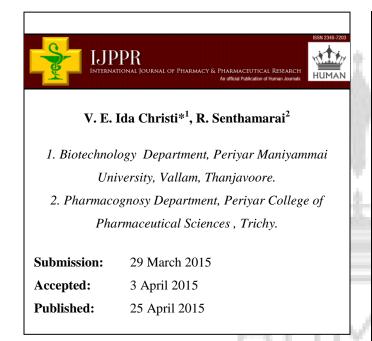
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Qualitative and Quantitative Pharmacognostical Studies on *Scoparia dulcis* Linn Leaf







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Keywords: Scrophylariaceae, Afflotoxins, Scoparia dulcis

ABSTRACT

Scoparia dulcis linn is called Sweet Broom belonging to the family Scrophulariaceae. It is a weed, branched herb with wiry stems, growing upto 1 m. It is traditionally used in treatment of diabetes, dysentery, earache, fever, gonorrhoea, headaches, jaundice, snake bite, stomach problems, toothache, and warts. In many culture, the plant is believed to have anti-cancerous properties. There is a good deal of research currently in progress on the chemical composition of the plant, and some of the phytochemicals have been identified and some have not been identified. One of the important particular constituent has been named scopadulcic acid B, and is used to inhibit cancerous growth. In the present study pharmacognostical parameters like morphology and microscopy of stem, leaf and root, physiochemical parameters like ash values, total fiber content, extractive values and moisture content determined. Preliminary were phytochemical study shows the presence of flavonoids, alkaloids, phenols and phytosterols. Inorganic elements such as Na, K, Ca, Fe, Zn, Cu, were quantitatively estimated. Total protein content, phenol content, flavonoid content, lipid content were estimated. Heavy metals like lead, arsenic mercury, nickel are estimated. Afflotoxins and pesticide residues were tested, and it shows absence of afflotoxins and others are in permissible limits. The present study gives basic pharmacognostical and preliminary phytochemical information about the plant for further work.

1. INTRODUCTION

The pharmacognostical and phytochemical study is a very important base for phytotherapy and for nutritional sciences because phytochemicals are complex mixtures that are incompletely characterized and have only recently been subject to scientific scrutiny. Inventorisation of herbal drugs used in traditional and modern medicines for a country like India, appears to be a stupendous task, where a number of well established indigenous or traditional systems, including Ayurveda, Unani, Siddha, Homoeopathy, Tibetan, Amchi, Yoga and Naturopathy are practised along with modern medicine for the management of total health care system (Joy.P 2001). In all these systems, a large number of plant drugs are used, although there may be some common plants. Another problem in correct identification of plants is that the plant drugs in those systems of medicine are known by their classical, Shastriya or vernacular names. It is not easy to correlate these names with acceptable scientific names (Henry A.N.1987). One plant species can have many vernacular classical names and one name may refer to different plant species. Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacturing of drugs and perfumery products (Bhandari 1990). Nowadays, some methodologies have been developed for this study. The WHO currently encourages, recommends and promotes traditional herbal remedies in National Health Care Programme because such drugs are easily available at low cost. They are generally mild without serious side effects, comparatively safer and their use will not require close supervision of an expert. One of the WHO assembly resolutions emphasized the need to ensure quality control of medicinal plant products using modern technique and establishment of required standards of quality for herbal medicines. Chemical screening of 'Scoparia dulcis' has shown that it is a source of novel phytochemicals in the flavone and terpene. Many of the active biological properties are attributed to these phytochemicals. The main chemicals being studied are scopadulcic acids A and B, scopadiol, scopadulciol, scopadulin, scoparic acids A, B, and C, and betulinic acid. It has been shown that the triterpenoids (Hayashi 1996,97). Leaf infusion used for bronchitis, cough, diarrhea, fevers, kidney diseases, haemorrhoids, anti diarrheic, and antiseptic (Latha.M. 2004). The leaf decoction used for cleaning wounds and fever. The leaf decoction mixed with maternal milk as an antiemetic for infants. It is also used as antipyretic and in poultices for migraine headaches. Ecuadorians take the tea of leaf for pain and swelling (Gamble

J.S.1935). Brazilians add the root to the bath when "cleaning their blood". They apply strained leaf juice for eye ailments; and to infected wounds (erysipelas). Indigenous tribes in Nicaragua use a hot water infusion of the leaves or the whole plant for stomach pain, for menstrual disorders, (Metcalf C.R.1950, 1979) as an aid in childbirth, as a blood purifier, for insect bites, fevers, heart problems, liver and stomach disorders, venereal disease, and as a general tonic.

