



Morpho-anatomical and preliminary phytochemical studies of leaves of *Gynandropsis pentaphylla* Linn.

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Abstract

Gynandropsis pentaphylla Linn (Fam: *Cleomaceae*) is also known as Arkapushpika, Suryavarita in Sanskrit, Churota, Arkahuli in Hindi, Velai keerai, Thaivelai in Tamil, belonging to the family of *Cleomaceae* is an annual, erect, branched, 0.6-1.2 m in height, stems and branches striate, white spreading hairs. Leaves 3-5 foliolate, petioles 5-7.6 cm long, and seeds muricate dark brown. The plant has been traditionally used as an anthelmintic and rubefacient. Leaves are applied externally over the wounds to prevent the sepsis. The plant also used in the treatment of malaria, piles, rheumatism and in tumour. The decoction of the root is used to treat fevers. The juice of the root is used to relieve scorpion stings. The leaves, applied as a poultice, are used as a vesicant and rubefacient in the treatment of rheumatism. The juice of the leaves is a remedy for pain in the ear. The whole plant is used in the treatment of scorpion stings and snake bites. The present investigation deals with the qualitative and quantitative microscopic evaluation of the leaf material and establishment of the quality parameters including physicochemical and phytochemical evaluation. The microscopical studies revealed the presence of mesophyll tissue, venation pattern, anomocytic stomata and glandular trichomes etc.. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, tannins, glycosides, phenolic compounds, flavanoids, saponins, protein and amino acids, steroids, etc was done by standard methods.

Key-Words: *Gynandropsis pentaphylla*, Microscopical characters, Physicochemical analysis

Introduction

India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value. The use of medicinal plants in traditional medicine is well known in rural areas of many developing countries^{1,2}. According to World Health Organisation (WHO) more than 80% of the World's Population rely on traditional medicine for their primary health care needs and have found a place in day-to-day life³. Medicines obtained from plants are relatively safer than synthetic alternative^{4,5}. According to the world Health Organization the macroscopic and microscopic description of medicinal plants is the first step towards the establishing the identity and degree of purity of such materials and should be carried out before any tests are undertaken⁶.

The plant *Gynandropsis pentaphylla* Linn (Fam: *Cleomaceae*) (Syn. *Gynandropsis gynandra* L. *Cleome gynandra* L.), is commonly known as 'Hurhur' and 'Karaila' in India and 'Cat's whiskers' in English⁷. It is an herb indigenous to the tropical and subtropical regions. The leaves and seeds of cat's whiskers are used in indigenous medicine in many countries. Cat's whiskers grow as a weed in most tropical countries. It has been used for several years in Indian traditional medicine as an anthelmintic and antimicrobial agent⁸. Leaves are applied externally over the wounds to prevent the sepsis. The decoction of the root is used to treat fevers. Leaves with a high percentage of vitamin C is taken as a pot herb in soups, fresh or dried. The leaves are used as disinfectants. Inhalation of the leaves also relieves headaches; leaf juice and oil, for earache and eye wash. Stems are used as analgesic and anti-inflammatory agent⁹. A decoction or infusion of boiled leaves and/or roots is administered to facilitate childbirth, treat stomach-ache, constipation, and conjunctivitis or thread-worm infection. The seeds and roots also have anthelmintic properties¹⁰. The whole

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plant is also used in the treatment of malaria, piles, rheumatism and in tumor¹¹. Seeds have been reported to have antihelmintic properties and rubefacient. The methanol extract possess very good antioxidant property¹². The plant *Gynandropsis pentaphylla* also possess anti-inflammatory and lysosomal stability actions in adjuvant induced arthritic rats^{13,14}. In the present study, includes macroscopic and microscopic evaluation, fluorescence analysis of powder, physico-chemical values, preliminary phytochemical screening of different extracts.

Material and Methods

Plant material

The leaves of *Gynandropsis pentaphylla* Linn were collected from Yercaud foot hills, Salem, Tamil nadu, in the month of March 2012. The plant material was taxonomically identified and authenticated by the botanist Dr.A. Balasubramanian, Director, ABS Botanical garden, Salem, Tamil Nadu, India. A voucher specimen has been kept in our museum for further reference. The leaves were separated and shade dried at room temperature for 10 days and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve no.40.

Macroscopic evaluation

The organoleptic and macroscopic characters of *Gynandropsis pentaphylla* Linn leaves like colour, odour, shape, size, taste, ect., were evaluated.

Instruments used

Photographs of different magnifications were taken with Nikon Labphot2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background^[15].

