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## Pharmacognostical and phytochemical evaluation of *Cassia alata* Linn

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#### Abstract

Medicinal plants are basic source of medicine in Indian traditional system medicines such as Ayurveda, Homeopathy and Siddha system of medicine. Medicinal plants are commonly used in treating, curing and preventing various ailments and are considered to play a beneficial role in health. *Cassia alata* Linn belongs to the family Caesalpiniaceae, is the one of promising medicinal plant used to treat various type of disease. The objective of present study is used to investigate the pharmacognostical and phytochemical evaluations of *Cassia alata* furnish useful information about its right identity and also give the exact information of presence of secondary metabolites in it. The identification and quality standardization of the plant, it is necessary that study of anatomical features and physicochemical standards of the plant. A detailed morphological evaluation, physicochemical evaluation and phytochemical screenings are mandatory for facilitate to avoid any misidentification of the plants. For this reason, the current research consists of anatomical and structural evaluations of the leaf, stem and powder microscopy along with the assessment of physicochemical parameters, fluorescence analysis and preliminary phytochemical screening.

**Keywords:** *Cassia alata* Linn, secondary metabolites, phytochemical analysis, flavonoids

#### 1. Introduction

Plant which have one or more of its parts having substances that can be used for treatment of diseases are called medicinal plants<sup>1</sup>. Medicinal plants are more significant to the health of individual and community. Medicines derived from plants are advantages and widely famous due to the safety, easy availability and low cost. Herbal medicine may include whole parts of plants are mostly prepared from different part of the plants. They are administered orally, inhaled are directly applied in the skin<sup>2</sup>.

The medicinal benefit of these plants lies in presence of secondary metabolites or phytochemicals that produce definite physiological action on the human body<sup>3</sup>. Some of the most important classes of secondary metabolites or phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compound and many more<sup>4</sup>. These natural compounds formed the basis of modern prescription drugs as we known today<sup>5</sup>. Use of the plants based drugs for a curing various ailments is as old as human civilization and is used in all cultures throughout history. The ancient man started to differentiate between useful and harmful are poisonous plant by trail and errors.

*Cassia alata* Linn belongs to the family Caesalpiniaceae. It is native to many tropical countries. It is an annual or biannual shrub with a nasty smell, 1–4 m tall, preferring sunny and moist areas. The leaves are yellowish-green, broad, with 5–14 leaflet pairs, the distal ones often larger and with a notched apex. Zygomorphic flowers are a bright yellow and form a generally simple erect raceme, evoking a dense golden spike or rod. They contain 7 stamens, 2 of which are much longer and a pubescent ovary. The fruit is a 10–16×1.5 cm tetragonal pod, winged on the angles, brown when ripe and containing numerous (up to 60) diamond-shaped brown seeds<sup>6</sup>. It is known as Ringworm shrub and winged senna in English; Dadrughna and Dvipagsti in Sanskrit; semaiagathi and Vandugolli in Tamil<sup>7-8</sup>.

Ethnomedicinally, the leaves are used as a purgative, expectorant, astringent and as a mouthwash<sup>9</sup>. The leaves are also specific for the treatment of ringworm and eczema<sup>10</sup>, scabies, athlete's foot<sup>11</sup>, herpes<sup>12</sup> and insect bites<sup>13</sup>. Various extracts and different parts of *Cassia alata* have been reported to own many pharmacological activities such as laxative<sup>14</sup>, antifungal<sup>15</sup>, wound healing<sup>16</sup>, hypoglycaemic<sup>17</sup>, anti-bacterial<sup>18</sup>, anthelmintic<sup>19</sup>, analgesic<sup>20</sup>, anti-inflammatory<sup>21</sup>, Abortifacient<sup>22</sup> and Anti-lipogenic activity<sup>23</sup>.

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## 2. Materials and Methods

### 2.1 Plant Material

Fresh specimens of leaflets and stem of *Cassia alata* were collected from Cuddalore districts of Tamil Nadu during a month of November 2017. This was subsequently authenticated and identified by Prof. P. Jayaraman, Plant Anatomy Research Centre, Chennai, Tamil Nadu and India. The voucher herbarium specimen (PARC/2018/3711) has been preserved in our laboratory for further reference.

### 2.2 Preparations of plant materials

The freshly collected aerial part of the plant was air-dried under shade at room temperature for 2 weeks. After drying the plant materials were pounded separately using mortar and pestle into smaller particles and then subjected grounded to fine powder using an electric blender and passed through a 22 mesh sieve. The powdered samples were stored in air-tight containers and kept at room temperature until required.

### 2.3 Preparation of various extracts from *Cassia alata*

The aerial parts of *Cassia alata* were dried and powdered. The plant powdered materials were successfully extracted with petroleum ether (40-60 °C) by hot continuous percolation method in Soxhlet apparatus [24] for 24 hrs. Then this marc was dried and then subjected to chloroform extraction (60 °C) for 24 hrs, then marc was dried and then it was subjected to methanol extraction (80 °C) for 24 hrs. The extracts were filtered and concentrated using rotary flash evaporator and residues were dried in desiccators over sodium sulphite below 60 °C.

$$\left. \begin{array}{l} \text{Percentage yield with} \\ \text{reference to crude plant} \\ \text{material} \end{array} \right\} \frac{\text{Weight in grams of extracts obtained}}{\text{Weight in grams of plant material taken}} \times 100$$

The percentage yield was calculated for the extracts and major compounds with reference to the crude material taken using the formula given below.

