
PHYTOCHEMICAL AND PRELIMINARY TOXICITY STUDY OF *SESBANIA*
GRANDIFLORA (LINN.) FLOWERS

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Abstract

Sesbania grandiflora Linn. (Family: Fabaceae) is widespread distributed West Bengal, Assam, Karnataka and North-Eastern. The present study intended with various phytochemical screening and toxicity studies were carried out on the flowers of *Sesbania grandiflora*. Preliminary phytochemical evaluation of the methanolic and aqueous extracts of revealed that presence of corbohydrate, proteins, amino acids, saponins, flavonoids, alkaloids, tannins and glycosides. The acute toxicity study was performed to determined LD₅₀ of methanolic extract 200-400 mg/kg, and aqueous extract 250-500 mg/kg.

Keywords: *Sesbania grandiflora*; Phytochemical constituents; Acute toxicity

1. Introduction:

Extractability of plant parts provides an idea regarding the amount of extract present in a definite quantity of drug. The extractability also serves as a tool for quality control of a plant-drug. The toxicity studies drug extracts provide preliminary information regarding the useful properties likely to possessed by the extract and at the same time provide the LD₅₀. Different signs and symptoms during gross observational studies of a drug give an idea regarding the type of drug action and dose to be employed. Therefore, on the basis of toxicities, the therapeutic dose and route of administration of drug can also be known.¹ Photochemicals with biological activity have had great utility as pharmaceuticals and pharmacological actions. These type of activities of herbal drugs are due to the presence of various active principals or phytoconstituents like alkaloids, glycosides, reducing sugar, tannins, saponins, resins, phytosterols, flavonoids, organic acids, essential oils, fixed oils etc. Although in recent times, synthetic drugs are used extensively in modern medicine systems. However many modern medicines are developed through the clues obtained from phytochemicals. More over the phytochemicals are even today are

important resources for medicinal uses. The plant products are becoming more popular than the synthetic drugs due to their low toxicity and long standing experience of exposure of these drugs in ethnic medicine system like Ayurveda.

Sesbania grandiflora (Linn.) belongs to the plant family **Fabaceae** is found in tropical Asia and North Australia. In India it is found at West Bengal, Assam, Karnataka and North-Eastern. It is cultivated as an ornamental plant, grows wild in hedges and shady forests². A short-lived, quick growing, soft-wooded tree, 6.9m height 0.6m in girth; leaves pinnate, 15-30 cm long; leaflets 42-62 liner oblong; flowers 6-10 cm long with showy, fleshy, white, crimson, red or pink petals. Pods 30 cm or longer, rather flat and somewhat 4-cornered, non-torulose, septate with swollen margins and 15-20 pale-coloured seeds^{3,4}.

The present study is designed to identify the phytochemical constituents of the flower and to evaluate the toxicity of various extracts in female albino mice.

2. Methodology

2.1. Plant Material: Flowers of *Sesbania grandiflora* (Linn.) were collected in the month of October and November from Bhopal (M.P.) and Authenticated by Prof. Madhuri Modak plant specimens, Department of Botany, M.V.M. College, Bhopal, Madhya Pradesh, India and voucher specimen has been deposited at the museum of our college.

2.2. Preparation of Extracts: The flowers of *Sesbania grandiflora* (Linn.) were collected and shade dried. The dried flowers were coarse powdered and the powder was packed in to soxhlet column and extracted successively with petroleum ether (60 – 80°C), methanol (60°C) and distilled water. The extracts were concentrated by using rotary flash evaporator under reduced pressure. The dried extracts were stored in airtight container in refrigerator below 10°C. The percentage yield of the each extract shown in **Table No. 1.**

2.2.1. The solution of methanolic and aqueous extracts was prepared using distilled water. Preliminary Phytochemical Screening

The preliminary photochemical screening was carried out on methanolic and aqueous extracts of *Sesbania grandiflora* (Linn.) flowers for the detection of various photochemicals.

The preliminary phytochemical screening was carried out and the results are shown in **Table No. 2.**

2.2.1.1. Test for alkaloids⁵:

(a) Dragendroff's Test: To 2-3 mL. filtrate and few drops Dragendroff's Reagent. Reddish brown colored precipitate is formed.

