## e - ISSN 2249-7544 Print ISSN 2229-7464



# INTERNATIONAL JOURNAL OF

PHYTOPHARMACY RESEARCH

www.phytopharmacyresearch.com

# PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES OF CROSSANDRA INFUNDIBULIFORMIS L. (ACANTHACEAE) LEAF

## \*K. Sumalatha

\*Assistant professor, Department of Pharmacognosy, Bhaskar Pharmacy College, Banjara Hills, Hyderabad - 500 034.

## ABSTRACT

Phytochemical and Pharmacognostic investigation was carried out on the leaves of *Crossandra infundibuliformis* L. The assignment such as macroscopy, anatomical studies and preliminary phytochemical screening were performed since the species was not noted for its pharmacognosy in part. Macroscopic studies is a technique of qualitative evaluation based on the study of morphological and sensory profiles of leaves of *Crossandra infundibuliformis* L. Microscopic studies is a technique of qualitative evaluation and used to confirm the structural details of drugs from the leaves of *Crossandra infundibuliformis* L. and also study of the phytoconstituents by application of chemical methods. The perusal of literature also revealed that no pharmacognostic work had been carried out on the leaves of *Crossandra infundibuliformis* L. For this reason we have investigated the Phytochemical and pharmacognostic profiles of leaves of *Crossandra infundibuliformis* L.

Keywords: Crossandra infundibuliformis L., Phytochemical Screening, Pharmacognostic Studies.

#### **INTRODUCTION**

Evaluation of a crude drug means its identification and determination of its purity and quality. Quality control of a crude drug can be attempted by different methods of evaluation depending upon the morphological and microscopical studies of the crude drugs and ash values are helpful in determining the quality and purity of a crude drug. Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant. To ensure reproducible quality of herbal medicines, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication followed by creating numerical values of standards for comparison. Pharmacognostical parameters for easy identification like leaf constants, microscopy & physico chemical analyses are few of the basic protocol for standardization of herbal drugs.

The macroscopical or morphological description of a crude drug includes size, shape, nature of inner and outer surfaces, type of fracture and organoleptic characters like colour, odour, taste, etc. The microscopical study is one of the important aspects of its

histological evaluation. The arrangement of tissues, types of cells and cell contents are revealed by suitable histological study of crude drug with the aid of an microscope. Certain microscopical characters like stomata, trichomes, calcium oxalate crystals, starch grains, fibers, vessels, etc., are important anatomical characters [1-4].

#### MATERIALS AND METHODS

#### Collection and Authentication of Plant Material

Leaves of *Crossandra infundibuliformis* L. (Acanthaceae) was collected from Tirupati, Chittoor (dist), Andhra Pradesh, India in the month of August, 2010. Care was taken to select the healthy plant material. The taxonomical identification and authentication of the plant was done by Prof. Dr .P. Jayaraman, Director of Plant Anatomy Research Centre (PARC), Chennai, Tamilnadu. The voucher specimen PARC/2009/428 was preserved in department of Pharmacognosy, SVCP for future reference.

#### Macroscopical Studies

The fresh leaves *Crossandra infundibuliformis* L.(Acanthaceae) was studied for its morphological characters and photographed under orginal environment.

Corresponding Author: K. Sumalatha Email:- sumampharmacy@gmail.com

## Microscopical Studies

#### Preparation of specimen

The fresh sample were cut into small pieces and fixed in FAA solution (Formalin 5ml + Glacial acetic acid 5ml + 70% Ethanol 90ml). After fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol (TBA) as per the standard procedure. After complete dehydration, the specimens were embedded in paraffin wax.

## Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary microtome (thickness  $10-12\mu$ m). Dewaxing and staining of the sections were done by customary procedure. Sections were stained mostly with toluidine blue.

*Staining* : For anatomical studies the following staining schedules were followed

**1. Tannic Acid** - Ferric Chloride counterstained with 0.5% alcoholic safrain. This schedule was found to be quite satisfactory for all young plant tissues in which the primary walls were stained.

2. Alcoholic safrain - (0.5%) counterstained with 0.25% fast green. This schedule gives good result for studying the histology of different tissues of the plant organs especially the cell inclusions.

3. **Toludine Blue** – **O** stain was prepared by dissolving 0.25g of the stain in the mixture of benzoic acid 0.25g, sodium benzoate 0.29g and distilled water 200ml with  $p^{H}$  of 4.2 – 4.4. Since Toluidine blue is a polychromatic stain, the staining results were remarkably good and the dye render pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies, etc. After dewaxing, the slides were stained for 5 – 10 minutes and then dehydrated [5-7].