### 2.4 Macroscopical observation of fresh plant of *Cassia alata*

The fresh plant of *Cassia alata* was used for macroscopical study. The size shape, colour, taste, and odour are observed. The powder of the plant are sieved and investigated in different organoleptic features by repeated examination. Morphological studies are performed according to the prescribed procedure [25, 26]

### 2.5 Microscopical analysis of leaf and stem part of *Cassia alata*

The microscopy of the plant studied according to the prescribed procedure. Transverse sections of leaf and stem are prepared and stained with Safranin and Fast green as per the procedure [25]. Powder microscopy was performed according to the prescribed procedure [27, 28]. Photographs of different magnifications were taken with a Nikon Labphot2 Microscopic Unit. For normal observations bright field are used. For the study of starch grains, crystals and lignified cells, polarized light is used. Since these structures have birefringent property, under polarized light they appear bright against a dark background [29].

### 2.6 Determination of behaviour of plant powder of *Cassia alata*

Behaviour of powdered plant material with different chemical reagents is determined under natural light.

### 2.7 Fluorescence analysis of powdered leaves of *Cassia alata*

Powdered leaves were subjected to analysis under violet light after treatment with various chemical and organic reagents. Three parameters are taken into account i.e. observation under long UV light (365 nm), Short UV light (254 nm) and normal day light. Similarly extracts were also subjected to UV chamber and fluorescence was observed and consistency was noted as an additional character for identification [30-32].

### 2.8 Phytochemical study

Freshly prepared extracts were subjected to phytochemical screening for the detection of various constituents using conventional protocol. Total ash, Water soluble ash, Acid insoluble ash, Alcohol soluble extractive value and Water soluble extractive value of *Cassia alata* were determined as per standard procedures [36].

### 2.9 Preliminary phytochemical screening of various extracts from *Cassia alata*

The extract was subjected to preliminary phytochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to the establishing and providing the identity of a substance. The various extracts of *Cassia alata* were subjected to the following chemical tests separately for the identification of various active constituents.

#### 2.9.1 Tests for alkaloids [33]

A small portion of the solvent free extract was stirred with few drops of dilute hydrochloric acid and filtered. The filtrate was tested for various alkaloidal reagents; Dragendroff's reagent: A little amount of the filtrate was treated with Dragendroff's reagent (potassium bismuth iodide solution). Appearance of orange colour indicated the presence of alkaloids.

**Mayer's reagent:** The filtrate was treated with Mayer's reagent (potassium mercuric iodide solution). Appearance of cream colour indicated the presence of alkaloids.

**Hager's reagent:** The test sample was treated with Hager's reagent (picric acid). Appearance of yellow precipitate indicated the presence of alkaloids.

**Wagner's reagent:** A little amount of the extract was treated with Wagner's reagent (iodine and potassium iodide solution). Appearance of brown precipitate indicated the presence of alkaloids.

**Ammonium Rinker Test:** A little amount of the extract was treated with Ammonium Rinker solutions with HCl. Appearance of flocculent pink precipitate indicated the presence of alkaloids.

#### 2.9.2 Tests for glycosides [25]

A small quantity of the extract was added with glacial acetic acid and a small amount of ferric chloride solution, followed by the addition of con. H<sub>2</sub>SO<sub>4</sub>, formation of red ring at the junction of two liquids indicated the presence of glycosides.

A small quantity of the extract was hydrolysed with dilute hydrochloric acid for a few minutes by heating in water bath and the hydrolysate was subjected to Legal's and Borntrager's tests to detect the presence of different glycosides.

**Legal's test:** To the hydrolysate, 1 ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with NaOH solution. Appearance of pink to red colour shows the presence of cardenolides.

**Borntrager's test:** To the hydrolysate, 1ml of chloroform was added and shaken well. The chloroform layer was separated and to this added equal quantity of dilute ammonia solution. Appearance of a pink colour in the ammonical layer indicated the presence of anthraquinone glycosides.

### 2.9.3 Tests for detection of steroids <sup>[34]</sup>

**Liebermann Burchard Test:** To the sample, 1ml of acetic anhydride and acetic acid were added followed by few drops of concentrated sulphuric acid. Colours changing from violet to blue indicated the presence of steroids.

**Salkowski Test:** The sample of the extract in a dry test tube, few tin granules, 1 ml of thionyl chloride were added and shaken well. Appearance of a pink colour indicated the presence of steroids.

**Hirschonn Reaction:** To the sample of the extract tetra nitro methane was added. Appearance of yellow colour indicated the presence of steroids.

### 2.9.4 Test for Flavonoids <sup>[26]</sup>

Small quantity of the extract was shaken with few ml of water and the resulting mixture is subjected to the following tests:

**Florescence test:** Few mg of the sample was dissolved in alcohol and a drop of the resulting solution is placed on What-Mann filter paper and observed under UV light. Appearance of florescence indicated the presence of flavonoids.

**Shinoda's Test:** The sample was dissolved in 5ml of alcohol (95%) and treated with few drops of con. HCl and 0.5 gm of magnesium metal. Development of a pink colour within a minute indicated the presence of flavonoids.

### 2.9.5 Tests for tannins and phenolic compounds <sup>[34]</sup>

Small quantity of the extract was dissolved in water, warmed and filtered. The resulting filtrate is used for the following tests:

**Ferric chloride test:** Small quantity of the filtrate was treated with freshly prepared neutral ferric chloride solution. Presence of violet colour indicated the occurrence of phenols.

**Lead acetate test:** Small quantity of the filtrate was treated with 10% lead acetate solution. Presence of white precipitate indicated the occurrence of phenolic compounds.