(b) Hager's Test: To 2-3 mL. filtrate with few drops Hager's Reagent gives yellow Colored precipitate is formed.

(c) Mayer's Test: To 2-3 mL. filtrate with few drops Mayer's Reagent gives cream Colored precipitate is formed.

(d) Wagner's Test: To 2-3 mL. filtrate with few drops Wagner's Reagent gives

Reddish brown colored precipitate is formed.

(e) Tannic Acid Test: To 2-3 mL. filtrates with few drops tannic acid solution gives Buff colored precipitate are formed.

2.2.1.2. Test for carbohydrates⁶:

a) Molish's Test: To 2-3 mL. aqueous extract, add few drops of α - naphthol solution in alcohol. Shake and add conc. H_2SO_4 from sides of the test tube. Violet ring is formed at the junction of two liquids.

b) Fehling's Test: Mix 1 mL. Fehling's A and Fehling's B solutions, Boil for 1 min. add equal volume of test solution. Heat in boiling water bath for 5-10 min. First a yellow, then brick red ppt is observed.

c) Benedict's test: Mix equal volume of Benedict's reagent and test solution in test tube. Heat in boiling water bath for 5-10 min. solutions appears green, yellow or red depending on amount of reducing sugar present in test solution.

d) Barfoed's Test: Mix equal volume Barfoed's reagent and test solution. Heat for 1-2 min. boiling water bath and cool. Red ppt is observed.

2.2.1.2. Test for glycosides⁷:

Test for cardiac glycosides:

a) Legal's Test: To aqueous alcoholic extract, add 1 mL. Pyridine and 1 mL. Sodium nitropursside. Pink to red color arrears.

b) Keller-killani Test: To 2-mL. extract, add glacial acetic acid. One drops 5% $FeCl_3$ and conc. H_2SO_4 Reddish brown color appears at the junction of the two liquid layers and upper layer appears bluish green.

c) Baljet Test: few drop of alcoholic extracts and add few drops of baljet reagent. Solution shows orange or red colour.

Test for Anthraquinone glycosides:

a) Borntrager's Test: To 3-mL. extract, add 5 mL. Dilute H_2SO_4 . Boil and filter, to cold filtrate, add equal volume benzene or chloroform. Shake well. Separated the organic solvent. Add ammonia ammoniacal layer turns pink or red.

b) **Modified Borntrager's Test:** To 5 mL. extract, add 5 mL. 5% FeCl_3 and 5 mL. dilute HCL. Heat for 5 min. in boiling water bath. Cool and add benzene or organic solvent, shake well, separated organic layer, equal volume dilute ammonia ammonical layer shows pinkish red color.

2.2.1.3. Test for saponins:

Foam Test: Shake the drug extract or dry powder vigorously with water, persistent foam observed.

2.2.1.4. Test for fats and oils: -

Place a thick section of drugs on glass slide. Add a drop of Sudan red III reagent. After 2 min. wash with 50% alcohol. Mount in glycerin. Observe under microscope. Oil globules appeared.

(a) To thin sections add a drop of 1% osmeic acid. After 1 min., observe under microscope. Oil drops appear black.

(b) Filter paper gets permanently stained with oils.

(c) Extracts gives red color with 2-3 drops of tincture alkana.

(d) Saponification Test: Evaporate extract to get 120 mL. of oil. To oil add 25 mL. 10% NaOH. Boil in boiling water bath for 30 min. cool. Add excess Na_2SO_4 solution. Soap forms and rise to the top. Filters add H_2SO_4 . Evaporate collect residue. It contains glycerol. Dissolve residue in ethanol. With ethanolic solution performing following tests.

(1) To ethanolic solution, add few crystals of KHSO_4 . Heat vigorously. Pungent odour of acrylic aldehyde is produced.

(2) To ethanolic solution, add few drops of CuSO_4 . And NaOH solution. Clear blue solution is observed.