## Photomicrograph

All permanent slides, after staining were dehydrated by using graded series of Ethanol + Xylol and mounted in DPX. Photomicrographs were done on NIKON – Lab hot – 2 microscope using Konica colour film (100 ASA). For normal observations bright field was used. For the study of crystals and starch grains, the sections were photographed under polarized light. Magnifications of the figures are indicated by scale bars. Descriptive terms of various observations are as found in standard Anatomy books [8].

#### Physicochemical Parametrs Determination of ash value

Ash values are helpful for determining the quality and purity of a crude drug especially in powdered form. On incineration, crude drugs normally leave an ash consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. Ash value determination furnishes a basis of judging the identity and gives the information related to its calculation with inorganic matter. Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand.

## I. Determination of total ash

About 3 g of powdered drug was weighed and placed in a silica crucible, which was previously ignited and weighed. Powdered drug was spread uniformly in a fine layer at the bottom of tarred silica crucible. Crucible was kept inside the muffle furnace and the temperature increased to make crucible dull red hot until free from carbon. Crucible was cooled, kept in a desiccator and weighed. Same procedure was repeated to arrive at constant weight. The percentage of total ash obtained was calculated with reference to the air dried drug. Total ash value of powdered leaves was recorded.

## **II.** Determination of acid insoluble ash

Total ash obtained above was boiled with 25 ml of dil. HCl for 5 minutes. Insoluble ash was collected on ashless filter paper and washed with hot water. Insoluble matter was transferred into tarred silica crucible, ignited and weighed. Procedure was repeated to get constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug. Acid insoluble ash values of powdered leaves were recorded.

## III. Determination of water soluble ash

Total ash obtained was boiled with 25 ml of water for 5 minutes. Insoluble matter was collected on an ashless filter paper, washed with hot water and ignited at temperature not exceeding  $450^{\circ}$ C. Weight of the insoluble matter was subtracted from the weight of the ash. Difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug [9].

## **Determination of Extractive Values**

Extractive values of a crude drug determine the amount of active constituents extracted with solvents from a given amount of plant material.

## I. Alcohol soluble extractive value

About 5 g of coarse powder (60-80 mesh) of the crude drug (shade dried) was weighed and macerated in iodine flask with 100 ml of 70% v/v alcohol, for a duration of 24 hrs with frequent shaking and finally allowed to stand for 18 hrs. Solution was filtered rapidly, taking precaution against loss of alcohol, 25 ml of filtered solution was evaporated to dryness at  $105^{\circ}$ C in a tarred flat bottomed petri dish. The percentage of alcohol soluble extract was determined with reference to the shade dried drug.

## II. Water soluble extractive value

About 5 g of coarse powder (60-80 mesh) of crude drug (shade dried) was weighed and macerated in

iodine flask with 100 ml of chloroform-water, for a duration of 24 h, with frequent shaking and finally allowed to stand for 18 h. Solution was filtered rapidly, 25 ml of filtered solution was evaporated to dryness at  $105^{0}$ C in a tarred flat bottomed petri dish. The percentage of water soluble extract was determined with reference to the shade dried drug.

## Loss on drying

About 1 gm of the powdered leaf of *Crossandra infundibuliformis* L. (Acanthaceae) was accurately weighed in a tarred petri dish, previously dried under specified conditions as per IP. The powder was distributed as evenly as practicable, by gently side shaking. The dish was dried in an oven at 100-105<sup>o</sup> C for 1 hour,cooled in dessicator and again weighed. The loss on drying was calculated with reference to the air dried powder drug.

#### Fluorescence analysis

The powder prepared from the leaves were treated with various solvents like 1N Hydrochloric acid, Acetic acid, Iodine, Ferric chloride, Ammonia, and Water to evaluate the fluorescence analysis in visible/day light, long UV(365nm) and short UV (254nm) light. Various extract of leaves were also subjected to fluorescence analysis. For fluorescence analysis, powdered drug was sieved through 60 mesh and observations were carried out according to the standard procedure.

## PHYTOCHEMICAL INVESTIGATION

Many medicinal plants occurring in India are yet to be subjected to various chemical investigations, which may help in the discovery of several new drugs. To investigate such chemical constituents from plants, phytochemical screening is required. Broadly, chemical constituents in plants may be divided into two major viz., primary and secondary chemical groups constituents. Primary constituents are the basic metabolites of plants such as carbohydrates, proteins, lipids, cellulose and chlorophyll which are distributed in almost all the plants. Secondary chemical constituents are selective and vary considerably from plant to plant and even within the species or varieties of same genus. Secondary chemical constituents are chiefly responsible for the biological activities of plants.