**Gelatin test:** Small quantity of the filtrate was treated with 1% w/v solution of gelatin in water containing 10% sodium chloride. Appearance of cream precipitate indicated the presence of phenolic compounds.

### 2.9.6 Tests for proteins and free amino acids <sup>[34]</sup>

Small quantity of the extract was shaken with few ml of water and the resulting mixture is subjected to the following tests:

**Biuret Test:** The sample was treated with equal volume of 5% sodium hydroxide solution and 1% copper sulphate reagent and an appearance of pink colour indicated the presence of proteins and free amino acids.

**Millon's Test:** The sample was treated with few drops of Millon's reagent (Mercuric nitrate solution), appearance of pink colour indicated the presence of proteins.

**Ninhydrin Test:** The test sample was treated with 0.1% w/v solution of ninhydrin in n-butanol. Appearance of purple colour indicated the presence of amino acid.

### 2.9.7 Test for Carbohydrates <sup>[35]</sup>

#### Molisch's Test

A small quantity of the plant extract was dissolved in 4ml of distilled water and filtered. The filtrate was subjected to Molisch's test for the presence of carbohydrate. Formation of violet colour ring at the junction of two liquids indicated the presence of carbohydrates.

**Fehling's Test:** A small quantity alkaline solution of the hydrolysed plant extract was slowly added a mixture of equal parts of Fehling's solution A and B. Formation of brick red coloured precipitate of cuprous oxide indicates the presence of carbohydrates.

**Selwinoff's Test:** A small quantity of hydrolysed plant extract was added with crystal of resorcinol and added to the solution and warmed on a water bath with an equal volume of concentrated hydrochloric acid. A rose red colour indicates the presence of ketosugar.

**Bial's Test:** A small quantity of hydrolysed plant extract was heated in a test tube with an equal volume of hydrochloric acid containing a little phloroglucinol. The formation of a red colour indicates the presence of pentosugar

### 2.9.8 Test for Volatile Oils

Thin section of extract was treated with alcoholic solution of Sudan III develops red colour in the presence of volatile oils. Thin section of *Cassia alata* was treated with tincture of alkana, which produces red colour, indicates the presence of volatile oils.

### 2.9.9 Test for saponins (Kokate, 2000)

**Foam test:** 1g of the extract was shaken vigorously with 20 ml distilled water in a graduated cylinder for 15min. The persistent froth (1cm height) for 1 hour indicating the presence of saponins glycoside.

### 2.10 Determination of Physicochemical Properties <sup>[36]</sup>

**Ash Values:** Air dried powdered samples of the whole plants of *Cassia alata* was investigated for total ash, acid insoluble ash and water-soluble ash values by following procedure.

**(a) Total Ash Value:** About 2gm of the *Cassia alata* was weighed accurately and spread as a fine layer at the bottom of a tarred silica crucible. The crucible was incinerated at 500 – 600 °C in a muffle furnace until free from carbon. Then the crucible was cooled & weighed, the percentage total ash content was calculated.

**(b) Acid Insoluble Ash Value:** The ash obtained from the total ash was boiled with 25ml of HCl for 5 min. The insoluble ash was collected on ash less filter paper, washed with hot water. The filter paper along with the residue was transferred to a tarred silica crucible and ignited at a temperature not exceeding 600 °C to constant weight. The percentage of the acid insoluble ash was calculated.

(c) **Water-Soluble Ash Value:** The total ash obtained was boiled with 25ml of water for 5 min. The insoluble matter is collected on an ash-less filter paper, washed with hot water and ignited for 15 min at a temperature not exceeding 450°C. The weights of insoluble matter were subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water-soluble ash was calculated.

**2.11 Extractive Values**

(a) **Water Soluble Extractive Value:** 15gm of coarsely powdered air-dried *Cassia alata* was accurately weighed, in a glass-stopper conical flask. 300ml of water was added shaken vigorously and reweighed the flask. The flask was allowed to stand for 1 hr then the flask was attached with a reflux condenser and boiled gently for 6 hour; cooled and weighed and filter rapidly through a dry filtered and weighted.

(b) **Alcohol soluble extractive value**

15gm of coarsely powdered air-dried *Cassia alata* was accurately weighed, in a glass-stopper conical flask. 100 ml of alcohol (90%v/v) is added shaken vigorously intermediately for 24 hours and reweighed the flask. The flask evaporated, cooled and weighted.

$$\text{Extractive value} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

**3. Results**

**3.1 Results of successive solvent extraction & percentage calculation of various extracts of *Cassia alata***

The successive solvent extraction of consistency, colour and percentage yield of *Cassia alata* showed that the consistency of petroleum ether (PEECA), chloroform (CECA) and methanol extracts (MECA) were waxy, oily and viscous respectively. The colour of various extracts like petroleum ether, chloroform and ethanol like green, greenish brown and Greenish brown correspondingly. The percentage of various extracts of aerial plant of *Cassia alata* was 1.20%w/w, 2.95%w/w and 3.20%w/w respectively and the results were tabulated in the Table 4.1.

