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# STUDIES ON THE PHYTOCHEMICALS IN LEAVES OF *SESBANIA GRANDIFLORA*(L.) POIRET AND IT'S CYTOTOXIC ACTIVITY ON ROOT TIP OF *ALLIUM CEPA* L.

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***Abstract***

Qualitative phytochemical screening in dried leaf powder of *Sesbania grandiflora* showed the presence of carbohydrates, alkaloids, flavonoids, saponins, terpenoids, steroids, tannins and cardiac glycosides. Quantitative phytochemical analysis of the leaves revealed the total amount of carbohydrates (49.6mg/g), reducing sugars (1.24mg/g), protein (73.1mg/g), Total chlorophyll (1.30mg/g), chlorophyll a (0.52 mg/g), chlorophyll b (0.78mg/g), carotenoids (0.012 mg/g), phenol (33.55mg/g) and tannin (1.23mg/g). Mitotic study in the pre-treated roots of *Allium cepa* L. in aqueous leaf extract of *S. grandiflora* revealed various types of chromosomal aberrations such as nuclear lesions, metaphase clumping, diagonal metaphase, anaphase clumping, strap shaped nuclei and binucleated cells. The mitotic index of root cells decreased with increase in concentration of aqueous leaf extract of *S.grandiflora*. Mitotic index were low (7.78%) in roots treated with 100% concentration of aqueous leaf extract when compared to those (16.67%) in 50% concentration of leaf extract, indicating it's cytotoxic activity at higher concentrations. Lower concentration of 50% concentration enhanced the mitotic activity when compared to control (11.5%). The study recommends the use of the leaves as vegetable due to it's high protein and carbohydrate content. But precaution has to be taken to avoid high doses of the leaves when used for medicinal purposes.

**Key words:** *Sesbania grandiflora*, Phytochemicals, cytotoxic**,** *Allium cepa*

## Introduction

*Sesbania grandiflora* (L) Poiret, a member of Papilionaceace is a fast growing perennial medicinal tree commonly called 'vegetable humming bird tree' or 'agati'. The juice of leaves are considered as antihelminthic and tonic and is used to treat worms, biliousness, fever, gout, itchiness and leprosy (Duke , 1983). The young leaves and the tender pod of the plant are used to supplement meals and as vegetables (Galeano *et al*., 2003). As a fast-growing N-fixing legume, it is used for the reforestation of eroded areas and to improve soil fertility (Heering and Gutteridge,1992). The present study aims to screen the phytochemicals of leaves of *Sesbania grandiflora* to assess the edibility of the leaves and it's nutritional and medicinal properties. The work also aims to assess it's cytotoxic activity on the root meristematic cells of *Allium cepa* L.

## Materials and methods

### SOXHLET EXTRACTION

The leaves of *Sesbania grandiflora* were collected from different places in Kollam district, Kerala. They were separated and washed well under tap water and then in distilled water to remove soil and other debris. They were then air-dried in shade. After drying, the leaves were ground to powder using blender and stored in cool dry air-tight containers for further studies. For phytochemical analysis of the leaves, both aqueous and ethanolic extracts were taken separately using Soxhlet's extraction apparatus. In each solvent the extraction was continued for a minimum of 8 hours until no plant residues was left in the recycled solvents. The excess solvents were separated by distillation. The aqueous and ethanolic extracts were concentrated separately on a hot plate. They were stored at 4ºC for phytochemical studies. The phytochemical screening were done by qualitative and quantitative methods using standard procedures .Harborne (1998) , Sadasivam and Manickam (1996).

### PHYTOCHEMICAL STUDY (QUALITATIVE)

#### Test for Carbohydrate (Molisch's Test)

Two drops of freshly prepared Molisch's reagent was added to 2ml each of ethanolic and aqueous extracts taken in two different test tubes. They were mixed properly and 1ml of concentrated sulphuric acid was added along the sides of the test tubes. Presence of reddish violet colour at the junction of the liquids indicated the presence of carbohydrate.