Anatomical studies

Collection of specimens

plant specimens were collected from Yercaud foot hills, Salem, Tamil Nadu, India. Care was taken to select healthy plants and for normal organs. The required samples of leaves were cut and removed from the plant and fixed in FAA (Formalin-5ml + acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24hrs of fixing, the specimens were dehydrated with graded series of tertiary -butyl alcohol as per the schedule given by Sass, 1940^[16]. The specimens were castled into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 μ m. De-waxing of the sections was done by customary procedure. The sections were

stained with Toluidine blue as per the method published by O'Brien *et al*^[17]. For studying the venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jefferey's maceration fluid was prepared. Glycerin mounted temporary preparations were made for macerated/ cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerin medium after staining. Different cell component were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labhot 2 Microscopic Unit. For the study of crystals, starch grains and lignified cells, polarized light were employed. Under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale -bars. Descriptive terms of the anatomical features are as given in the standard anatomy books.

Power microscopy

The dried leaves were powered and studied under microscope. A pinch of powder was taken in a microscopic slide, 1-2 drops of 0.1% w/v phloroglucinal solution and a drop of concentrated hydrochloric acid were added and covered with a cover slip. The characteristics features of cell components were observed and their photographs were taken using photomicrography.

Physicochemical analysis

The dried powdered leaves was subjected to physicochemical analysis including fluorescence analysis^[18], moisture content, total ash, water soluble ash, acid insoluble ash, sulphated ash, alcohol soluble extractive and water soluble extractive^[19] to determine the quality and purity of the plant materials.

Preparation of extracts

The powdered material of *Gynandropsis pentaphylla* was extracted separately by using soxhlet apparatus with different solvents^[20] and aqueous for cold maceration. After extraction, the extracts were concentrated under reduced pressure.

Preliminary phytochemical screening

The dried powdered leaves were extracted with petroleum ether (60-80°C), chloroform and alcohol using soxhlet apparatus and aqueous extraction by cold maceration. The solvents were completely removed and reduced pressure by using vacuum evaporator. All the extracts were screened qualitatively for the

presence of various groups of phytoconstituents using different chemical tests. ^[15]

Results and Discussion

Macroscopic study

It is an annual, erect, branched, 0.6-1.2 m in height, stems and branches striate, white spreading hairs. Leaves 3-5 folioate, petioles 5-7.6 cm long, and glabrescent; leaflets obovate, subserrulate; pods linear. Annual, a foot or more high, branching, covered with long and short, and mostly glandular, clammy hairs. Lower and middle leaves on long petioles, upper subsessile, and much smaller. Leaflets 1/2-1 inch long, 4 lines wide (Figure 1 & 2). Pedicels 1/2 inch long, lengthening in fruit. Petals white or pale rosy. Pods 2 inches long or more, on a stipe 1/2 inch long, glandularly rough, tipped with a short style and broad stigma. Seeds reniform, rough with little pustules. Seeds muricate dark brown. Seeds finely longitudinally striated, with slightly cristate transverse ridges. Flowers 1-2 cm across, mostly white or pinkish; pedicels generally 1-2 cm long.

Microscopic study of leaf

The leaf consists of a prominent midrib and fairly thick lamina. (Fig.3.1, 2). The midrib is wide and circular with narrow adaxial groove. (Fig.3.1). It is 600µm thick and 720µm wide. The epidermal layer is thin with small Squarish cells with memberanous cuticle. The ground tissue consists of about six layers of homogenous, thin walled angular compact cells (Fig.3.2).

The vascular system is three starnded comprising a central median layer strand and two smaller lateral strands, one on either side of the median strand (fig. 3.2). The strands are collateral with solitary or clustered wide, angular, thick walled cells. (Fig.3.3). The xylem elements are 20µm wide. Phloem elements are in diffuse distribution, spread along the lower part of the xylem strand.

The lateral veins are slightly dilated and circular with central core of collateral vascular strand exsheathed all around by a layer of wide bundle sheath cells. The sheath cells have accumulation of chloroplasts in the liner end of the cell lumen.(Fig.4.2). These cells are called Kranz-tissue. The vascular strand includes a conical mass of xylem elements and a thick arc of phloem elements.

Lamina (Fig.4.1)

The leaf-blade (lamina) is smooth and uniform on both sides. The adaxial epidermis is wide comprising barrel shaped or rectangular thin walled cells. It is apostomatic (with out stomata). The abaxial epidermis is comparatively thin, with cylindrical, thin walled cells; this layer is stomatiferous (having stomata). The

mesophyll tissue not well differentiated into palisade and spongy tissues. One or two layers of cells in the adaxial part are slightly vertically elongated. The remaining cells are small, lobed and loosely arranged. The lamina is 160µm thick.