**Table 3.1:** Results of the successive solvent extraction - consistency, colour and percentage yield of *Cassia alata*

| Parameters                  | Extracts   |                |                |
|-----------------------------|------------|----------------|----------------|
|                             | Pet. ether | Chloroform     | Methanol       |
| Consistency                 | Waxy       | Oily           | Viscous        |
| Colour (Visible/ Day light) | Green      | Greenish brown | Greenish brown |
| Percentage Yield (% w/w)*   | 1.20       | 2.95           | 3.20           |

\*Mean ± SEM (n = 3)



**Fig 3.1:** Aerial parts of *Cassia alata* Linn

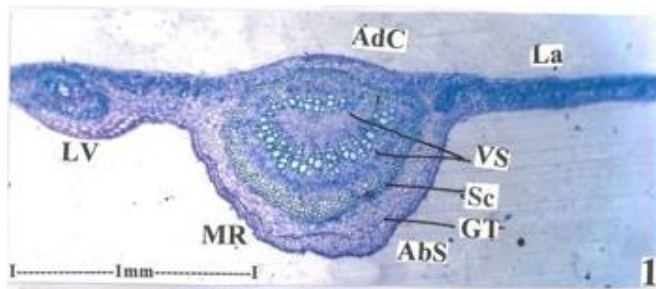
**3.2 Results of transverse section of leaf, stem and powder characters of *Cassia alata***

**Leaf**

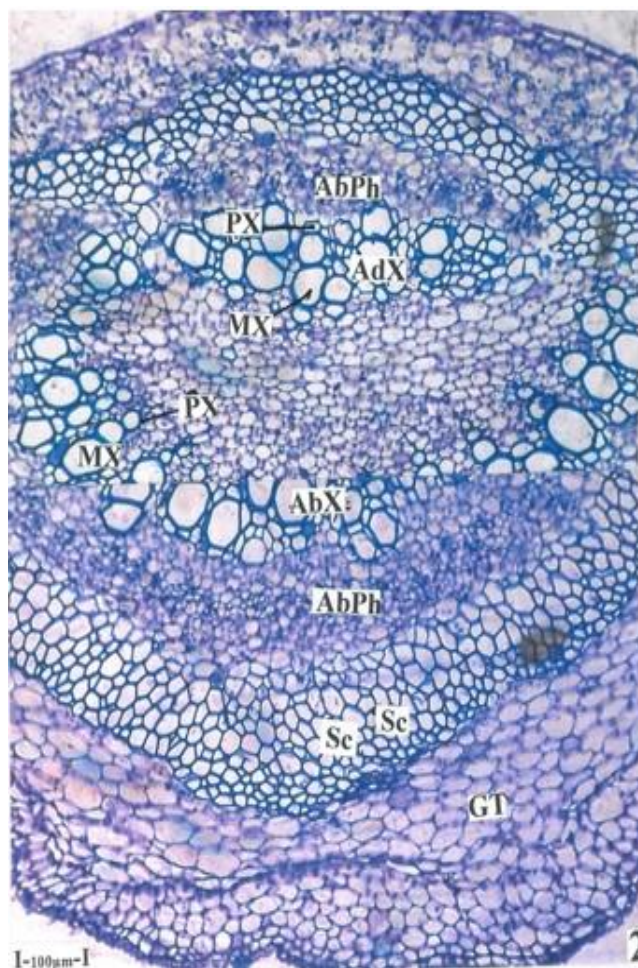
The leaf consists of a thin lamina and a thick midrib. The midrib is planoconvex. The adaxial side is flat abaxial side is widely convex. The midrib is 800µm thick and 1mm wide (Fig 3.2.1).

The midrib consists of a thin epidermal layer of rectangular, thin walled cells. Liner to the epidermis apparently matous zone, which narrow on the adaxial side and their along the abaxial side (Fig 3.2.2). The parenchyma cells are angular thin walled and compact.

**3.3 Results of Microscopical analysis of leaf of *Cassia alata***

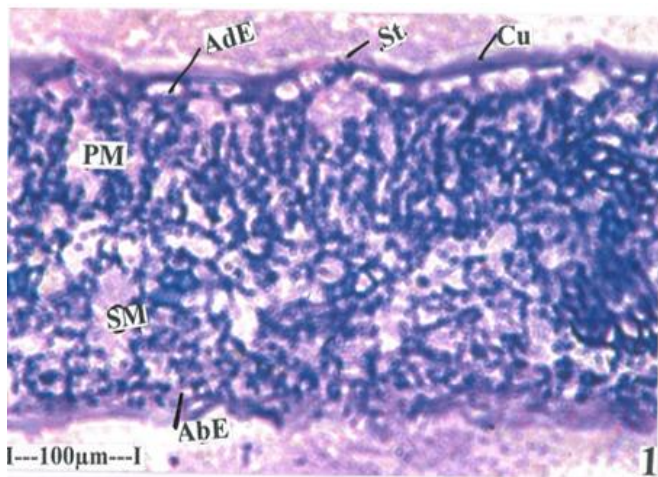


**Fig 3.2.1.** Transverse section through Midrib

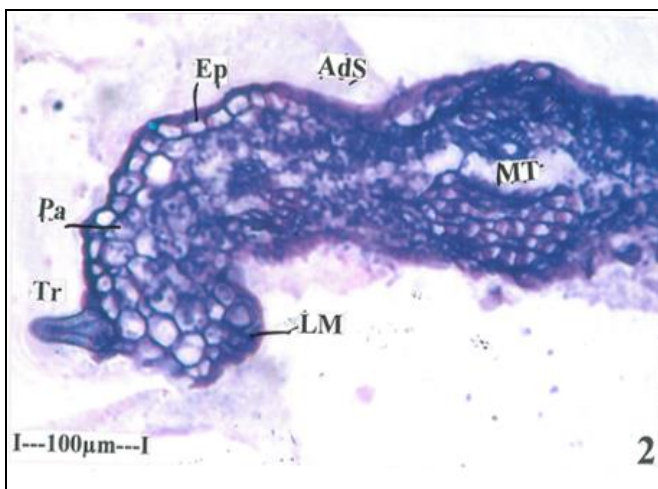


**Fig 3.2.2:** Transverse section of Midrib entire view enlarges

Abs- Abaxial side, Adc- Adaxial Cone, Abph- Abaxial phloem, Adx- Adaxial xylem, Abx- Abaxial xylem, GT- Ground tissue, La-Lamina, LV- Lateral vein, MR- Midrib, MX- Meta Xylem, PX- Proto xylem; Sc- Sclerenchyma, VS- Vascular strand.