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**Test for Alkaloids (Mayer's test)**

0.5g of extract powder of ethanol and water was treated with 5ml of 1% HCl in separate boiling tube on a water bath. Then the solutions were filtered. 1ml each of filterates were taken in a test tube and few drops of freshly prepared Mayer's reagent was added. An organic white precipitate indicated the presence of alkaloids.

**Test for flavonoids (Sulphuric acid test)**

1ml each of ethanolic extract and aqueous extract were treated separately with few drops of sulphuric acid . Formation of orange colour indicated the presence of flavonoids.

**Test for cardiac glycosides (Keller- Klliani test)**

To 10 ml each of aqueous and alcoholic extracts taken in different test tubes, 3ml of 5% glacial acetic acid was added. To this a drop of 1% ferric chloride solution was added to them. They were transferred to different tubes containing 2 ml of concentrated sulphuric acid. A Reddish brown colour at interface indicated the presence of cardiac glycosides.

**Test for saponin (Foam test)**

10ml of aqueous and ethanolic extracts were shaken vigourously in different test tubes for 15 minutes. Presence of persistent froth of 1cm height indicated the presence of saponin.

**Test for steroids (Salkowski test)**

1ml of aqueous and alcoholic extracts were dissolved separately in 10ml of chloroform in different test tubes. Equal volume of concentrated sulphuric acid was added by sides of the test tubes .The upper layer turns red and sulphuric acid layer showed yellow .This indicated the presence of steroids.

**Test for tannin (Ferric chloride test)**

2ml of aqueous and ethanolic extracts were taken separately in two test tubes. A few drops of 5% Ferric chloride solution was added to both tubes. Blue colour indicated the presence of condensed tannin.

**Test for terpenoids Salkowski test)**

1ml of plant extracts ( Ethanolic and aqueous ) were separately treated with 2ml of chloroform and 1ml of concentrated Sulphuric acid .Reddish brown colour at the interface indicated the presence of terpenoids.

**PHYTOCHEMICAL STUDIES (QUANTITATIVE)**

**Estimation of Carbohydrates (Anthrone method)**

Weighed 100 mg of powdered leaves of *Sesbania grandiflora* in a boiling tube. Hydrolysed by keeping it in a boiling water bath for 3 hours with 5 ml of 2.5 N HCl and cooled to room temperature. Neutralized it with solid sodium carbonate until the effervescence ceased. Made up the volume to 100 ml and centrifuged. Collected the supernatant and 1.0 ml aliquot for analysis was taken. Prepared the standard (Glucose stock-100mg dissolved in 100ml distilled water). Pipetted out 0.2ml, 0.4ml, 0.6ml, 0.8ml, and 1.0 ml of the working standard (10ml stock diluted to 100ml with distilled water) in different test tubes. Distilled water served as the blank. Made up the volume to 1.0 ml in all tubes including the sample tube by adding distilled water. Then added 4 ml of freshly prepared Anthrone reagent. Heated for 8 minutes in a boiling water bath. Cooled rapidly to get the green to dark green colour and absorbance read at 630 nm using UV/Vis Spectrophotometer. Standard graph of glucose prepared by plotting concentration of the standard on the X- axis and absorbance on Y- axis. From the graph calculated the amount of carbohydrate present in the sample and applied in the formula.

**Calculation**

Amount of carbohydrates present in 100 mg of sample =

$$\frac{\text{mg of glucose}}{\text{volume of test sample}} \times 100$$

**Estimation of reducing sugar (DNS method)**

Weighed 1.0 g of the leaf powder of *S.grandiflora* and extracted sugar with 10 ml of distilled water. Centrifuged and collected the supernatant . Prepared