These phyochemical screening facilitate the quantitative estimation of qualitative separation of pharmacologically active chemical compounds. *Solvents used*: All the solvents used for the study were of analytical grade.

## Extraction of plant material

The air dried powdered drug (170)gm of *Crossandra infundibuliformis* L. (Acanthaceae) leaf was extracted separately with petroleum ether, methanol,

chloroform maintained at a temperature range from  $60^{\circ}$ C -  $70^{\circ}$ C using soxhlet apparatus for about 48hrs. The extraction was carried out until the solvent in siphon tube becomes colourless. The aqueous extract was prepared by macerating the powdered drug using double distilled water for about 48hrs. Each extract was concentrated by distilling off the solvent and evaporated to dryness on the water bath, dried under vaccum dessicator. The percentage yield was calculated and consistency of the extracts was noted.

#### PRELIMINARY PHYTOCHEMICAL SCREENING

The concentrated extracts were subjected to chemical tests as per the methods mentioned below for the identification of various active chemical constituents [10-13].

#### Detection of Alkaloids

Small portions of solvent-free chloroform, alcohol and aqueous extracts were stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal reagents.

**a.** *Mayer's test:* The filtrate was treated with potassium mercuric iodide (Mayer's reagent) and the formation of cream coloured precipitate indicates the presence of alkaloids.

**b.** *Dragendorff's test:* The filtrate was treated with potassium bismuth iodide (Dragendorff's reagent) and formation of reddish brown precipitate indicates the presence of alkaloids.

**c.** *Wagner's test:* The filtrate was treated with solution of iodine in potassium iodide (Wagner's reagent) and formation of brown precipitate indicates the presence of alkaloids.

**d.** *Hager's test* The filtrate was treated with a saturated solution of picric acid (Hager's reagent) and formation of yellow precipitate indicates the presence of alkaloids.

#### Detection of Carbohydrates and Glycosides

Small quantity of alcohol and aqueous extracts were dissolved separately in distilled water and filtered. The filtrate was subjected to various tests to detect the presence of different carbohydrates.

**a.** *Molisch's test:* The filtrate was treated with alcoholic solution of  $\alpha$  -Naphthol and a few drops of conc. sulphuric acid were added through the sides of the test tube. The formation of violet ring at the junction of the liquids indicates the presence of carbohydrates.

**b.** *Fehling's test*: The filtrate was treated with few ml of dilute hydrochloric acid and heated on a water bath for 30 minutes. After hydrolysis the solutions were

neutralized with sodium hydroxide solution. To the neutralized solutions, equal quantities of Fehling's A & Fehling's B solutions were added and heated on a water bath for a few minutes. Formation of red-orange precipitate indicates the presence of reducing sugars.

**c.** *Benedict's test:* The filtrate was treated with 5ml of Benedict's reagent and heated on a water bath for a few minutes. The formation of red-orange precipitate indicates the presence of reducing sugars.

Another small portion of extract was hydrolysed with dilute hydrochloric acid for a few hrs (2 to 4 h) in a water bath and subjected to various tests to detect the presence of different glycosides.

**d.** *Liebermann-Burchard's test:* Hydrolysates were treated with a few drops of acetic anhydride, boiled and cooled. Few drops of sulphuric acid were added through the sides of the test tube. Formation of a brown ring at the junction of two liquids and green colour in the upper layer indicates the presence of cardiac glycosides.

**e.** *Legal's test:* Hydrolysates were treated with sodium nitroprusside in pyridine and methanolic alkali. Formation of blood red colour indicates the presence of cardiac glycosides.

## **Detection of Phytosterols**

The prepared extracts were refluxed separately with solution of alcoholic potassium hydroxide till complete saponification took place. Saponified mixtures were diluted with distilled water and extracted with solvent ether. Ethereal extract was evaporated to dryness and the residue subjected to Liebermann-Burchard's test.

#### a. Liebermann-Burchard's test

Ethereal residues were treated with a few drops of acetic anhyride; boiled, cooled, and 1 ml of sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two liquids and green colour in the upper layer indicates the presence of steroids and triterpenoids.

#### Detection of Fixed oils and Fats

**a.** *Spot test:* A small quantity of prepared extracts were pressed separately between two filter papers. Formation of oil stains on the filter paper was observed for the presence of fixed oil.