2.2.1.5. Test for flavanoids⁷:

a) **Shinoda Test:** To dry powder or extract, add 5 mL. 95% ethanol few drops conc. HCL and 0.5 g. magnesium turning pink colored observed.

b) To small quantity of residue, add lead acetate solution. Yellow colored precipitate is formed.

2.2.1.6. Addition of increasing amount of sodium hydroxide to the residue shows

yellow coloration, which decolorizes after of acid.

2.2.1.7. Test for tannins and phenolic compounds⁵:

a) **Ferric chloride solution Test:** To 2-3 mL. of aqueous extract, add few drop of 5% ferric chloride solution gives deep blue-black color.

b) **Lead acetate solution Test:** To 2-3 mL. of aqueous extract, add few drop of Lead acetate solution gives white ppt.

c) **Iodine solution Test:** To 2-3 mL. of aqueous extract, add few drop of dilute iodine solution gives transient red color.

d) **Acetic Acid solution Test:** To 2-3 mL. of aqueous extract, add few drop of Acetic Acid solution gives red color solution.

e) **Bromine water Test:** To 2-3 mL. of aqueous extract, add few drop of Bromine water Decolouration of bromine water.

2.2.1.8. **Dilute HNO_3 Solution Test:** To 2-3 mL. of aqueous extract, add few drops of dilute HNO_3 Solution gives reddish to yellow color.

2.2.1.9. Test for protein and free amino acids:

Test for protein:

a) **Biuret Test:** To 3 mL. of test solution and 4% NaOH and few drops of 1% CuSO_4 Solution. Violet or pink color appears.

b) **Million's Test:** Mix 3 mL. Test solution with 5 mL. Million's Reagent. White ppt. warm ppt turns brick red or the ppt dissolves giving red colored solution.

c) **Xanthoprotein Test:** Mix 3 mL. Test solution with 1 mL. conc. H_2SO_4 , white ppt is formed. Boil precipitate turns yellow. Add NH_4OH . Ppt turns orange.

D) **Test for protein containing sulphur:** Mix 5 mL. Test solution with 2 mL. 40% NaOH and 2 drops 10% lead acetate solution. Boil solution turns black or brownish due to PbS formation.

E) Test for amino acid⁸:

a) **Ninhydrin Test:** Heat 3 mL. Test solution and 3 drops 5% Ninhydrin

solution in Boiling water bath 10 min. purple or bluish color appears.

- b) **Test for tyrosine:** Heat 3 mL. test solution and 3 drops Million's Reagent. Solution shows dark red color.
- c) **Test for cystine:** To 5 mL. test solution and few drops of 40% NaOH and 10% lead acetate solution. Boil black ppt. of lead acetate sulphate is formed. (Singh S.P. et al. 2004)

2.2.1.10. Test for steroid⁷:

- a) **Salkowski Reaction:** To 2 mL. of extract, add 2 mL. Chloroform add 2-mL. conc. H₂SO₄. Shake well chloroform layer appears red and acid layer shows greenish yellow fluorescence.
- b) **Liebermann-burchard Reaction:** Mix 2 mL. extract with chloroform. Add 1-2 mL. acetic anhydride and 2 drops conc. H₂SO₄ from the side of the test tube, first red, and then blue finely green color appears.

2.2.1.11. **Liebermann Reaction:** Mix 3 mL. extract with 3 mL. acetic anhydride. Heat and cool. Add few drops conc. H₂SO₄ Blue color appears.

2.2.1.12. Test for gums and mucilages⁹:

Test for gums:

Hydrolyse Test solution using dilute HCL. Performing Fehling's or Benedict's Test. Red color is developed.

Test for mucilages:

- Powdered drug material shows red coloration with ruthenium red.
- Powdered drug swells in water or aqueous KOH.