the standard (Glucose stock-100mg dissolved in 100ml distilled water). Pipetted out 0.2ml, 0.4ml, 0.6ml, 0.8ml, and 1.0 ml of the working standard (10ml stock diluted to 100ml with distilled water) in different test tubes. Made up the volume to 2.0 ml with distilled water. (Distilled water for control sample).Pipetted out 0.2ml of sample solution to a test tube and made up the volume to 2.0 ml with distilled water.Added 3 ml of DNS reagent to sample and control tubes. Heated the contents in a boiling water bath for 5 min. When the contents of the tubes were still warm added 1.0 ml of 40% Rochelle salt solution. Cooled the test tubes and read the absorbancy at 510 nm using UV/Vis Spectrophotometer. Control sample is devoid of sample. Prepared Standard graph of glucose by plotting concentration of the standard on the X- axis and absorbance on Y- axis. Calculated the amount of reducing sugar present in the sample as in formula.

**Calculation**

Amount of reducing sugar present in the sample (mg/g) using standard graph

$$\frac{\text{Conc. of Standard} \times \text{OD of Sample} \times \text{Total Volume of Extract}}{\text{OD of Standard} \times \text{Volume of Sample} \times \text{Weight of Tissue}}$$

**Estimation of protein (Bradford method)**

1.0 g of of fresh leaves of *S.grandiflora* was taken in a mortar and ground well with 15 ml of 0.1 M phosphate buffer with pH 7. Filtered the homogenate and centrifuged the filtrate at 10,000 rpm for 10 min. and used the supernatant for estimation. Pipetted out 0.02ml, 0.04ml, 0.06ml, 0.08ml and 0.1ml of BSA stock solutions (100mg Bovine Serum Albumin in 100ml distilled water) in test tubes. Made up the volume to 1.5 ml with 0.1 M phosphate buffer. (0.1M $PO_4$ buffer as control). Pipetted out 0.02 ml of the sample and made upto 1.5 ml with 0.1 M phosphate buffer. Mixed with equal volume of Coomassie brilliant blue reagent and incubated them for 5 min at room temperature. Measured the absorbance at 595 nm against a blank containing reagent and buffer without protein using UV/Vis Spectrophotometer. Prepared Standard graph of BSA by plotting concentration of the standard on the X- axis and absorbance on Y- axis. Calculated the amount of protein present in the sample as in formula.

**Calculation**

The protein concentration was calculated from the standard graph of BSA drawn by Bradford Method

Total soluble protein (mg/g)

$$= \frac{\text{Conc of Standard} \times \text{OD of the Sample} \times \text{Volume of the Extract}}{\text{OD of the Standard} \times \text{Volume of Sample} \times \text{Weight of tissue}}$$

**Estimation of chlorophyll and carotenoids (Arnon, 1949)**

100mg of the leaf material of *Sesbania grandiflora* was taken and ground in mortar and pestle using 20ml of 80% acetone. Filtered the homogenate and centrifuged at 5000 rpm for 5min. Transferred the supernatant to a 50ml volumetric flask. Ground the pellet with 20ml of 80% acetone, centrifuged and transferred the supernatant to the same volumetric flask. Repeated the procedure till the pellet was colourless. Made up the volume of the supernatant to 50ml with 80% acetone in a volumetric flask. Read the absorbance of the supernatant at 645, 663 and 480nm against a blank (80% acetone) using UV/Vis Spectrophotometer. Chl a, Chl b , total chlorophyll and carotenoids were calculated using the formula:

Total chlorophyll ( mg/g) =20.2

$$(A\,645) + 8.02(A\,663) \times \frac{V}{1000\,W}$$

Chlorophyll a (mg/g) = 12.7

$$(A\,663) - 2.69(A\,645) \times \frac{V}{1000W}$$

Chlorophyll b (mg/g) = 22.9

$$(A\,645) - 4.68(A\,663) \times \frac{V}{1000\,W}$$

Amount of total carotenoid (mg/g) =

$A_{480+}[(0.114 \times A_{663})-(0.638 \times A_{645})] \times v/1000xw$

(A= Absorbance at specific wavelengths, V=Total volume of the extract W= weight of tissue)