Powder microscopy

The powder of the leaf shows the following inclusions:

Epidermal glandular trichomes: (Fig.5.1, 2)

Multicellular, Multiseriate, cylindrical glandular trichomes are abundant in the powder. The trichome has straight or curved cylindrical stalk iwhich may be short or long (fig 5.1, 2). The terminal part of the stalk is swollen into elliptical or spherical secretary head, (Fig.6.2). The secretary head is multicellular with darkly staining cells. When viewed from above the head appears circular (Fig.6.1), measuring 40µm in diameter. The height of the trichome varies from 70µm to 170µm.

Epidermis (Fig.7.1)

Small pieces of epidermal peeling are frequently seen with powder. The epidermal cells are thin walled with many undulate anticlinal walls, the epidermal cells are amoeboid in outline. Stomata are abundant. They are anomocytic type (without specific subsidiary cells). The guard cells are 20×25 µm in size.

Venation

Small pieces of lamina are seen in the powder which shows the venation pattern (Fig 7.1, 2). The major veins and the vein-lets are equally thick; the veins are straight. The venation is densely reticulate (Fig7.1). The vein-islets are distinct formed by thick, straight veins. The vein terminations are simple or forked once or twice and short and thick (Fig 7.2.).

Fluorescence analysis

The fluorescence analysis of leaf powder with different chemical reagents is mentioned in Table 1.

Physicochemical analysis

In physicochemical analysis, various parameters such as moisture content, foreign matters, total ash, Acid insoluble ash, water soluble ash, sulphated ash and various extractive values were determined in triplicate as mentioned in Table 3.

Extraction of plant material

The dried and coarse powdered leaves of *Gynandropsis pentaphylla* linn. was extracted with solvents of increasing polarity successively by soxhlet apparatus whereas aqueous extract was obtained by cold maceration. The percentage yield of dried and coarse powdered leaves of *Gynandropsis pentaphylla* linn. was found to be 4.86%, 3.76%, 11.46%, 25.42% respectively with petroleum ether, chloroform, ethanol and aqueous.

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, tannins, glycosides, phenolic compounds, flavanoids, saponins, protein and amino acids, steroids, etc (Table 6).

Conclusion

The present study is used to investigate the pharmacognostical, physical constants and preliminary phytochemical screening of *Gynandropsis pentaphylla* Linn. leaves provided useful information about its correct identity and evaluation. The macroscopical studies revealed the morphological character of different parts of plant. The microscopical studies revealed the presence of lamina, mesophyll tissue, venation pattern, anomocytic stomata. Midrib, xylem, phloem and glandular trichomes etc. Various physicochemical parameters such as ash values, extractive values, foreign organic matter, moisture content and fluorescence analysis were determined. Exhaustive extraction of the plant material was done with petroleum ether, chloroform, ethanol and chloroform water. Phytochemical screening is also useful to isolate the pharmacologically active principles present in the drug. The other parameters observed are also useful for the future identification of the plant and serves as a standard monograph for identification and evaluation of plant.

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Fig. 1: A portion of plant with inflorescence



Fig. 2: Leaves of *Gynandropsis pentaphylla* Linn.

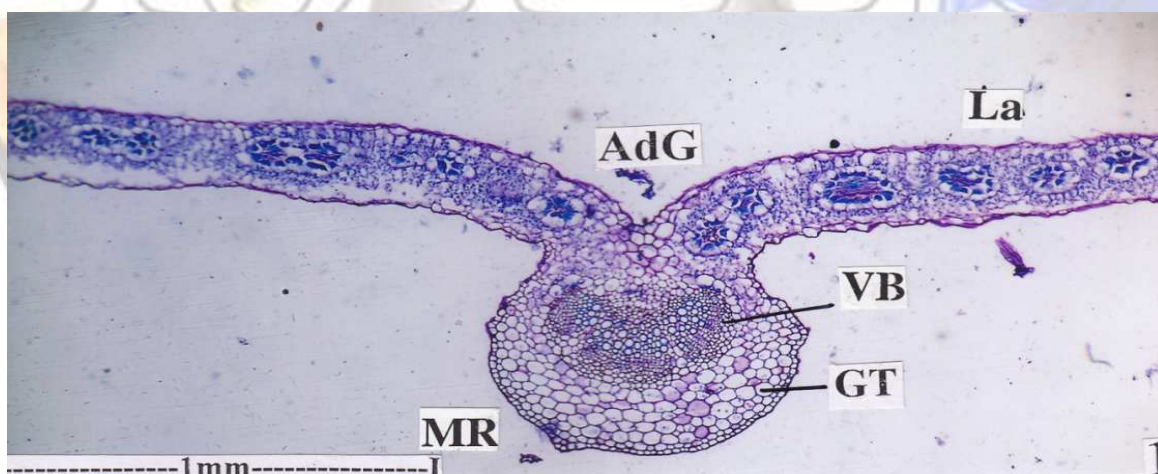


Fig. 3.1: T. S. of leaflets with midrib and Lamina. (AdG: Adaxial Groove; La: lamina; GT: Ground Tissue; MR: Midrib; VB: Vascular Bundle)