2. MATERIALS AND METHODS

2.1. Plant materials

'Scoparia dulcis' plants are collected from Kanyakumari District, Tamilnadu, India, and authenticated by Dr. G.V.S.Murthy, Botanical survey of India, Coimbatore. V. No. is BSI/SRC/5/23/2012-13 Tech/496. The plants were collected, cleaned, the leaves were separated, shade dried and powdered, then passed through 40 mesh size packed in an air tight container for further use.

2.2. Chemical and solvents

All the chemicals were obtained from S.D. Fine, Mumbai and all are analytical grade.

2.3. Pharmacognostical study

The plant is a native of tropical America and introduced into India. It is a small much branched glabrous annual herb with wiry stems in favourable condition; it can grow upto 60 cm height. The leaves are in opposite or in whorls of three, oblanceolate 2-3 cm long, serrate margins pointed at both ends and narrowed below the short stalk (Henry A.N.1987). The plant has quadrangular, grooved stem. The flowers are bisexual, solitary or few together, with long pedicels. Sepals 5 imbricate, white 4 stamens. The fruit is a small, globose capsule with many minute seeds (Bhandari M.M.1990).

2.4. Preparation of transverse section of leaf, stem and root

Care was taken to select healthy plants as normal organs. The required samples of different organs were cut and removed from the plant and processed. The specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 mm. Dewaxing of the sections was done by customary procedure (Johensen.1940). The sections were stained with

Toluidine blue as per the method published by (O' Brien et al. (1964). Since Toluidine blue is a polychromatic stain.

For studying the leaf constants microscopy like stomatal morphology, venation patellae to the surface and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) were prepared and epidermal peeling by partial maceration employing 'Jeffrey' maceration fluid (Sass, 1940) were prepared. Different cell component were studied and measured. Photographs of different magnifications were taken with NIKON Labphoto 2 microscopic Unit. For normal observations bright field was used. Descriptive terms of the anatomical features are as given in the standard Anatomy books (Esau, 1964). The results are the triplicate values were calculated and expressed in (Table no.1).

2.5. Preparation of extract

About 1kg of drugs powdered and extracted with methanol by cold maceration method for 7 days. Then the extracts were filtered and the last traces of the solvent were evaporated under reduced pressure in a rotary evaporator. The yield of the dry extracts was calculated.

2.6. Determination of ash values

About 2 g accurately weighed powdered drug from the three samples were incinerated in a silica crucible at a temperature not exceeding 450°C for 4 hours in a muffle furnace until free from carbon. It was then cooled and weighed. The % w/w of ash with reference to the air-dried drug was calculated. The acid insoluble ash, water soluble ash and sulphated ash was done according to the standard procedure (Dr. C. K. Kokate, 1994). Average of the triplicate values were calculated and mentioned in the (Table No. 2).

2.7. Determination of extractive value

Accurately weighed 5 g of air-dried powdered drug was macerated with 100 ml of 90 % alcohol of the specified strength in a closed flask for 24 h, shaken frequently during first 6 h and allowed to stand for 18 h. It was then filtered rapidly, taking precautions against loss of the solvent and 25 ml of the filtrate were evaporated to dryness in a tared flat-bottomed shallow dish and dried at 100°C to constant weight. The % w/w of alcohol soluble extractive value was calculated with reference to the air-dried drug. The same procedure was repeated with different solvents like

chloroform, petroleum ether, benzene and water according to the standard procedure (Dr. C. K. Kokate, 4th edition1994). The values are mentioned in the (Table No. 3).

2.8. Determination of total fibre content

About 3 gm of the finely powdered crude drug leaf are weighed and extracted with petroleum ether at room temperature. Then the drug was dried from that 2 gm of drug was taken for the estimation. The drugs were separately boiled with 300 ml of dilute sulphuric acid for 30 minute. Filter the extract material through a muslin cloth and wash with boiling water. Then boil the material with 200 ml dilute sodium hydroxide for 30 minutes. Filter through muslin cloth and wash with boiled water 25 ml of alcohol successively. After washing the residue transfer to silica crucible, which was previously weighed (W1). Dry the residue for 2 to 3 hours for 130° C and cool the crucible in the desiccators and weigh again (W2). Incinerate the residue for 30 minute at 100°C and cool it to room temperature in a desiccators and weigh again (W3). Then calculated by using the following formula: (W2-W1) - (W3-W1) / weight of sample x 100 (Kanderwal K.R 2005).