**Estimation of total phenol (Folin- Ciocalteu method)**

1 g of leaf sample of *S.grandiflora* was ground with 10 ml of 80% ethanol in mortar and pestle. Filtered the homogenate and centrifuged at 1000 rpm for 20 minutes. Evaporated the supernatant to dryness in a petriplate by keeping it in hot air oven at $60^0$C .The dried precipitate was then dissolved in 5 ml of distilled water. Pipetted out 0.1 ml of the suspension in a test tube and made up the volume to 3ml with distilled water. Prepared standard stock solution of Catechol by dissolving 100mg in 100ml distilled water which was diluted -

10 times to prepare working standard. Pipetted out 0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of working standard solution in different test tubes and made up the volume to 3ml with distilled water. Added 0.5 ml of Folin -Ciocalteau reagent to each test tube and after 3 min. added 2 ml of 20% Sodium carbonate and mixed well .Placed the tubes in boiling water-bath for 1 minute and then cooled the tubes. Read the absorbancy at 650nm against a reagent blank using UV/Vis Spectrophotometer. Standard graph of Catechol was prepared by plotting concentration of the standard on the X- axis and absorbance on Y-axis. From the graph the amount of phenol present in the sample was calculated.

**Estimation of tannin (Folin- Denis method)**

Weighed 0.5 g of powdered leaf material of *S.grandiflora* and transferred to 250ml Erhlenmeyer flask. Added 75 ml distilled water. Heated the flask gently and allowed to boil for 30 min. Centrifuged at 2000 rpm for 20 min and collected the supernatant in 100 ml volumetric flask and made up the volume. Transferred 1 ml of the sample extract to a 100ml volumetric flask containing 75ml water. Added 5ml of Folin- Denis reagent, 10 ml of sodium carbonate solution ( 35g in100ml distilled water) and diluted to 100 ml with distilled water. Shaken well and read the absorbance at 700nm after 30 min using UV/Vis Spectrophotometer. Prepare a blank with water instead of the sample. Dissolved 100mg tannic acid in 100ml distilled water (Stock standard). Diluted 5 ml of stock standard to 100ml with distilled water (working standard= 50µg/ml). Prepared standard graph by pipetting out 0.25ml, 0.5ml, 1.0ml., 1.5ml and 2ml of tannic acid solution. Calculated the tannin content of the sample as tannic acid equivalent from the standard graph.

**Cytotoxic studies on root meristematic cells of Allium cepa L.**

Uniform sized bulbs of *Allium cepa* were planted in sterilized sandy soil without manure to prevent cellular alterations. Geminated bulbs with healthy roots of about 1-2cm length were collected at peak mitotic period (9AM to 10AM). They were carefully washed in distilled water. For cytotoxic studies, they were treated by suspending them in different concentrations (50% and 100%) of aqueous leaf extract of *Sesbania grandiflora* for 2 hours. Treatment in distilled water served as control samples. Micropreparations of the Control and treated root tips were done by excising a few healthy root tips and washing them thoroughly in distilled water and fixed immediately in ethanol and glacial acetic acid mixture (2:1) for I hour. After hydrolysis in 1N HCl, they were thoroughly washed with distilled water and squash preparations were made with improved techniques using 2% Acetocarmine (Sharma and Sharma, 1980). Chromosomal aberrations were observed under 40X magnification and photomicrographs taken using OLYMPUS CH 20i Binocular stereomicroscope. To calculate Mitotic Index (MI) of control and treated samples, total number of dividing cells and the total number of cells were counted from five different fields of view using high power (40X) of Olympus microscope. MI (%) were calculated using the formula Total number of dividing cells x 100/ Total number of cells in field.

**Results and Discussion**

The qualitative screening of phytochemicals in the leaf powder of *Sesbania grandiflora* (L.) Poiret revealed the presence of carbohydrates, alkaloids, flavonoids, cardiac glycosides, saponins, steroids, tannins and terpenoids in ethanolic extract while flavonoids and cardiac glycosides were absent in aqueous extract.