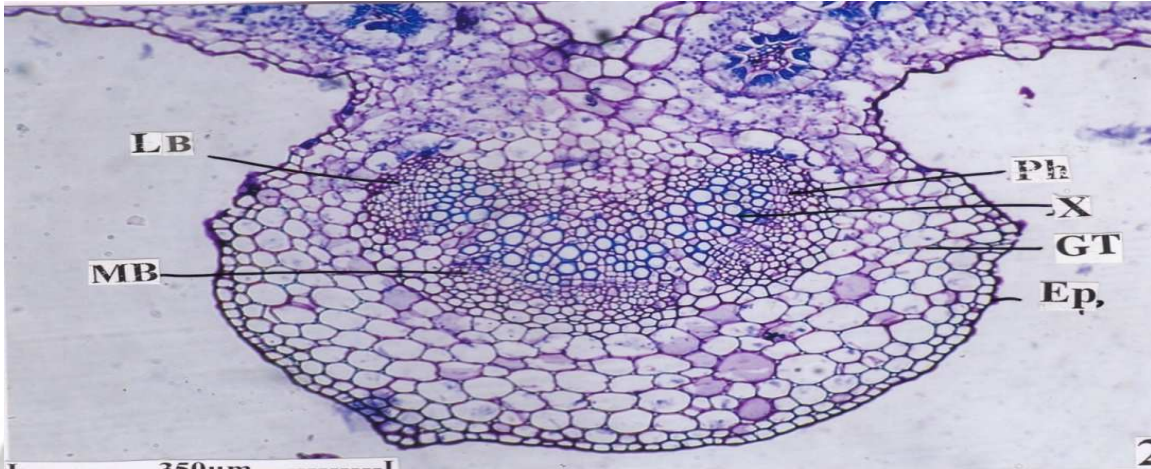


Fig. 3.2: T. S. of Midrib. (Ph: Phloem; X: Xylem; Ep: Epidermis; GT: Ground Tissue; MB: Median Bundle; LB: Lateral Bundle)

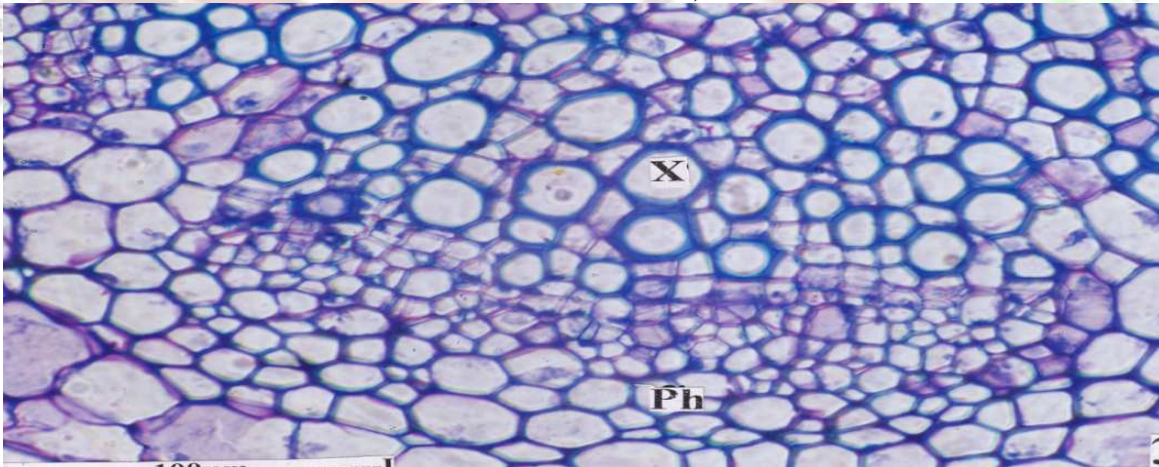


Fig. 3.3: Vascular strand of the Midrib. (Ph: Phloem; X: Xylem)