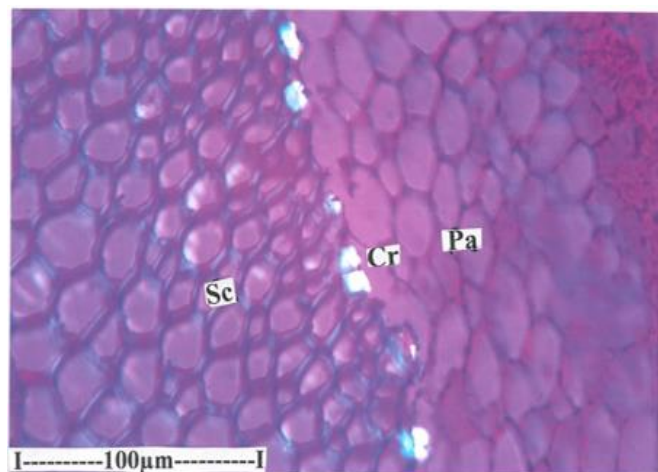


**Fig 3.3.1:** Transverse section of Lamina



**Fig 3.3.2:** Transverse section of leaf margin

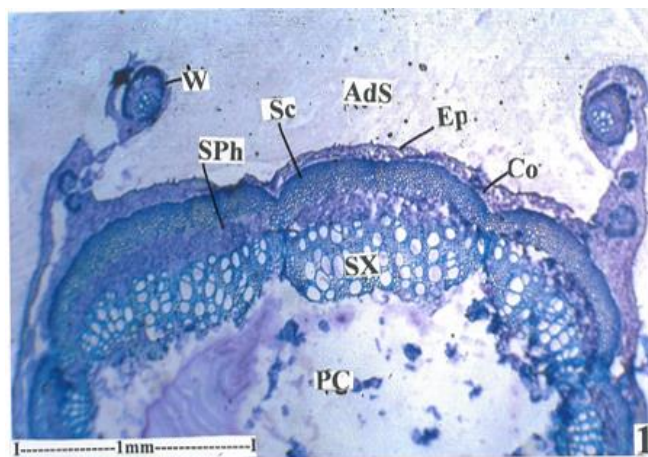
AbE- Abaxial epidermis; AdE- Adaxialepidermis; Ep- Epidermis; Cu- Cuticle; LM- Leaf Margin; MT- Mesophyll Tissue; PM- Palisade Mesophyll; Pa- Parenchyma; SM- Spongy Mesophyll; St- Stoma; Tr- Epidermal Trichome.



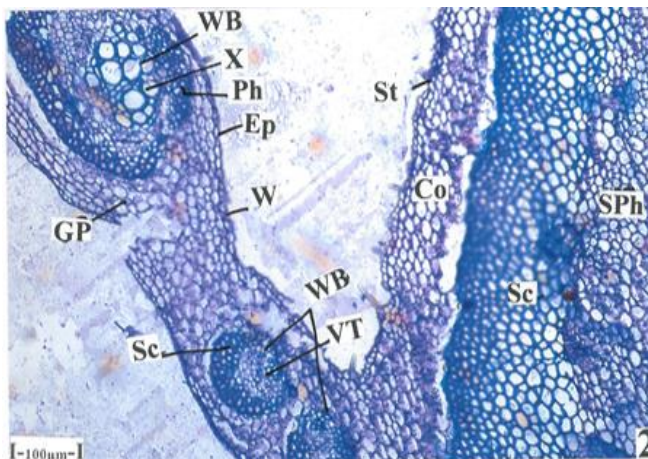
**Fig 3.4:** Distribution of crystals along the fibre band of the stem (As seen under polarized light)

Cr- Crystal; Pa- Parenchyma; Sc- Sclerenchyma  
The vascular system is wide and occupies the major portion the midrib. The vascular system consists of a thick continuous plano convex cylinder of sclerenchyma cells. The sclerenchyma cylinder encloses the hallow cylinder vascular tissues (Fig 3.2.2).

The vascular system includes a wide cup shaped abaxial part and slightly convex adaxial part. The vascular strands collateral with thin layer of meta-xylem and proto-xylem elements and thick continuous layer of phloem (Fig 3.2.2). The xylem elements are angular and fairly thick walled. The central core of the midrib includes compact thin walled parenchyma cells.