2.9. Preliminary phytochemical analysis

The extracts prepared with different solvents were taken and standard methods of chemical identification tests (Dr. C. K. Kokate, 1994) were used to detect the nature of phytoconstituents present in them .

3. QUANTITATIVE ESTIMATION

3.1 Total flavonoid content

The flavonoid content was determined by the use of a slightly modified colorimetric method described previously by (Zhishen et al. 1999). A 0.5 ml aliquot of appropriately (2mg/2ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5 % Sodium nitrite solution. After 6 min, 0.15 ml of 10 % Aluminium chloride solution was added and allowed to stand for 6 min, and then 2 ml of 4 % Sodium hydroxide solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate

and the results were expressed as Rutin equivalent. Values are means of three independent analysis \pm standard deviation (n = 3) RE – Rutin equivalent.

3.2. Total phenolic content

The total phenolic content in methanolic extract of the plants was estimated by Folin-Ciocalteau reagent (Siddhuraju P and Becker K., 2003) method Gallic acid stock solution (90 µg/ml) was prepared by dissolving 100 mg of gallic acid in 100 ml of ethanol. Various dilutions of standard gallic acid were prepared from this stock solution. Folin-Ciocalteau reagent was prepared by mixing Folin's reagent with Phenol reagent (1:1), and diluted 1:1 in distilled water, before use. Calibration curve was plotted by mixing 1 ml aliquots of 10, 20, 30, upto 90 µg/ml gallic acid solutions with 5.0 ml of Folin-Ciocalteu reagent (diluted tenfold) and 4.0 ml of sodium carbonate solution (75 g/l). The absorbance was measured after 30 min at 20°C at 765 nm. One ml of and methanol extract (1.0 g/100 ml) was mixed separately, with the same reagents as did in construction of total phenolic compound in both the extracts separately by using formula; C = C1 × V/m. Where; C = Total content of phenolic compounds in mg/g, in GAE (gallic acid equivalent); C1 = the concentration of Gallic acid established from the calibration curve in mg/ml; V = the volume of extract in ml; M = the weight of plant extract in gm (Lowry1957).

3.3 Estimation of protein

The dried and powdered sample was extracted by stirring with ethanol (1:5 w/v) at 25°C for 24 h and centrifuged at 7,000 rpm for 10 min (Loganayaki et al., 2011). 0.25 ml of the methanolic solution of the samples was taken. The volumes in all the tubes were made up to 1.0 ml with distilled water. 5.0 ml of alkaline copper reagent was added to each tube. Mixed well and allowed to stand for 10 min. Then 0.5 ml of Folin-Ciocalteau reagent was added. Mixed well and incubated at room temperature for 30 minutes. A reagent blank was also prepared. After 30 minutes the blue colour developed were read at 660 nm. The results were expressed as mg/g dry matter (Chunk Ok 1980).

3.4 Estimation of total lipid content

10 g of the sample was used to extract lipids with 150 ml of petroleum ether for 16 hr, at a solvent condensation rate of 2–3 drops/sec according to (Chung OK et al., 1982) AACC

Approved Method 30-25 with minor modifications of sample size and extraction time. The obtained extract was concentrated and evaporated at room temperature to dryness. The weight of extract gives the total lipid content, which was expressed as mg/g dry matter.

3.5 Estimation of inorganic and heavy metals

The metals present in the plant material were estimated by the method explained in the book (P. parimoo 1998 and A. H. Beckett, 1997). The dried leaf powder was incinerated and prepared the ash. The ash was used for the estimation. 1 gm ash is dissolved in diluted hydrochloric acid and filtered. This solution was taken for the estimation of sodium, potassium by flame photometric method and calcium, copper, manganese, magnesium, zinc and iron was estimated by Atomic absorption method. Then this sample was used for the estimation of heavy metals using atomic absorption spectra method.

