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Research Article

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Pharmacognostical Study and Localization of Tannin in *Pentatropis Capensis Linn.f.* (Buullock) Leaf-An Extra Pharmacopoeial Plant

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ABSTRACT

Pentatropis capensis Linn. f. (Bullock) is a straggler twining on thorny shrubs and trees, belonging to the family Asclepiadaceae, commonly known as Kakanasa, distributed throughout Bengal and Western Peninsula. Till there was no data regarding the detailed pharmacognostical study and localization of tannin content of leaf and its powder. This study deals with the detailed pharmacognostical and preliminary phytochemical evaluation, and identification of area of distribution of tannin material in leaf. The pharmacognostical evaluation shows stomatal index of upper epidermis and lower epidermis are 23 and 37 respectively, palisade ratio is 4 and histochemical tests confirmed the presence of lignin, starch and calcium oxalate crystal. Location of tannin is confined microscopically on palisade parenchyma cells of upper epidermis. The phytochemical screening shows the presence of carbohydrate, flavonoids and tannins.

Key words: Pentatropis capensis, Tannin content, Leaf, Stomatal index, Flavonoids.

INTRODUCTION

Nature has a treasure of medicines to treat all kinds of ailments. But there is a lack of standardization or documentation of identification characters which is the major backslaps of herbal medicines. Therefore the correct and scientific identification of these plants is quite necessary to get the full therapeutic impact of the drugs. A gross study of the plants with its microscopical, external and internal morphological is possible only by the study of pharmacognosy[1].

The tannins are broadly classified into two groups based on the complexity of their chemical structures, i.e. (i) Hydrolysable Tannins and (ii) Condensed Tannins [2]. These hydrolysable tannins give blue-black color with aqueous solutions of iron salts. Tannins of the leaves are more abundant in the palisade parenchyma and a high frequency of tannic deposits is also seen in parenchyma cells of the midrib.

Tannin has a role as growth regulator and allellopathic agents as well as anti-pathogenic and anti-herbivoric substances. Tannins may share the function of representing function that reduce the digestibility of plant tissue and hence minimize losses to herbivores and pathogens. It may be taken in consideration that condensed tannins may combine to polysaccharides and contribute to hardening of cell walls and retard the digestion and decomposition of conducting tissues [3].

Pentatropis capensis Linn. f. (Bullock), family Asclepiadaceae is commonly known as Kakanasa in Ayurveda and Uppilankodi in Siddha. Though controversy exists with the name of kakanasa, many of the ayurvedic vaidyas accepted *Pentatropis capensis* as Kakanasa. Ayurvedic classics like Charaka samhita mentioned its use in preparation of Chyavanprash (rejuvinative), Trayausanadi ghrta in cough and also used to prepare ghrta with other drugs which is an excellent remedy for helping conception [4]. Various floras have been confirmed it as anti-fungal, antiseptic and keratolytic and utility in gonorrhoea, rheumatism, skin diseases, constipation, diarrhoea, acidity, fever, body pain and cold. Ground material of the roots and infusion made from the roots are given in fever and hydrocele [5], [6]. Tribal and rural folklore of India are using it in various conditions viz. Headache, constipation, running nose, body pain [7].

It is a twining glabrous perennial straggler twining on thorny shrubs or tree; having simple leaf with 5-8 mm. long petioles, flowers are small greenish to purplish in colour, corolla segments lanceolate obtuse, fruits 7.3x 0.9 cm, tapering, ovate- lanceolate or ovate- oblong, thickly margined. It occurs in semi arid thorny forest throughout Bengal, Deccan, peninsula, from Mumbai to southwards [8].There is lack of scientific evaluation of *Pentatropis capensis* due to the existing controversy and being extra pharmacopoeial plant. The present study was designed for pharmacognostically localization, and localization of tannin in *Pentatropis capensis* leaf.

MATERIALS AND METHODS

Collection and Authentication

The whole plant was collected personally from Talabar Chakri and Herbal garden of Gujarat Ayurved University of Jamnagar district, Gujarat, India during morning time in the month of September when the plant was in flowering stage.

For authentification of the plant drug, twig containing leaves and flowers were collected. The specimen were disease free with all parts intact without any injuries and herbarium was prepared and were sent to central National Herbarium (CNH), Botanical Survey of India (BSI), Shibpur, Howrah, West Bengal, India. The samples were identified to be *Pentatropis capensis* Linn. f. (Bullock), family Apocynaceae (according to Angiosperm Phylogeny Group I, II, III classification) and the authentification certificate was issued by P. Venu, scientist ‘F’ & HOO, CNH, BSI, Kolkata, West Bengal, India.