**b.** Saponification test: A few drops of 0.5 N alcoholic potassium hydroxide were added to a small quantity of petroleum ether or benzene extract along with a drop of phenolphthalein. Mixture was heated on a water bath for 1 to 2 hrs. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

## **Detection of Saponins**

Foam test: About 1 ml of alcohol and aqueous

extracts were diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Formation of any froth above the surface was observed for the presence of saponins.

#### **Detection of Phenolic compounds and Tannins**

Small quantities of the prepared extracts were diluted separately in water and were tested for the presence of phenolic compounds and tannins.

**a.** *Ferric chloride test:* To the test solutions, a few drops of 5% ferric chloride solution were added. Formation of a bluish-black or greenish-black colour indicates the presence of phenolic compounds and tannins.

**b.** *Gelatin test:* To the test solutions a few drops of 1% gelatin solution in 10% sodium chloride were added. Formation of white precipitate indicates the presence of tannins.

**c.** *Lead acetate test:* To the test solution, a few drops of 10% lead acetate solution were added. Formation of white precipitate was observed for the presence of tannins. Formation of a yellow precipitate indicates the presence of flavonoids.

**d.** Aqueous bromine test: To the test solution, a few drops of aqueous bromine solution were added. Formation of a yellow precipitate indicates the presence of tannins.

#### **Detection of Proteins and Free Amino Acids**

Small quantities of alcohol and aqueous extracts were diluted separately in water and tested for the presence of proteins and free amino acids by subjecting the extracts to various tests.

**a.** *Millon's test:* To 2 ml of the test solutions, 2 ml of Millon's reagent were added and heated. Formation of white precipitate that gradually turns red indicates the presence of proteins and amino acids.

**b.** *Biuret test:* To the test solutions, a few drops of 0.7% copper sulphate solution were added. Formation of a purplish violet colour indicates the presence of amino acids.

**c.** *Ninhydrin test:* To the test solutions, a few drops of ninhydrin solution were added in a water bath. Formation of a bluish colour indicates the presence of amino acids.

#### **Detection of Gums & Mucilage**

About 10 ml of aqueous extract was added to 25 ml of absolute ethanol with constant stirring. Precipitate was examined for its swelling properties and for the presence

of carbohydrates.

#### **Detection of Flavonoids**

*Shinoda test:* To the test solutions, a few fragments of magnesium metal were added along with concentrated hydrochloric acid, and heated. Formation of magenta colour indicates the presence of flavonoids.

Alkaline reagent test: To the test solutions a few drops of sodium hydroxide solution was added. Formation of an intense yellow colour that turns less intense on addition of acid indicates the presence of flavonoids.

#### **RESULTS AND DISCUSSION** Macroscopical Features of the Leaf *Anatomy of the leaf*

The leaf consists of thick circular midrib, as seen in cross-seational view, and thin lamina which arises as adaxial lateral wings from the midrib (Fig: 1.4).

The midrib is 1.2mm in diameter. It has a thin distinct layer of epidermis comprising small squarish cells. The ground tissue consists of outer zone three or four layers of collenchyma cells; remaining portion is parenchymatous; the cells thin walled, circular and less compact.

#### Transeverse section of midrib – Enlarged

The vascular system consists of a central, wide, deeply urn-shaped strand and two small, less conspicuous adaxial strands situated of the adaxial side and inner to the attachment of the lamina (Fig 1.5). The main central strand consists of thin layer of circular of circular solitary, thick walled xylem element and a thin layer of phloem engheathering the xylem strand. The vascular strand is covered by thin layer of thick walled cells so that, the vascular appears as closed cylinder.

The adaxial accessory strands are circular with a central group of small xylem elements surrounded by phloem elements (Fig 1.5).

#### Lateral vein

The lateral vein is plano convex in sectional view, the adaxial side is flat and the abaxial part is convex (Fig 1.6). It is  $170\mu m$  thick. The epidermal layer

of the lateral vein consists of broad, rectangular or circular cells with thick cuticle. The cells within the abaxial part are wide, circular and compact. The vascular strand is single, circular with collateral xylem and phloem.

#### Lamina

The lamina is  $90\mu$ m thick. It is differentiated into adaxial and abaxial sides. The adaxial epidermis is thick with rectangular cells and measure  $15\mu$ m thick. The abaxial epidermis is thin and bears glandular trichomes (Fig 1.7, 1.8). The trichome is capitate type. It has a single, short stalk cell and a spherical head of four cells (Fig 1.7). The trichome is  $20\mu$ m in height and  $15\mu$ m in thickness.

The mesophyll tissue is differentiated into adaxial palisade zone of single row of short, wide cylindrical cells and abaxial zone of about four layers of lobed spongy parenchyma cells (Fig -1.8).