3.6. Test for the presence of aflatoxins

The presence of aflatoxins B1, B2 and G1, G2 in the sample was detected as follows (Trees G 1990). 10 μ l of the Aflatoxin solution and the test solution was separately applied onto a suitable thin-layer chromatographic plate coated silica gel mixture. The chromatogram developed in an unsaturated chamber containing a solvent mixture of chloroform, acetone and isopropyl alcohol (85: 10: 5). After drying the spots on the plate was located by examination under UV light at 365 nm and the standard applications of the Aflatoxin solution appeared clearly as separated blue fluorescent spots and the spot obtained from the test solution showed no spot corresponding to any of the spots obtained from the applications of the Aflatoxin solution (K. R. Brain 1995). As per the method cited in Ayuvedic Pharmacopoeia of India; volume II and part II.

3.7 Pesticides residues estimation

The plant was estimated for the presence of pesticides residue by using Mass Spectroscopic method (Willard Merritt 1986) and compared with that of some standard pesticides like Aldrin, Dieldrin, Diazone, Baygon, Parathion, Chlordane, Carboryl etc. The results show the absences of pesticide residues.

RESULTS AND DISCUSSION

Scoparia dulcis leaf, stem and root show the following microscopical characters.

The leaf is dorsiventral, mesomorphic, amphistomatic and proximally veined. Midrib of the leaf is flat on the adaxial side and broadly semicircular on the abaxial side. The midrib is 330 µm in vertical plane and 380 µm in horizontal plane. The epidermal layer of the midrib consists of dilated, circulated or angular cells which are $25 - 30 \mu m$ thick. Beneath the adaxial epidermis, the palisade mesophyll extends across the vascular bundle forming a transcurrent zone. The lower part of the midrib has parenchymatous with wide, angular or circular thin walled cells. Larger vascular bundle cells are present in the midrib region of the leaf. The vascular strand is single, wide and planoconvex in sectional view measuring 110 µm thick and 210 µm wide. The xylem elements are in several parallel vertical files and the cells are angular, wide and thick walled. Phloem elements occur in this are beneath the xylem. The lamina is distinctly bifacial. The stomata occurs both in the upper and lower epidermal layer. The adaxial epidermis consists of thin walled cylindrical cells which are 15 -20 µm thick (Fig.1). The abaxial epidermis is slightly thin with cylindrical cells. The palisade zone consists of two layers of short, wide, cylindrical cells. The spongy mesophyll has four or five layers of spherical or lobed, loosely arranged cells. Glandular trichomes are seen in the epidermal surface. The gland has a short, wide stalked cell and a wide, peltate secretory head cell. The glands occur inside a shallow epidermal pit. The gland is 40µm in height and 35 µm wide. The trichome consists of densely cytoplasmic cells which are secretory in function. The glandular trichomes are seen in the form of circular plate. It has two rows of rectangular cells. Transverse section of the leaf revealed that the epidermal cells consist of straight anticlinal walls. Beneath every upper epidermal cell there are about 3.08±0.45 palisade parenchyma cells are present; they are very green, tightly packed and two layered. Below the palisade parenchyma there are loosely arranged spongy parenchyma cells are present. The adaxial as well as abaxial epidermal layers are stomatiferous. The epidermal cells are lobed and amoeboid in outline due to wavy anticlinal walls. The stomata are anomocytic type and have no distinct subsidiary cells (Fig.2). Some of the stomata tend to be anisocytic which possess three unequal subsidiary cells encircling the guard cells.

The stem is four angled with thick latent wings the angles. The stem is 1.7 mm thick. The wings are 250 µm thick and 150 mm in height. (Fig.3). The stem consist of a thin continuous layer of

epidermis with small spindle shaped cells. The epidermis is 15 mm thick. Cortex is 50 mm wide. It comprises outer zone of three or four layers of chlorenchyma cells and inner zone of about three layers of parenchyma cells. The inner boundary of cortex consist of unistratose, small sclerenchyma cells. The wings consist of small angular compact thick walled cells (Fig.3). The vascular cylinder is uniformly thick, closed cylinder enclosing the pith, it includes the layer of phloem elements. Xylem consists of compact radial files of xylem vessels and fibers. The pith is wide. It includes large, thick walled compact angular parenchyma cells. In a slightly thicker (older) stem the outer cortex consists of narrow zone of palisade cells followed by inner cortex of compact parenchyma cells (Fig 3.3). The secondary xylem cylinder has increased in thickness comprising more number of xylem elements in the radial files of xylem cylinder. The epidermis of young stem bears glandular type of trichomes (Fig 3.1). The gland is peltate type. It consists of a short stalk cells, horizontally flat, circular plate of body. The body cells have dense cytoplasm . The glands is 30 mm in height and 60 mm in diameter. Stomata with prominent ledges are looking like breaks (Fig. 3.1).