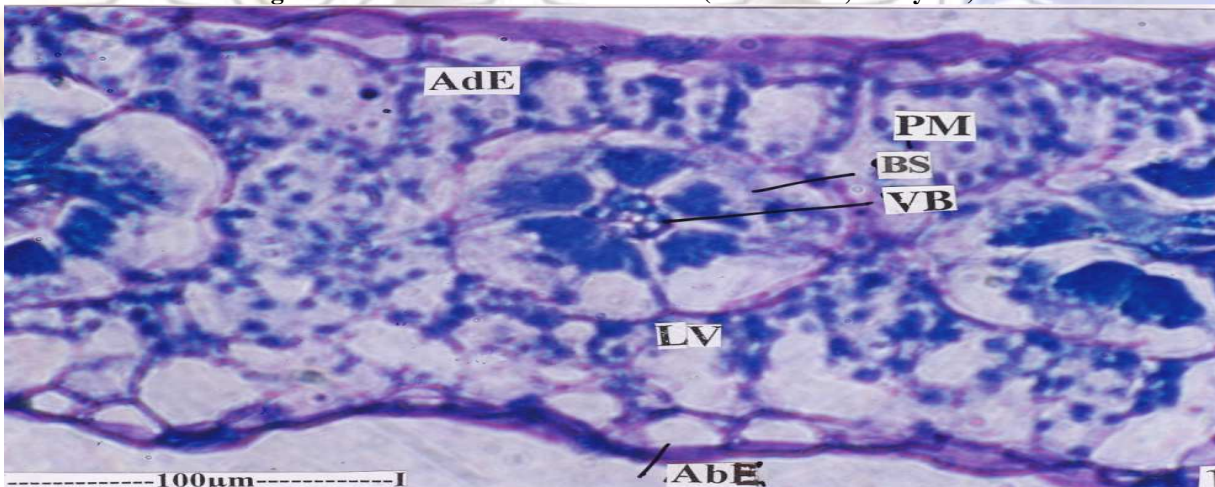


Fig. 4.1: T. S. of Lamina. (AbE: Abaxial Epidermis; AdE: Adaxial Epidermis; BS: Bundle Sheath Cells; LV: Lateral Vein; ; PM: Palisade Mesophyll; VB: Vascular bundle)

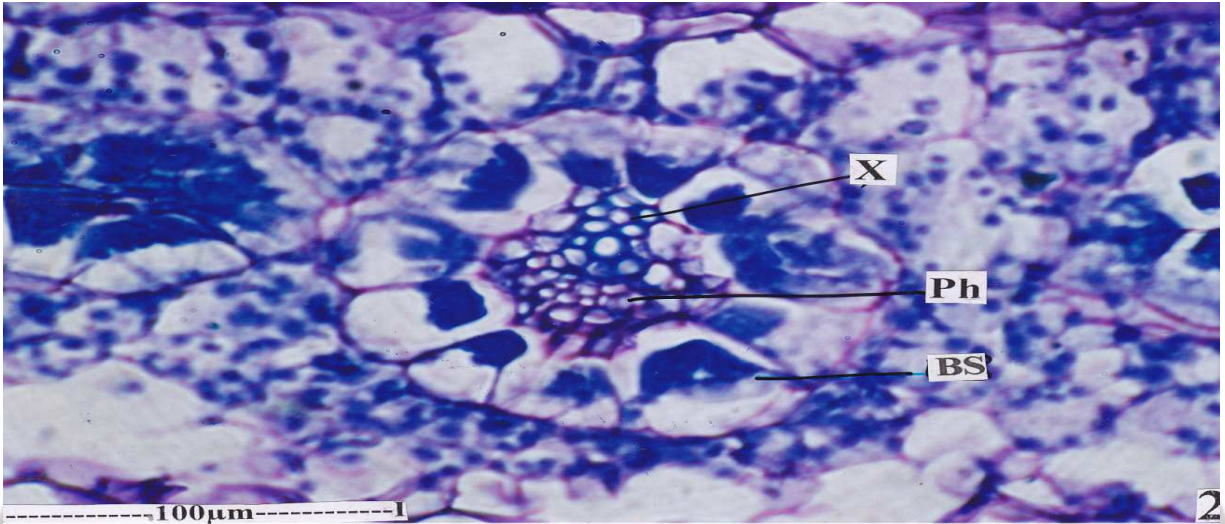


Fig. 4.2: One Vascular bundle with 'Kranz type' of bundle sheath cells. (BS: Bundle Sheath Cells; Ph: Phloem; X: Xylem)

