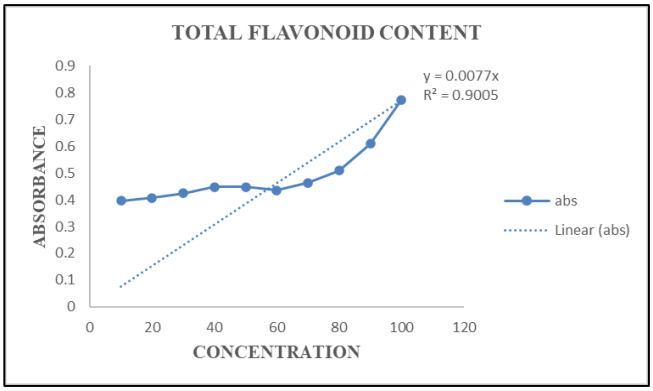

Fig 2: Concentration response curve for standard qurecetin at different Concentration

Table 5: Result of Total Flavonoid content of *Sesbania grandiflora* bark extract

Sr.no	Sample	Absorbance	Concentrationug/ml
1	Methanolic extract	0.398	56.85
		0.42	60
2	Aqueous extract	0.118	16.85
		0.135	19.28

4. Discussion and Conclusion

From preliminary phytochemical screening of extracts of the plant *Sesbania grandiflora*, it was found that in petroleum ether extract contains steroids and triterpenoids, chloroform extract contains proteins, steroids, alkaloids, and triterpenoids etc. while methanolic extract contains glycosides, alkaloids, flavonoids, tannins, saponins and phenolic compounds, in the aqueous extract there is presence of glycosides, alkaloids, flavonoids, tannins, saponins and phenolic compounds.

The phenolic constituent of plant are one of the major group of compounds acting as primary antioxidant or free radical terminators, it was responsible to determine their total amount in plant extract. The content of total phenolic in methanolic and aqueous extract expressed in gallic acid equivalents (GAE) varied between 54.83, 58.06, 33.87, 54.19 µg/ml in metanolic and aqueous extract of plant *Sesbania grandiflora* respectively. (Table No. 3). According to our study the high phenol content in plant *Sesbania grandiflora* extract can explain its high free radical scavenging activity. The total phenolic content of test extract were calculated using standard curve of gallic acid (y =0.0031x) graph. No. 3.1

The Total flavonoid content was determined by Aluminium chloride colorimetric method for methanolic and aqueous extract of bark of plant *Sesbania grandiflora* by using UV Spectrophotometer at absorbance at 510 nm. Total flavonoid content were calculated by using the standard curve of Querecetin (Y=0.0077x) graph no. 3.2. And were expressed in as Querecetin equivalent (QE) per mg of plant extract. The methanolic extract of plant *Sesbania grandiflora* was found to contain higher amount of flavonoid content as compared to aqueous extract of plant *Sesbania grandiflora*. (Table no.4). Flavonoid plays important role in antioxidant. According to our study high content of flavonoid in plant can express its high radical scavenging activity.

5. References

1. Khandelwal KR. “Practical pharmacognosy, Technique and Experiments”, Nirali prakashan 2002;(9):149-159.
2. Shukla Shruti, Mehta A, John J. Antioxidant activity and Total phenolic content of ethanolic extract of *Caesalpinia bonducella* seeds, Food and Chemical Toxicology, 2007;4(2):1848-1851.
3. Chaiyan Boonyuen, Sunanta Wangkarr, Oranart Suntornwat, Rasamee Chaisukasant. Antioxidant Capacity and Phenolic Content of *Mimusops elengi* Fruit Extract, Kasetsart J (Nat. Sci.), 2009;43:21-27.
4. Lydia J, Sudarsanam D. Total phenol and Total Tannin content of *Cyperus rotundus* L, and its medicinal significance, Reaserch Pharm and Tech 2012;5(12):1500-1502.
5. Jia Zhishen, Tang Mengcheng, Jianming Wu. The determination of flavonoid content in *Mulberry* and their scavenging effects on superoxides radicals, Food Chemistry 1999;64:555-559.
6. Tiwari Preeti, Patel Rakesh K. Total phenolic and flavonoids and Antioxidant potential of *Draksharishta* prepared by traditional and modern methods, Research J. pharmacognosy and Phytochemistry 2012;4(5):244-249.
7. Wagner H, Bladt S. “Plant Drug Analysis Thin Layer Chromatography”, Springer New Delhi 2007;(2):196-197,353.
8. Egon Sthal “Thin-Layer Chromatography - A laboratory Handbook”, published by Springer, New Delhi 2007;(2):317-321.

9. Rangari VD, "Pharmacognosy and Phytochemestry", published by Career publication, Nashik 2012;(3):129-138.
10. Jayaprakasam R, Francis Saleshier M. Development And Validation Of HPTLC AND HPLC Methods For The Standerdization of stigmesterol From The Extract of Acalypha Indica and Its Formulation**,** World Journal Of Pharmacy And Pharmaceutical Science 2014;3(5):729-741.
11. Vineeta V Khanvilkar, Preeti N Gunjal. Chromatographic Method Development and Validation for Simultaneous Estimation of Stigmasterol and Embelin. World Journal of Pharmacy and Pharmaceutical Science 2015;4(8):1613-1631.