Roots of different thickness were tried, the root measuring 1.6 µm thick consists of 650 µm in diameter and large radially elongated air chambers in the cortical region. The outer cortex is 2 or 3 layered, the air chambers in circle separated from each other by their radial partition filaments. (Fig. 4.1, 4.2). The xylem cylinder is dense and compact and consists of wide cylinder wide solitary and diffusing distributed vessels and narrow thick walled Fibres (Fig. 4.2). Phloem is in thin continuous cylinder. The slightly thicker root has less distinct periderm and two circles of air chambers separated by thick multilayered partition filaments. The partition filaments are broken at certain places. The vascular cylinder consists of a layer of parenchymatous endodermis a distinct cylinder of phloem and 800 µm thick xylem cylinder. The vessels are either solitary or in multiples of two or three. The vessels frequency is more and the xylem fibres are thick walled and occur in radial rows (Fig. 4.2). In a thickest root, which is 2.1 mm in diameter, the aerenchymatous cortex is thicker with larger air-chambers (Fig 4.1). The vascular cylinder is 850 µm wide. The endodermal layer is thick and distinct. The phloem cylinder is 8-10 layers and continuous around the xylem cylinder. The vessels are numerous, solitary and diffusely distributed. They are circular or angular, wide and thin walled (Fig. 4.1, 4.2). Xylem fibres are also wide and thick walled.

Table 1. Leaf constants

Parameters	Range		
Palisade ratio	2.49-3.66		
Stomata number upper surface	4.25-9.74		
Stomata number lower surface	9.18-17.41		
Stomata index upper surface	6.24-13.87		
Stomata index lower surface	8.59-19.32		
Vein islet number	22.99-28.00		
Vein termination number	26.32-30.07		
Epidermal cells upper surface	54.80-70.39		
Epidermal cells lower surface	68.43-100.56		
Stomata length	29.53-35.96		
Stomata breadth	12.95-17.04		

Table 2. Ash Values

Sr. No.	Parameters	Values % w/w
1	Total ash	8.25
2	Acid insoluble ash	0.31
3	Water soluble ash	2.24
4	Sulphated ash	4.24

Table 3. Extractive values

Sr. No.	Solvent Name	Values % W/W	
1	P.ether	7.4	
2	Benzene	9.4	
3	Chloroform	6.7	
4	Methanol	14.3	
5.	Acetone	4.3	
6.	Water	18.6	

PHYTOCHEMICAL ANALYSIS

Table 4. Quantitative estimation

Sr. No.	Parameters	Values % w/w		
1	Total Flavonoid Content	$1.95 \pm 0.030 \text{ (mg RE/g)}$		
2	Total Phenolic Ontent	70.16 ± 12.52 (mg TAE/g extract)		
3	Total Protein Content	$17.23 \pm 0.29 \text{ (mg/g dry matter)}$		
4	Total Lipid Content	60.00±0.45 (mg/g dry matter)		
5	Vitamin C Content	3.205 ± 0.41 (mg/g fresh matter)		

Inorganic metals: Inorganic elements such as Na 28ppm, K 58ppm, Ca 304ppm, Iron 11ppm, Zn 0.52ppm, Cu 0.2385ppm, Magnesium 77ppm, Manganese 0.02ppm were quantitatively estimated by atomic absorption spectral analysis. It shows all these elements are present in considerable quantity.

Heavy metals: Heavy metals like Mercury, Lead, Cobalt, Nickel, Arsenic and Cadmium were estimated by using atomic absorption spectrum, it shows the absence of all these heavy metals. So the plant is free from heavy metals, it can be taken internally.

Afflatoxins: Afflatoxins are very toxic materials, if it is present in herbal medicines, it may leads to danger disease to human beings. It was tested by TLC method by comparing with that of standard afflatoxins like G_1 , G_2 , and B_1 , B_2 . There are no sports related with the standards.