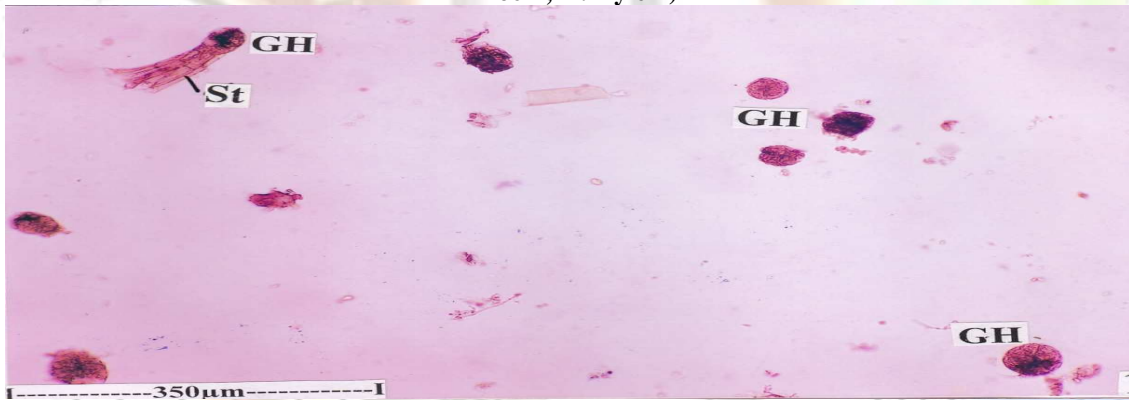


Fig. 5.1: Isolated glandular trichomes found in the leaf-powder. (St: Stalk; GH: Glandular Head)

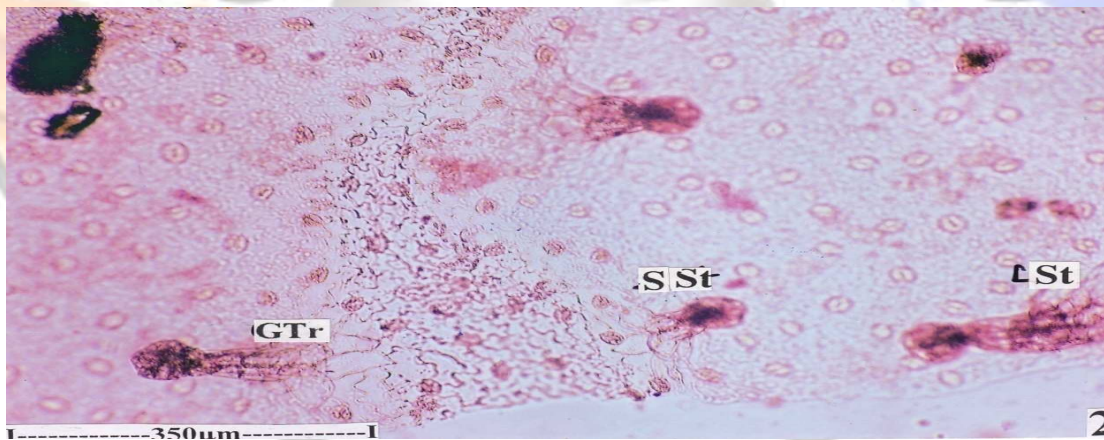


Fig. 5.2: Isolated glandular trichomes found in the leaf-powder. (GTr: Glandular Trichomes; SSt: Short Stalked Trichomes; St: Stalk)



Fig. 6.1: Glandular head as seen from above (GH: Glandular Head)

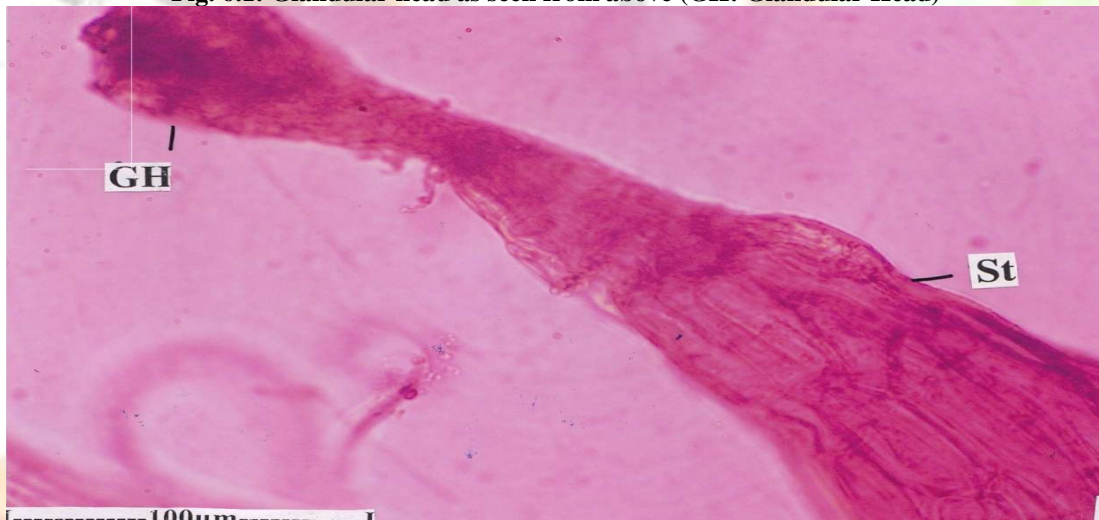


Fig. 6.2 One trichome entire view (GH: Glandular Head; St: Stalk)