The leaf margin is blunt and semi-circular with usual tissues of the lamina. The epidermal cells are more thick walled. A compact zone of palisade cells and larger intact spongy parenchyma cells are seen at the marginal part of the lamina (Fig - 1.9). The marginal lamina is 90µm thick.

## **Epidermal morphology**

Epidermal tissue

In surface view of the paradermal section of the lamina, the epidermal cells appear highly lobed and amoeboid in centline. The anticlinal walls are fairly thick and are wavy. The adaxial epidermis is apo stomatic (Having no stomata). (Fig 1.10)

Abaxial epidermis and stomata

The abaxial epidermis consists of smaller cells, they have thick, wavy anti clinal walls and the cells appear lobed in surface view. The stomata are diacytic type (Fig - 1.11).

The stoma has two subsidiary cells; one subsidiary cell is smaller than the other. The subsidiary cells occur at right angles to the guard cells. The guard cells are wide and elliptical with wide stomatal pore (Fig -1.12). They are 20µm long and 15µm wide [14-15].

 Table 1. Macroscopical features of fresh leaf of Crossandra infundibuliformis L. (Acanthaceae)

Characteristics	Observations
Condition	Fresh
Shape	Oblong
Colour	Thick green
Odour	Characteristic
Taste	Slight bitter
Size	12-15 cm height, 5-6 cm width
Texture	Firm
Margin	Entire
Surface	Smooth
Base	Slightly decurrent into stalk
Midrib	Parallel

S. No	Parameters	Average % W/W
I.	Ash values	
	a) Total ash	12.5
	b) Acid insoluble ash	3.8
	c) Water soluble ash	4.5
II.	Extractive values	
	a) Alcohol soluble Extractive	12.4
	b) water soluble extractive	8.9
III.	Loss on drying	4.85

## Table 2. Physicochemical parameters of leaf powder of Crossandra infundibuliformis L. (Acanthaceae)

#### **Fluorescence Analysis**

Table 3. Fluorescence analysis of leaf powder of Crossandra infundibuliformis L. (Acanthaceae)

Treatment of newdor	Ultra -vi		
Treatment of powder	short wave (254nm)	long wave (365 nm)	Visible light
Powder as such	Thick Brown	Brown	Green
In 50% H <sub>2</sub> SO <sub>4</sub>	Brown	Dark Brown	Green
In 50% HNO <sub>3</sub>	Brown	Blue	Green
In 5% KOH	Thick green	Green	Green
In methanol	Brown	Blue	Green
In 1N HCl	Brown	Light Brown	Green
In 1N methanolic NaOH	Dark green	Dark blue	Green
In ethanol (70% v/v)	Dark green	Blue	Green

#### Table 4. Fluorescence analysis of different extractives of the Crossandra infundibuliformis L. leaf

Solvent	Short UV	Long UV	Visible Light	
Petroleum ether( $60^{\circ}$ - $80^{\circ}$ )	Pale Green	Pink fluorescence	Bluish green	
Chloroform	Blue	Red	Blue	
Methanol	Green	Orange	Green	
Water	Light green	Light green	Brown	

Table 5. Colour, Consistency and Percentage yield of different extracts of Crossandra infundibuliformis L.(Acanthaceae) leaf

S.No	Extraction	Colour	Consistency	Yield% w/w
1.	Petroleum ether (60-80°C)	Greenish brown	Semi solid and sticky	22.5
2.	Methanol (60-80°C)	Dark green	Semi solid	17.9
3.	Chloroform (60-80°C)	Dark green	Semi solid	12
4.	Water	Light green	Sticky	8.5

## Table 6. Phytochemical screening of different extracts of Crossandra infundibuliformis L. (Acanthaceae) leaf

Chemical Tests	Pet.ether	Chlorofom	Methanol	Double distilled water	Powder
Alkaloids	-	_	_	_	_
Carbohydrates	+	+	+	+	+
Steroids	++	+	-	+	+
Flavanoids	+	-	+	+	+
Triterpenoids	-	-	-	-	-
Glycosides	-	-	-	-	-
Saponins	-	-	-	-	-
Tannins	-	-	-	-	-
Lipids	-	-	-	-	-
Gums&mucilage	-	-	-	-	-

(+) presence



Fig 1. Whole plant of Crossandra infundibuliformis L. (Acanthaceae)

Fig 2. Flowers of Crossandra infundibuliformis L. (Acanthaceae)



Fig 3. Leaf of Crossandra infundibuliformis L. (Acanthaceae)