Sr. No.	Chemical test	Aqueous extract	Methanol extract	Chloroform extract	Acetone extract	Benzene extract	E. acetate extract
1	Alkaloids						
	Dragondroff's test	+	+	+	+	+	+
	Mayer's test	+	+	+	+	+	+
2	Carbohydrates		6				
	Molisch's test	-	- 4	-	-	-	-
	Fehling's test	-	·	-	-	-	-
3	Proteins and Free Amino acids	- h-		- Á			
	Ninhydrin test	1	- 0		-	-	-
	Biuret test	(1)	-	1 / 1	-	-	-
4	TanninsandPhenolic	1	171	t de la			
	Compounds						
	Ferric chloride test	+	+	+	+	+	+
	Lead acetate test	+	+	+	+	+	+
5	Phytosterols		1.1	A A L			
	Libermann Burchard test	ПU	+ * /	₽\ I N	+	+	+
	Salkowwski test	+	+	+	+	+	+
6	Flavanoids						
	Shinoda test	+	+	+	+	+	+
	Ammonia test	+	+	+	+	+	+
7	Glycosides						
	Borntrager's test	-	-	-		-	-
	Modified	-	-	-	-	-	-
	Borntrager's						
	Keller-Killiani test	-	-	-	-	-	-
	Legals	-	-	-	-	-	-

 Table 5. Preliminary phytochemical analysis

Sr.	Treatment	Colour Development		
No.	Treatment	Short λ(254)	Long λ(365)	
1.	Powder as such	Yellow	-	
2.	Powder + dil. HNO ₃	Yellow	Yellowish brown	
3.	Powder + NaOH in methanol	Green	Yellowish brown	
4.	Powder + Acetic acid	Yellowish brown	Orange	
5.	Powder with NaOH in water	Yellowish green	Violet Shade	
6.	Powder with Ferric chloride	Fluorescent green	Violet shade	
7.	Powder + HCl	Yellow	Red	
8.	Powder + picric acid	Pale yellow	Orange red	
9.	Powder + iodine	Pale yellow	Light red	
10.	Powder with dil. H ₂ SO ₄	Yellow	Brown with violet shade	

CONCLUSION

The present study gives a clear qualitative pharmacognostical, quantitative physical and preliminary phytochemical studies of *Scopario dulcis* plant. Thus, it is evident that the present study would provide various resourceful information in relation to pharmacognostical identification of this plant leaves, stem and root. Furthermore, information regarding physicochemical characteristics of such plant leaves and nature of chemical constituents present in them, it would also be useful for standardization of such herbal drugs of folk medicinal practice of present era and enrichment of Ayurvedic Pharmacopoeia. It would also help scientists to utilize such needful information regarding the plants identity and characteristics in developing new poly herbal formulations.

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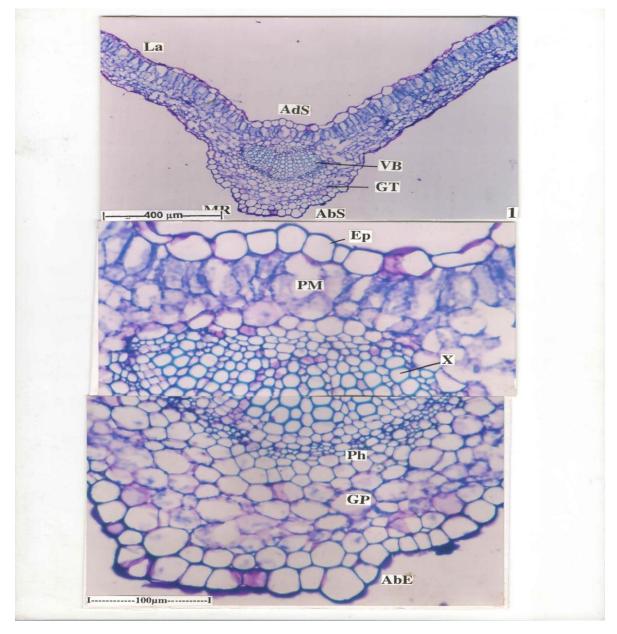
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Fig. 1. T. S. of leaf



AbE- Abaxial epidermis, AbS- Abaxial side, AdS- Adaxial side, Ep- Epidermis, GT- Ground Tissue, La- Lamina, MR- Midrib, Ph - Phloem, PM - Palisade Mesophyll, VB- Vascular Bundle, X- Xylem, SM- Spongy mesophyll, GTr- Glandular Trichome, MT- Mesophyll Tissue, St-Stomata, Ec- Epidermal cells