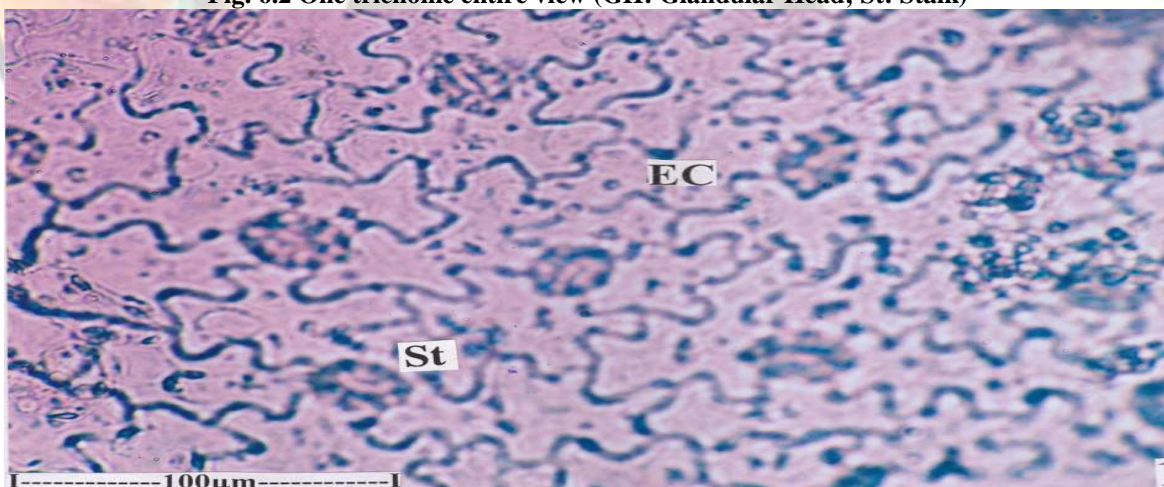


Fig. 7.1: Epidermis and stomata in surface view (EC: Epidermal Cells; St: Stomata)

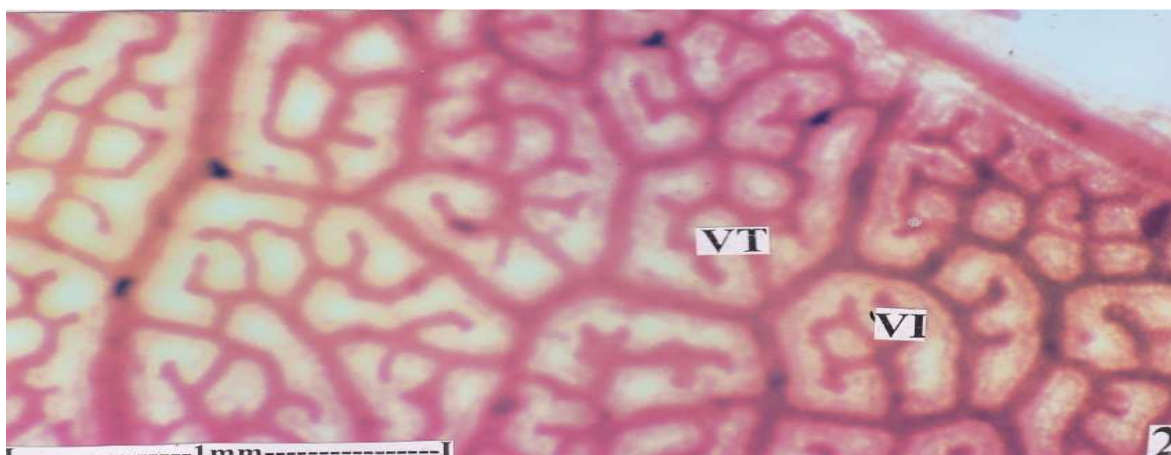


Fig. 7.2: Venation pattern (VI: Vein-Islet; VT: Vein Termination)