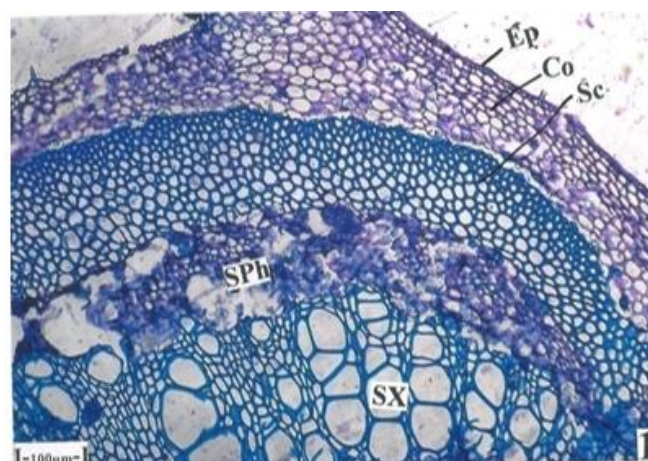


**Fig 3.5.1:** Transverse section of stem upper winged portion

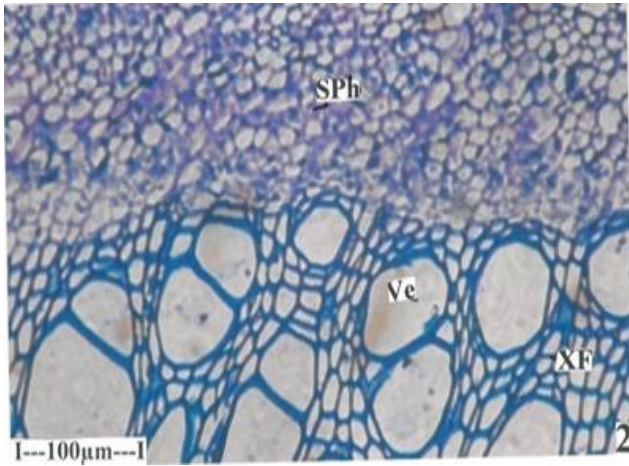


**Fig 3.5.2:** Wing enlarged

Ad- Adaxial side; Co- Cortex; Ep- Epidermis; Gp- Ground parenchyma; PC- Pith cavity; Ph- Phloem; Sc- Sclerenchyma; SPH- Secondary Phloem; St- Stem portion; SX- Secondary Xylem; VT- Vascular Tissue; W- Wing; WB- Wing Bundle; X- Xylem.

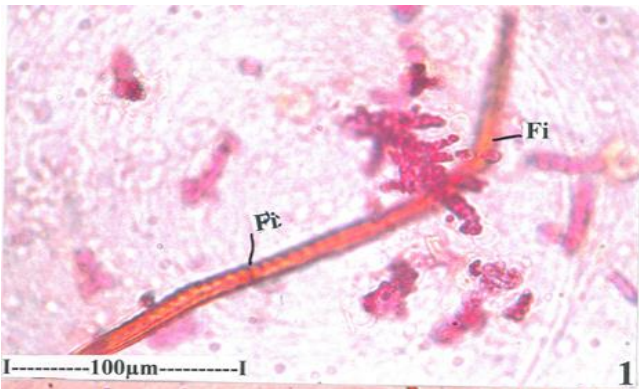


**Fig 3.6.1:** Transverse section of stem – one sector secondary Phloem

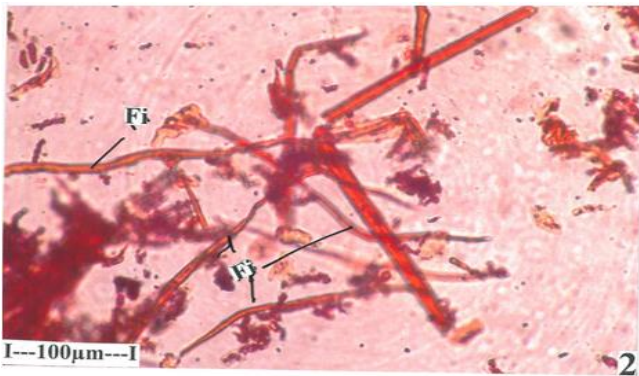


**Fig 3.6.2:** Transverse section of stem one sector and secondary xylem enlarged

Co- Cortex; Ep- Epidermis; Sc-Sclerenchyma; SPh-Secondary Phloem; SX-Secondary Xylem; Ve-vessels; XF-Xylem Fibres.