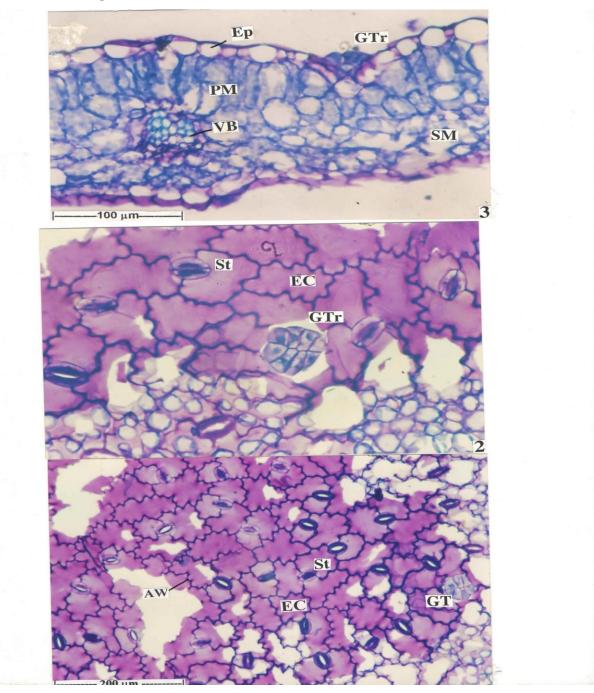
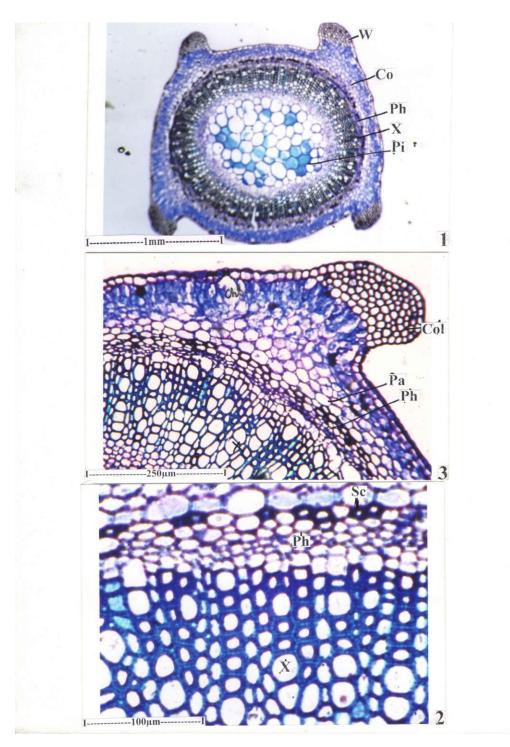
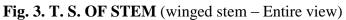


Fig. 2. C. S. Through lamina shows stomata, Trichome

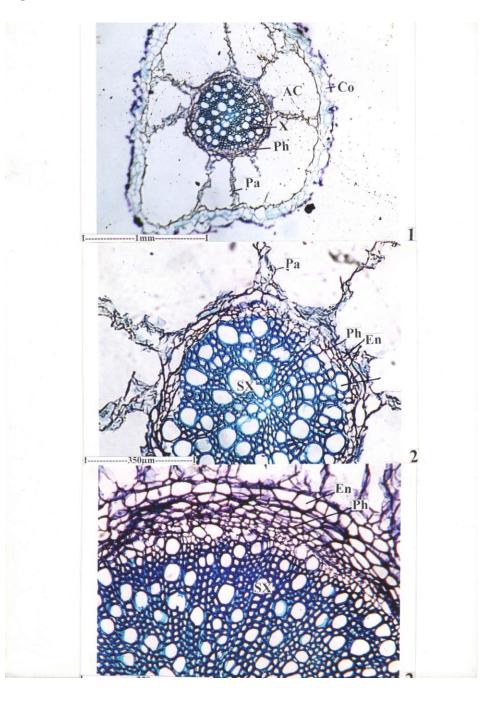
Gp- Ground plan, Gtr- Glandular Trichome, Ph- Phloem, Sc- Sclerenchyma, St- Stomata, X- Xylem





Co- Cortex, Col- colenchyma, PM- Palisade Mesophyll, Ep- Epidermis, Pa- Parenchyma, Pi-Pith, Ph- Phloem, Sc- Sclerenchyma, W- Wing, X- Xylem

Fig. 4. T. S. OF ROOT:



AC- Air chamber, EN- Endodermis, PA- Parenchyma, CO- Cortex, Ph- Phloem, SX-Secondary, X- Xylem, EN- Endodermis, PA- Parenchyma, PE- Periderm, Sph- Secondary phloem, SX-Secondary Xylem, Ve- Vessels