**Quantitative phytochemical analysis of the leaves of *S.grandiflora***

**Carbohydrates** (Anthrone method) – The optical density(OD) of the sample was 0.2238 and the corresponding concentration of the sample from standard graph was 0.0496mg/ml i.e. 49.6mg/g tissue as per given formula. The values obtained for preparation of standard graph of glucose are presented in Table 1.

**Reducing sugar** (DNS method) - The OD of the sample was 0.063. The corresponding concentration of the standard glucose from the standard graph was 0.152 at 0.06 mg/ml concentration (Table 2). The amount of reducing sugar in *Sesbania grandiflora* leaves was calculated as 1.24mg/g tissue.

**Protein** (Bradford method) - The OD of the sample was observed as 0.307. The OD of the standard (BSA) was 0.126 at 0.04 mg/ml concentration of BSA (Table 3). The total soluble protein in the

leaves of *Sesbania grandiflora* calculated as per formula was 73.095mg/g tissue.

**Total Chlorophyll**- The OD of the sample at 645nm and 663nm were 0.089 and 0.101 respectively. Therefore the total chlorophyll in leaves of *S. grandiflora* leaves was 1.30 mg/g tissue. Amount of Chlorophyll a and Chlorophyll b were 0.52mg/g tissue and 0.78 mg/g tissue respectively.

**Carotenoid** – The OD of the sample at 480nm, 645nm and 663nm were 0.069, 0.089 and 0.101 respectively. Therefore the amount of carotenoid in the leaves was 0.012 mg/g tissue.

**Phenol** (Folin- Ciocalteu method) - OD of the sample at 650 nm was 0.368. The concentration of the sample corresponded to 0.33547 mg/ml in standard graph (Table 4). The amount of phenol was 33.54mg/g tissue.

**Tannin** (Folin –Denis method) - OD of the sample at 700 nm was 0.3641. From the standard graph of tannic acid corresponding concentration of the sample was 61.4299 µg/ml (Table 5). The total tannin content in the leaf of *S. grandiflora* was calculated as 1.23 mg/g tissue.

## Cytotoxic assay

Mitotic study in the pre-treated roots of *Allium cepa* L. in aqueous leaf extract of *S. grandiflora* revealed various types of chromosomal aberrations (Figure 1-8) such as nuclear lesions, metaphase clumping, anaphase clumping, strap shaped nuclei, diagonal metaphase and binucleated cells. The mitotic index (MI) of root cells decreased with increase in concentration of leaf extract of *S.grandiflora*. Mitotic index was low (7.78%) in roots treated with 100% concentration of aqueous leaf extract compared to those treated in 50% concentration of aqueous leaf extract (16.67%), indicating it's cytotoxic activity at higher concentrations. The MI in control root tips was 11.5%. The lower concentration has shown to have enhancement effect on mitotic activity of onion root tip cells.

The phytochemical study is in accordance to the findings of Reji and Alphonse (2013). They reported the presence of carbohydrates, proteins, flavonoids, alkaloids, tannins, and glycosides in the leaves of *S.grandiflora*. The secondary metabolites indicate the high therapeutic value of the plant. High content of protein and carbohydrates supports their use as vegetable (Galeano *et al*., 2003). In order to initiate the search for drugs from plants, the antimitotic activity of the extract were studied by *Allium cepa* assay (Levan, 1949). The *Allium cepa* root meristem assay is widely considered as a practical and reliable system for screening environmental mutagens and carcinogens ( Fiskes – Jo, 1985;Stich *et al*., 1975). Chromosomal aberrations were observed in both concentrations of the treated root tips of *A.cepa.* Decrease in mitotic index of the root meristematic tissue of *A.cepa* with increase in concentration of the aqueous leaf extract indicated cytotoxic effect of the leaves of *S.grandiflora* at high concentrations.