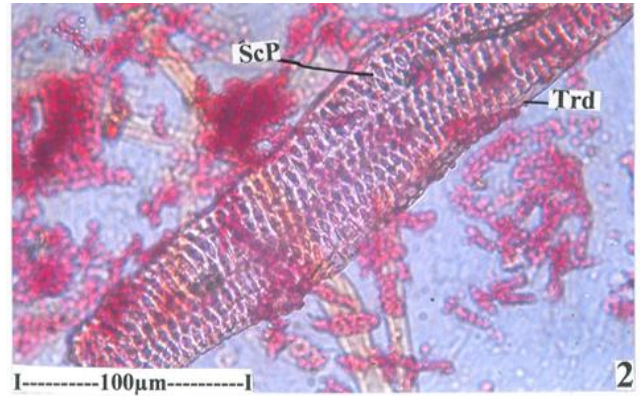
**Fig 3.7.1:** A fibre with single pits



**Fig 3.7.2:** Fibres without pits Fi- Fibre; Pi- Pits

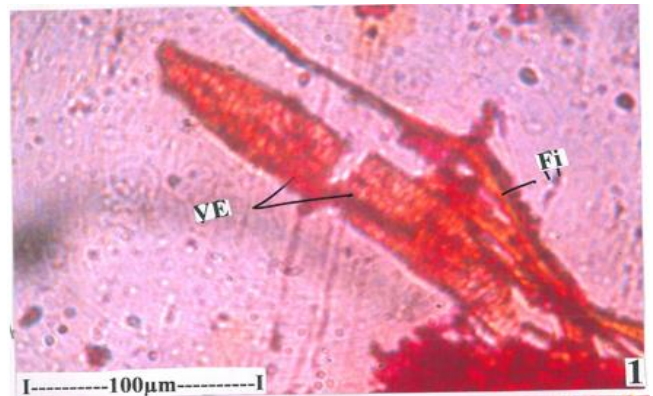


**Fig 3.8.1:** Unique type of tracheid

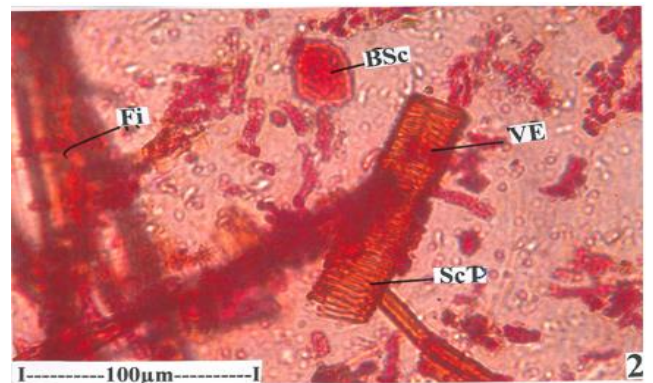


**Fig 3.8.2:** Unique type of tracheid with multiseriate scalariform form.

ScP- Scalariform pits; Trd- Tracheid.



**Fig 3.9.1:** Two Broken vessel elements



**Fig 3.9.2:** A vessel element with scalariform pits.

Fi- Fibres; BSc- brachysclereids; ScP- Scalariform pits; VE- Vessel Element

**Lamina**

The lamina is dorsiventral with difference between the abaxial and adaxial sides (Fig 3.3.1). The adaxial epidermal layer consists of fairly larger rectangular thick walled cells. The epidermis is also stomata ferous (Fig 3.3.1). The abaxial epidermis has the spindle shaped cells. The mesophylls tissue is differentiated into three horizontal layers of columnar palisade cells. The lamina is 300µm thick.

**Leaf margin**

The margin of part of the leaf becomes gradually thin and extreme end curved down. The curved leaf margin has thick walled, rectangular cells with thick cuticle (Fig 3.3.2). The mesophyll tissue consists to of compact circular parenchyma cells. The leaf margin is 150µm thin.

### Crystals

Calcium oxalate crystals are common in thin stem. The crystals are located along the border line of sclerenchyma zone (Fig.3.4). The crystals are druses. These crystals are spherical bodies with minute spiny surface.

### Stem

The stem is ovate in cross sectional view with broad adaxial parts and narrow abaxial part (Fig 3.5.1). These are two long wings, are an either lateral side. The wings are dump shaped and thick and dilated at the apex. These three vascular brudles in the wing of which the basal brudle is smaller and the middle brudle is larger; the upper brudle in quite larger (Fig 3.5.2). The vascular bundles of the wings have wide cluster of circular thick walled xylem elements and a thick are of phloem elements. (Fig 3.5.2).

The main stem consists of epidermal layer cortical zone and vascular cylinder (Fig 3.5.1 & 3.6.1) the epidermal layer consists of small rectangle cells. The cortical zone includes about 12 layers of rectangular thin wall compact parenchyma cells.

The vascular cylinder has thick continuous layer of sclerenchyma cells which unsheathe inner vascular tissues. The sclerenchyma cylinder consist of small thick wall compact cells in the periphery. The cells in the inner part are larger and comparatively thin wall. The vascular cylinder includes a circle of thick segments of xylem and phloem. The phloem segment include small circle of sieve elements and parenchyma cell. Inner to the phloem occurs several triangular segments of xylem. (Fig 3.6.1 & 3.6.2).

The xylem segment possess wide vessel which are in continuous radical files of angular thin wall cells. In between the xylem rows is seen xylem fibre which are small cells,

radially elongated thick wall and lignified cells. The vessels may be wide or narrow. The wide vessels are 60µm in diameter.

### Powder Microscopy

The powder preparation leaf shows the following inclusion:

Fibers with wide lumin having uniseriate vertical row of pits are occasionally in the powder (Fig 3.7.1). The fiber is 10µm thick. The end of the fibre is not very narrows. The cell walls are thick and lignified.

Some of the fibres are without pits. These fibres are thick wall. The cell wall is thick and lignified. These fibres are tapering at the ends. (Fig 3.7.2). The fibres are 10 µm thick. Tracheid rarely these occurs a unique type of tracheids in the powder. These tracheids are 50µm thick and 250µm thin. These tracheids have a vertical row of compact elliptical bordered pits. (Fig 3.8.1 & 3.8.2).

Vessel elements: Short cylindrical vessel elements are often seen in the powder. They are short and cylindrical with scalariform multiseriate pits on lateral walls. The end wall has wide circular horizontal end wall perforation at end walls. The vessel elements are 100µm long and 25µm wide (Fig 3.9.1 & 3.9.2).

Occasionally small cubical scleroids are seen in the powder. These cells are called brachysclereids (stone cells). These cells have dense circular simple pits. (Fig 3.9.2).

### 3.4 Results of behaviour of aerial part of the plant powder of *Cassia alata* with different chemical reagents

Behaviour of powder of *Cassia alata* with different chemical reagents was detected and results were shown in Table no. 3.2.

**Table 3.2:** Results of behaviour analysis of plant powder of *Cassia alata* with different chemical reagents

| Reagent                                      | Observation                | Inference              |
|--|----------------------------|------------------------|
| Powder + Iodine                              | Black colour observed      | Presence of starch     |
| Powder + HgCl <sub>2</sub>                   | Blue colour observed       | Presence of alkaloids  |
| Powder + Ammonia                             | Light Pink colour observed | Presence of glycosides |
| Powder + AgNO <sub>3</sub>                   | Slight precipitate formed  | Presence of proteins   |
| Powder + Picric acid                         | Colour changed             | Presence of alkaloids  |
| Powder + Water shaking                       | Foam not produced          | Absence of saponins    |
| Powder + Con. H <sub>2</sub> SO <sub>4</sub> | Black colour               | Presence of starch     |
| Powder +FeCl <sub>3</sub>                    | Bluish black colour        | Presence of tannins    |
| Powder + Con. HNO <sub>3</sub>               | Orange brown colour        | Presence of tannins    |

### 3.5 Results of fluorescence analysis of plant powder of *Cassia alata*

The colour changes, when observed under day light and UV-light by method and results were presented in the Table 3.3.