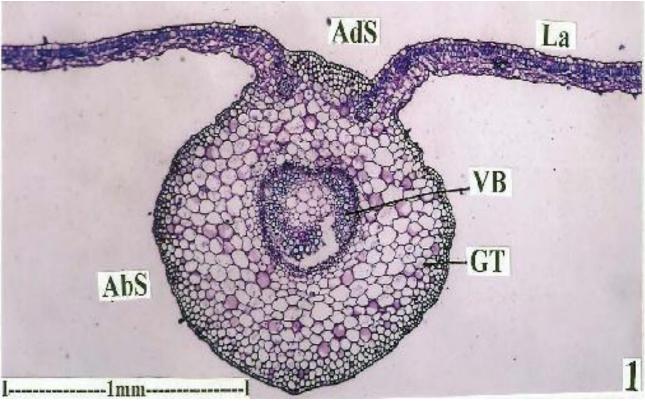
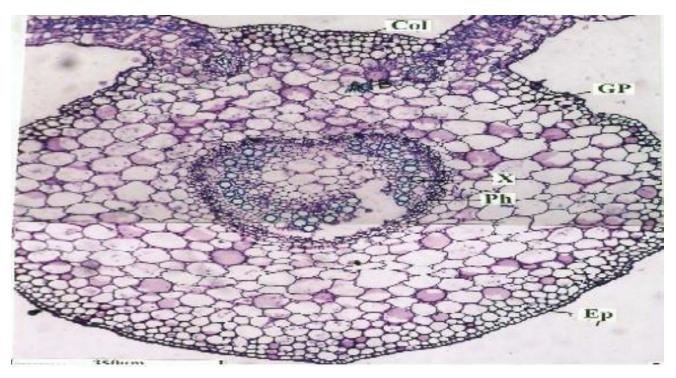


Fig 4. Transverse section of leaf through midrib with lamina

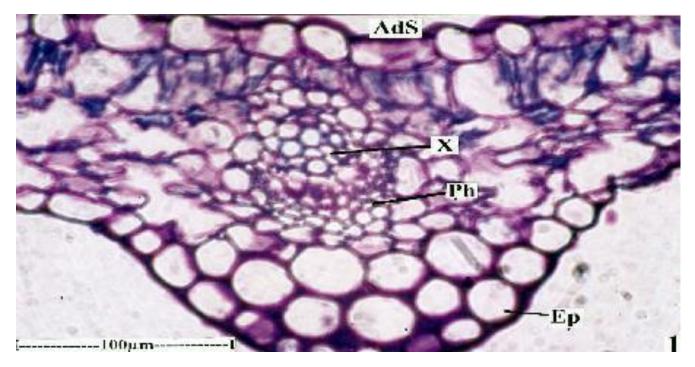
AbS – Abaxial side; AdS – Adaxial side, La – Lamina; VB – Vascular bundle

Fig 5. Transverse section of midrib – enlarged



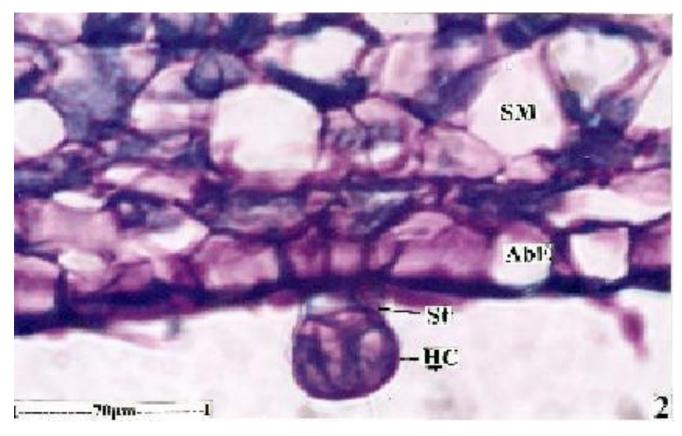
Col – Collenchyma; EP – Epidermis; GP – Ground Parenchyma; GT – Ground tissue; X – Xylem.

## Fig 6. Transverse section of lateral vein



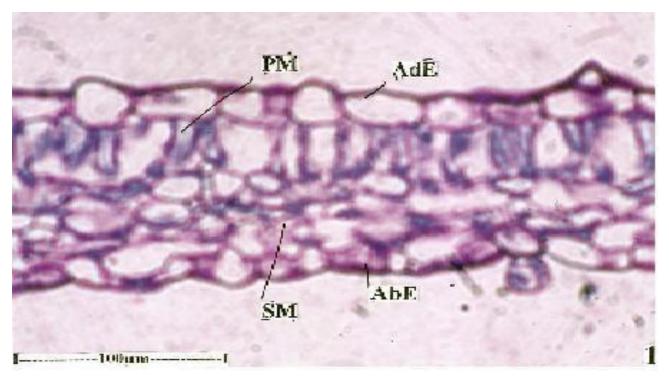
AdS – Adaxial side; EP – Epidermis; Ph – Phloem; X – Xylem.