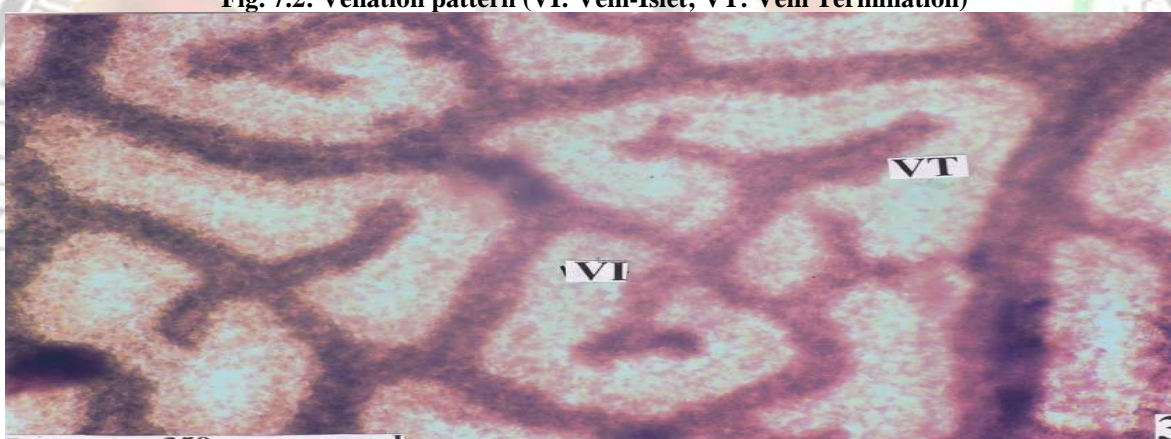


Fig. 7.3: Vein-islets and Vein Terminations (VI: Vein-Islet; VT: Vein Termination)

Table 1: Fluorescence analysis of *Gynandropsis pentaphylla* Linn leaf powder

Reagents	Day light	UV light
Drug powder	Pale green	Pale green
Drug powder + 1M NaOH	Greenish yellow	Green
Drug powder + alcoholic 1M NaOH	Dark green	Pale green
Drug powder + 1M HCL	Light brown	Faint green
Drug powder + 50% HNO ₃	Light Yellow	Pale green
Drug powder + 5% FeCl ₃	Dark green	Greenish yellow
Drug powder + 80% H ₂ SO ₄	Yellowish brown	Yellowish brown
Drug powder + water	Greenish yellow	Dark green
Drug powder + conc. H ₂ SO ₄	Black	Greenish brown

Table 2: Reaction of powdered drug with different reagents

Treatment	Colour
Powder as such	Pale green
Powder + conc. H ₂ SO ₄	Yellowish black
Powder + conc. HNO ₃	Yellowish brown
Powder + conc. HCL	Brown
Powder + 5% Iodine	Brownish yellow
Powder + 5M NaOH	Greenish brown
Powder + glacial acetic acid	Yellowish brown
Powder + 80% H ₂ SO ₄	Yellowish brown

Table 3: Physicochemical analysis of *Gynandropsis pentaphylla* Linn leaves

S/No.	Parameters	Value obtained on dry weight basis (%w/w)
1	Moisture content	13.26 ± 0.06
2	Foreign organic matter	8.70 ± 0.26
3	Total Ash value	4.06 ± 0.14
4	Acid insoluble ash	3.11 ± 0.12
5	Water soluble Ash	1.75 ± 0.35
6	Sulphated Ash	4.18 ± 0.05
7	Alcohol soluble extractive	10.46 ± 0.36
8	Water soluble extractive	17.37 ± 0.25

Table 4: Percentage yield of various extracts of *Gynandropsis Pentaphylla* Linn.

S/No.	Extracts	Nature of Extract	Colour of Extract	% yield (w/w)
1	Petroleum ether	Semi-solid mass	Dark brown	4.86
2	Chloroform	Semi-solid mass	Dark green	3.76
3	Ethanol	Semi-solid mass	Green	11.46
4	Aqueous	Semi-solid mass	Greenish brown	25.42

Table 5: TLC of leaves extract of *Gynandropsis pentaphylla* Linn.

S/No.	Name of the Extract	Solvent system	Spot observed in Iodine Chamber	R _f Values
1	Ethanolic	Toluene : Ethyl acetate (9:1)	2	0.48, 0.54
2	Aqueous	Chloroform : Methanol (9:1)	3	0.66, 0.56, 0.79

Table 6: Preliminary phytochemical screening of leaves of *Gynandropsis pentaphylla*

S.No.	Plant Constituents	Identification Test	Pet. Ether extract	Chloroform extract	Alcoholic extract	Aqueous extract
1.	Alkaloids	Mayer's Test	+	-	+	+
		Hager's test	+	-	+	+
		Dragendroff's test	+	-	+	+
		Wagner's test	+	-	+	+
2.	Carbohydrates	Molisch's Test	-	+	+	+
3.	Glycosides	Brontrager's test	-	-	-	-
		Legal's test	-	-	-	-
4.	Phenolic compound	fecl ₃ test	+	+	+	+
		Lead acetate test	+	+	+	+
5.	Tannins	fecl ₃ test	+	+	+	+
		Alkaline reagent test	+	+	+	+
		Lead acetate test	+	+	+	+
6.	Protein and amino acids	Million's test	-	+	+	+
		Ninhydrin test	-	+	+	+
		Biuret test	-	+	+	+
7.	Saponins	foam test	+	-	+	+
8.	Sterols	Liebermann's Burchard test	-	+	+	+
9.	Fixed oils and Fats	Spot test	+	+	+	+
10.	Flavonoids	Shinoda's test	-	-	+	+
		Alkaline reagent test	-	-	+	+