**Table 3.3:** Results of fluorescence analysis of plant powder of *Cassia alata*

| Reagent                                     | Long UV light (365nm) | Short UV light (254nm) | Visible/Day light    |
|---|-----------------------|------------------------|----------------------|
| Powder + 1N HCl                             | Black                 | Light green            | Olive green          |
| Powder + 50% HCl                            | Black                 | Light green            | Olive green          |
| Powder + 50% H <sub>2</sub> SO <sub>4</sub> | Black                 | Light green            | Greenish yellow      |
| Powder + 50% HNO <sub>3</sub>               | Black                 | Light green            | Light red            |
| Powder + 1N NaOH                            | Black                 | Green                  | Dark brown           |
| Powder + Alcoholic NaOH                     | Brick Red             | Dark green             | Light brownish black |
| Powder + Water                              | Black                 | Green                  | Light brownish black |
| Methanol                                    | Blackish brown        | Green                  | Greenish brown       |

### 3.6 Results of physicochemical properties of *Cassia alata*

The physicochemical parameters results were shown in the Table 3.4.

**Table 3.4:** Results of physicochemical parameters of *Cassia alata*

| S. No | Parameters                       | Result       |
|-------|----------------------------------|--------------|
| 1     | Total ash                        | 10.5±0.2156  |
| 2     | Acid insoluble ash               | 3.25±0.1241  |
| 3     | Water soluble ash                | 10.4±0.2052  |
| 4     | Loss on drying                   | 0.3±0.0235   |
| 5     | Water soluble extractive value   | 11.28±0.2576 |
| 6     | Alcohol soluble extractive value | 5.1±0.1683   |

Mean (w/w) ± SEM (n = 3)

### 3.7 Results of phytochemical analysis of various extracts of *Cassia alata*

The results of the phytochemical analysis of various extracts from *Cassia alata* were carried out for Petroleum ether extract, chloroform extract and methanolic extract separately. The results were given in the following table 3.5.

**Table 3.5:** Results of phytochemical analysis of various extract of *Cassia alata*

| Phytochemical                 | Pet. ether extract | Chloroform extract | Methanolic extract |
|-------------------------------|--------------------|--------------------|--------------------|
| Alkaloids                     | -                  | -                  | +                  |
| Glycosides                    | +                  | +                  | +                  |
| Steroids                      | -                  | -                  | +                  |
| Flavonoids                    | +                  | +                  | +                  |
| Tannins & phenolic compounds  | +                  | +                  | +                  |
| Proteins and free amino acids | -                  | -                  | +                  |
| Carbohydrates                 | -                  | +                  | +                  |
| Volatile oils                 | +                  | +                  | +                  |
| Saponins                      | -                  | -                  | -                  |

(+): presence (-): absence

### 4. Discussion

Development of standards is a fundamental part of developing the exact identity and quality of crude drug. Macroscopy, microscopy and physicochemical parameters of crude drugs which give the detail note on correct identity, purity and quality of medicinal plants. As there is no detail record on pharmacognostical and phyto chemistry on leaves and stem of *Cassia alata* Linn. Moreover the present study is undertaken to develop the some pharmacognostical and phytochemical standards. A significant percentage yield variations were also observed in successive Soxhlet extractions with different polarity solvents. Powder treated with different reagents showed presence of starch, glycosides & tannins and absence of saponins. The colour changes are observed under day light and UV-light (Short & Long wave length). The plant powder treated with different reagents shows characteristic fluorescence colour was observed. The physicochemical parameters like total ash was 10.5±0.2156; acid insoluble ash was 3.25±0.1241; water soluble ash was 10.4±0.2052; loss on drying was 0.3±0.0235; water soluble extractive value was 11.28±0.2576; and alcohol soluble extractive value was 5.1±0.1683. The preliminary phytochemical screening showed that PEEIB was rich in glycosides, volatile oil, tannins & phenolic compounds and flavonoids; CEIB was rich in glycosides, volatile oil, flavonoids, tannins & phenolic compounds and carbohydrates and MEIB was rich alkaloids, glycosides, steroids, flavonoids, tannins & phenolic compounds, proteins and free amino acids, volatile oil and carbohydrates. Among these three different fractions of plant

extracts, methanolic extracts are shows the presence of major classes of phytoconstituents. In the present study of *Cassia alata* exhibit the presence of various phytochemicals such as carbohydrates, flavonoids, alkaloids, tannins and phenolic compounds. Moreover all the three fractions are shows the presence of flavonoids, which are beneficial for human health. The major active nutraceutical ingredients in plants are flavonoids. Its possess the large range of pharmacological activities in the mammalian body such as antineoplastic, cardioprotective, antiatherosclerotic effects, antiulcer activity, antidiabetic effects, hepatoprotective activity, antiviral activity and antioxidant activity. In present exploration, various standardization parameters such as macroscopic, microscopic and phytochemical screening were carried out and which could be helpful in authentication of *Cassia alata* Linn. The results of present study will also serve as reference material in the preparation of monograph.

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