2.2.1.13. Test for starch:

- a) **Iodine Test:** Mix 3 mL. of test solution and few drops of dilute iodine solution. Blue colour appears. It disappears on boiling and reappears on cooling.
- b) **Tannic Acid test for starch:** with 20% Tannic acid. Test solution gives ppt. (Kokate C.K. et al. 2008)

2.3. Animals used

Wistar mice (24-30 g) of either sex procured from DRDE, Gwalior (Madhya Pradesh, India) were used for this study. They were maintained under standard conditions (temperature 22 ±20°C, relative

humidity 60±5% and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol. All the procedures were performed in accordance with Institutional Animal ethics committee constituted as per the direction of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), under ministry of animal welfare division. Government of India, New Delhi, India. Animal ethical clearance for performing the experiments on animals was obtained from the Institutional Animal Ethical Committee (IAEC).

2.3.1. Determination of acute toxicity (LD₅₀)¹¹.

The acute toxicity for methanolic and aqueous extracts of flowers was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment, fixed dose method was adopted as per OECD Guideline No. 423 of CPCSEA.

3. Results

3.1. Preliminary phytochemical screening

Preliminary phytochemical screening was carried out for the presence of carbohydrate, proteins, amino acids, saponins, flavonoids, alkaloids, tannins and glycosides for petroleum ether, ethanolic and aqueous extracts of flowers of *Sesbania grandiflora* (Linn.). Results are shown in Table No. 2

3.2 Determination of acute toxicity (LD₅₀)

As per GHS classification, methanolic and aqueous extracts of *Cocculus hirsutus* (leaves) and *Sesbania grandiflora* (flowers) were found to be of class 4 (>300 to 2000 mg/kg, b.w.) and class 5 (>2000 to 5000 mg/kg, b.w.); However, on the basis of mortality in either steps of acute oral toxicity test guidelines 423, different LD50 cut-off dose (in mg/kg, b.w.) were determined. Accordingly, the individual

effective lower dose and higher dose were determined (in mg/kg, b.w.) as 1/10th and 1/5th of the respective LD50 cut-off dose. Results have been summarized in **Table No.3**

No adverse effects were observed on the respiratory, circulatory, autonomic and central nervous systems and somatomotor activity. Also, the behavioural pattern was found to be normal throughout the study period. Apart from the moribund rats, none showed any sign of abnormal change in skin and fur, eyes and mucous membranes. Tremors and convulsions were also not observed in any of the survivor.

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Table No. 1. Percentage yield of crude extracts of *Sesbania grandiflora* (Linn.) flowers.

S. No.	Solvent	Colour and Consistency	Percentage yield (%)
1	Methanol	Blackish green and pasty	16.25
2	Aqueous	Brownish and pasty	8.77

Table No. 2. Preliminary phytochemical screening of *Sesbania grandiflora* (Linn.) flowers

S.No	Tests	Extracts	
		Methnolic extract	Aqueous extract
1.	Carbohydrates		
	Molisch's test	++	+
	Fehling test	++	+
	Benedict's test	++	+
	Barfoed test	+	+
2.	Proteins & amino acids		
	Biuret test	+	+
	Millons test	+	+
	Ninhydrin test	+	+
3.	Glycosides		
	Legal test	+	-
	Baljet's test	+	-
	Keller killani test	+	-
	Borntegers test	+	-
	Modified Borntegers test	+	-
4.	Flavonoids		
	Shinoda test	++	-
	Lead acetate test	+	-
	NaOH test	++	-
	H ₂ SO ₄ test	+	-
5.	Alkaloids		
	Wagner test	++	+
	Hager test	+	+
	Mayer test	++	+
	Dragendorff test	+	+
6.	Tannins and Polyphenolic		
	5%FeCl ₃ Solution	++	+
	Acetic acid test	-	-
	Lead acetate test	+	+
	Dil. Iodine solution test	+	+
	Dil. Potassium permanganate	++	+
	Dil. Nitric acid test	+	-
7.	Saponin		
	Foam test	++	+

+ present ; ++ more clarity; +++ better response

Table No.3. Acute oral toxicity *Sesbania grandiflora* (flowers)

S. No	Extract	GHS Category	Dose Calculations (mg/kg, b.w		
			LD50 Cut-off value	Lower effective dose	Higher effective dose
1	MESG	Category 4	>300 to 2000	200	400
2	AESG	Category 5	>2000 to 5000	250	500