## Conclusion

The phytochemical evaluation of the leaves of *Sesbania grandiflora* (L.) Poiret indicates the high therapeutic value of the plant due to their secondary metabolites. It also confirms their use as vegetable as indicated by their high protein and carbohydrate content. The high phenol content also indicates it's antioxidant property. Cytotoxicity in the form of chromosomal aberrations and mitotic inhibition were observed in meristematic root tip cells of *Allium cepa* L. treated with high concentration of crude aqueous leaf extracts of *Sesbania grandiflora.* This indicates the need for using the leaf of *Sesbania grandiflora* only at low concentrations even for medicinal purposes.

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**Table 1.** Values obtained for Standard graph of glucose (Anthrone method)

| Concentration of Glucose (mg/ml) | Absorbance (630nm) |
|---|---|
| 0.02 | 0.111 |
| 0.04 | 0.166 |
| 0.06 | 0.280 |
| 0.08 | 0.368 |
| 0.10 | 0.424 |

**Table 2.** Values obtained for standard graph of glucose (DNS method)

| Concentration of Glucose (mg/ml) | Absorbance (510nm) |
|---|---|
| 0.02 | 0.051 |
| 0.04 | 0.098 |
| 0.06 | 0.152 |
| 0.08 | 0.203 |
| 0.10 | 0.257 |

**Table 3.** Values obtained for standard graph of BSA (Bradford method)

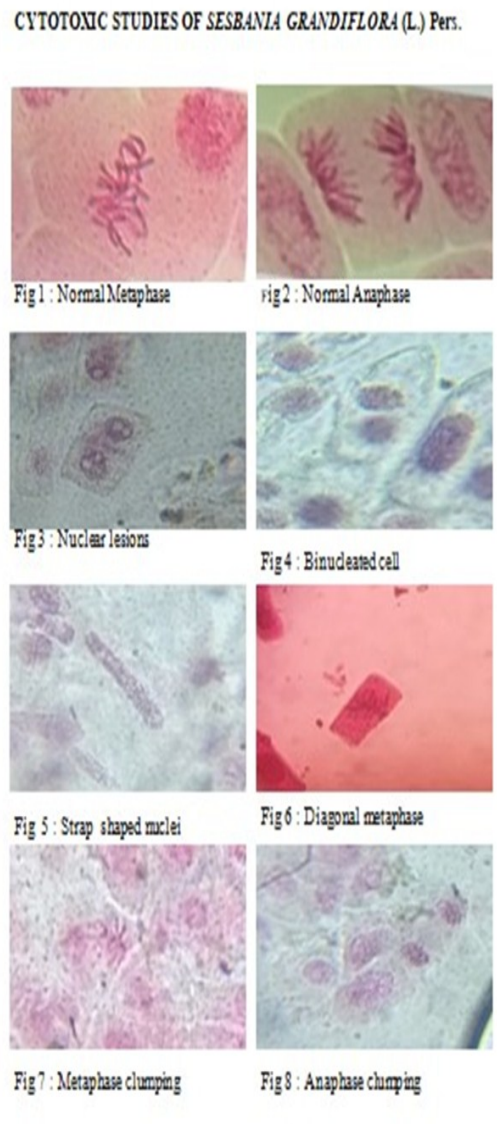
| Concentration of BSA (mg/ml) | Absorbance (595nm) |
|---|---|
| 0.02 | 0.083 |
| 0.04 | 0.126 |
| 0.06 | 0.189 |
| 0.08 | 0.225 |
| 0.10 | 0.237 |

**Table 4.** Values obtained for standard graph of Catechol (Folin-Ciocalteu method)

| Concentration of Catechol (mg/ml) | Absorbance (650nm) |
|---|---|
| 0.01 | 0.131 |
| 0.02 | 0.221 |
| 0.03 | 0.332 |
| 0.04 | 0.442 |
| 0.05 | 0.532 |

**Table 5.** Values obtained for standard graph of Tannic acid (Folin-Denis method)

| Concentration of Tannic acid (µg/ml) | Absorbance (700nm) |
|---|---|
| 15 | 0.080 |
| 25 | 0.114 |
| 50 | 0.280 |
| 75 | 0.420 |
| 100 | 0.595 |

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