Fig 7. Transverse section of lamina showing a glandular trichome on the abaxial epidermis



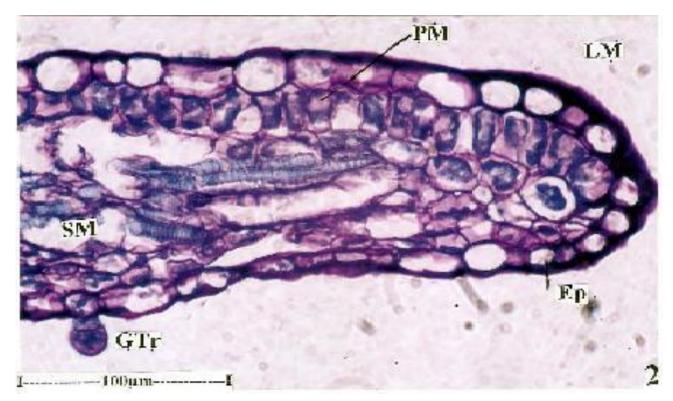
ABE – Abaxial Epidermis; HC – Head Cell; SM – Spongy Mesophyll; ST – Stalk

## Fig 8. Transverse section of lamina



AbE – Abaxial Epidermis; AdE – Adaxial Epidermis; PM – Palisade Mesophyll; SM – Spongy Mesophyll.

Fig 9. Transverse section of leaf margin



 $EP- Epidermis; \ GTr- Glandular\ Trichome; \ LM- Leaf\ Margin, \ PM- Palisade\ Mesophyll,\ SM- Spongy\ Mesophyll.$ 

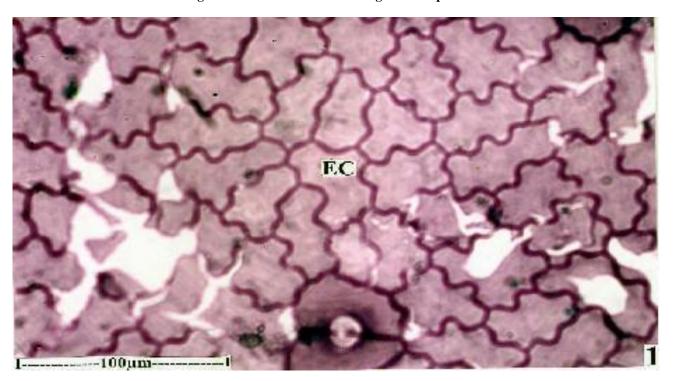
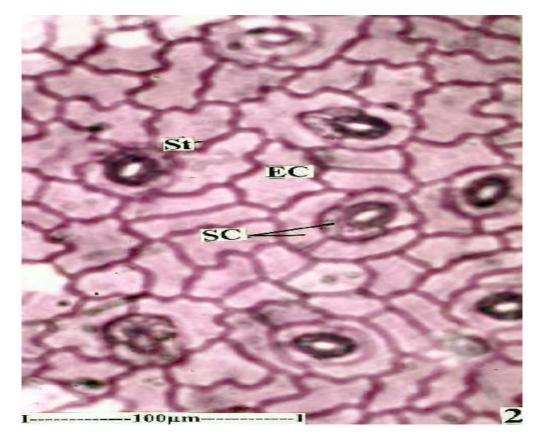


Fig 10. Paradrmal section showing adaxial epidermis

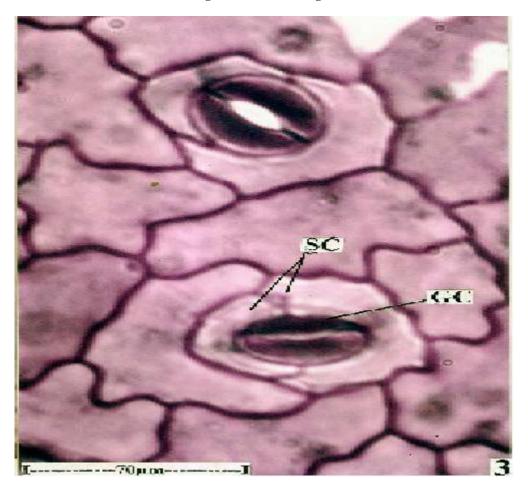
**EC** – Epidermal Cells

Fig 11. Abaxial epidermis with stomata



EC – Epidermal cells; SC – Subsidiary cells; ST – Stomata.

#### Fig 12. Stomata enlarged



GC- Guard Cell; SC- Subsidiary Cells

#### DISCUSSION AND CONCLUSION

The microscopic features are of diagnostic value in future identification of a crude drug. The microscopical study of the leaves of *Crossandra infundibuliformis* L. (Acanthaceae) showed the presence of specific features like midrib consists of adaxial strands, thick walled xylem and thin layer of phloem. The lateral vein is plano convex, the adaxial side is flat and abaxial part is convex. The lamina is 90um thick, the leaf margin is blunt and the epidermal cells are more thick walled. Compact zones of palisade cells are larger, intact spongy parenchyma cells are seen at the marginal part of lamina. In epidermal morphology, the epidermal cells are highly lobed and amoeboid in nature. The anticlinal walls are fairly thick and are wavy. The adaxial epidermis is apostomatic. The stoma are diacytic and consists of two subsidiary cells. The guard cells are elliptical with wide stomatal pore. The physicochemical parameters like ash values, extractive values, loss of drying were determined as per the standard monograph procedure. Flourescence analysis was performed for standardizing the drug and petroleum ether extract showed pink fluorescence under long U.V.

Petroleum ether, methanol, chloroform and water extracts of leaves of *Crossandra infundibuliformis* L. (Acanthaceae) were prepared and the percentage yield was calculated. Petroleum ether extract given higher yield. The Preliminary phytochemical screening of the leaves of *Crossandra infundibuliformis* L. (Acanthaceae) revealed the presence of steroids, flavonoids and carbohydrates.

#### REFERENCES

- 1. Sandhya S, Jaffery SAH, Vinod KR. Pharmacognostical studies on the leaf and root of *Physalis angulata* L. *International Journal of Pharma Research and Development* Online, 2(1), 2010, 1-8.
- 2. Selvamani P, Dhrubo Jyoti Sen and Jayanta Kumar Gupta. Pharmacognostical standardization of *Commiphora berryi* (Arn) Engl and phytochemical studies on its crude extracts. *African Journal of Pharmacy and Pharmacology*, 3(2), 2009, 37-46.

- 3. Wallis, Text book of Pharmacognosy, CBS publishers, Delhi, India, 1985, 572-575.
- 4. Bhattacharya S, Kamaruz Zaman M. Pharmacognostical evaluation of *Zanthoxylum nitidum* Bark. *International Journal of PharmTech Research*, 1(2), 2009, 292-298.
- 5. Johanson DA. Plant Microtechnique, Mc.Graw Hill Book. Co. New York, 1940, 523.
- 6. O'Brien TP, Feder N, Mc Cull ME. Polychromatic Staining of Plant Cell walls by Toluidine blue-O, *Protolpasma*, 1964, 364-373.
- 7. Sass JE. Elements of Botanical Microtechnique, Mc.Graw Hill Book. Co., New York, 1940, 222.
- 8. Esau K. Plant Anatomy, John Wiley & Sons, New York, 1979, 550.
- 9. Khandelwal KR, Practical pharmacognosy, Nirali Prakashan, 1<sup>st</sup> edition, 2006, 149-153.
- 10. Kokate CK, Purohit AP, Gokhale SB, Text book of Pharmacognosy, 27th edition, Nirali Prakashan, Pune, India, 2004.
- 11. Harborne JB, Phytochemical Methods, 3<sup>rd</sup> edition, Springer (India) Private Limited, New Delhi, 1998.
- 12. Sama V, Reddy YSR, Ramesh M, Swamy MM, Mahadevan N, Suresh B. Pharmacognostical Studies on *Dodonaea* viscosa leaves. *African Journal of Pharmacy and Pharmacology*, 2 (4), 2008, 83-88.
- 13. Amish AD, Kalidass C, Mohan VR. Pharmacognostical Investigation of *Hedyotis puberula* (G. Don) Arn. Asian Journal of Exp. Biol, Sci., 1 (2), 2010, 373-377.
- 14. Indian Pharmacopoeia, 4<sup>th</sup> edition, Vol. 2, Government of India, Ministry of Health and Family, Controller of Publication, New Delhi, 1996, A53-A57 & A100-A107.
- 15. Chase CR, Pratt RJ. Fluprescence of Powdered vegetable drugs with particular reference to development of a system of identification. *Journal of American Pharmaceutical Assolation*, 38, 1949, 324-331.