The voucher specimen no. is CNH/86/2012/Tech. II/ 917. After authentification, the required part or leaves were separated from the collected plant materials and shade dried at room temperature and they are pulverized in mixer grinder to coarsely powdered drug and passed through sieve 60 and stored in well closed container by keeping away from direct Sun light for the further use.

Macroscopic and microscopic analysis [9], [10]

The macroscopy and microscopy of the leaves were studied according to the standard method. For the microscopical studies, transverse sections were prepared and studied with stain and without stain. Organoleptic characters and powder microscopy were performed using fine powder of leaves.

Analysis of Tannin content

The free hand transverse section of the leaf through midrib treated with ferric chloride and observed under microscopy.

Determination of Stomatal Index [11]

Leaf fragments were observed under microscope for the presence and quantification of epidermal cells, stomata (type and distribution), palisade cells, Vein-islet number and vein termination number. Stomatal index was calculated as the percentage of number of stomata present per epidermal cells and each stoma was counted as one cell.

Histochemical tests

To detect the presence of various constituents of the leaf drug, sections and powder of leaves were treated with various reagents like ruthenium red (for mucilage), $FeCl_3$ (for tannin) and iodine (for starch grains) etc [12].

Preliminary Phytochemical Screening [13], [14], [15]

Different phytochemical screening of the extracts, obtained from the dried whole plant of *Pentatropis capensis* by successive solvent extraction, was carried out according standard methods.

RESULTS

I. Macroscopic Characteristics:

P. capensis is a slender twinning herbs or under shrubs. Leaves are (4.7 – 5.7cm. x 1.8-2.4cm.) simple, opposite, superimposed in arrangement, exstipulate with 4-6 mm. long petiole, broadly ovate to cordate, obtuse in apices and rounded or cordate base and venations are reticulate and unicostate in nature.

II. Microscopic Characteristics of Leaf:

A) Transverse section of Petiole

In transverse sectional view, the petiole is circular with shallow wide upper concavity. It consists of a thin layer of thick walled spindle shaped epidermal cells and circular or angular thin walled compact parenchymatous ground tissue. The epidermal cell contains multicellular warty trichromes and plenty of rosette crystals of Ca- oxalate. There is a wide and thick arc shaped main vascular strand. Thin parallel lines of xylem elements observed along with small discrete groups of phloem on the outer and inner side of the xylem strand. The xylem elements are lignified and thick walled.

B) Transverse section of Leaf through midrib:

The leaf is dorsiventral with less prominent midrib and thick lamina having zerophytic features. The midrib is slightly thicker than the lamina. The mesophyll tissue is differentiated into upper two to three layer of thin vertical cylinders of palisade cells and lower part loosely arranged spongy parenchyma layer. The collenchyma cells are absent in midrib section. The vascular bundle is small, collateral and is placed in the central part of the midrib. It consists of a few short parallel files of thick walled xylem and a thin arc of phloem.

The upper and lower epidermal cells are polygonal in surface view with fairly thick straight anticlinal walls. The periclinal walls are smooth with faint cuticular markings. It is amphistomatic, having stomata on both sides of the lamina. The stomata are dense in lower epidermis than upper epidermis. The stomata are mostly paracytic type where rarely animocytic stomata also found.

III. Detection of Tannin in Leaf:

About 85% of the palisade parenchyma turns into dark blue colour after ferric chloride treatment, indicating the presence of tannin in upper epidermis i.e. during photosynthesis the upper epidermis has major role in physiological

activity as compared to lower epidermis. The secondary metabolites, synthesis by the salicylic and tannic components, are converted to tannin.

IV. Quantitative microscopy of Leaf:

Quantitative microscopy of leaf showed in table I and II, that size of adaxial stomata is 8.2 X 5.6 µm. and abaxial stomata is 7.8 X 3.6 µm, the stomatal index of upper epidermis is 23 and lower epidermis is 37 which found dense stomata at lower epidermis than upper epidermis. The reason behind dense epidermis may be to overcome intensity of light and temperature and reduce evaporation.

Table I: Measurement of different leaf components

Sr. No.	Parameter	Length	Width
1.	Upper epidermal cell	15.6 µm	11.8 µm
2.	Lower epidermis cell	14.2 µm	11.8 µm
3.	Stomata of upper epidermis	8.2 µm	5.6 µm
4.	Stomata of lower epidermis	7.8 µm	3.6 µm
5.	Trichromes	68 µm	7 µm

Table II: Measurements of different leaf cellular component

Sr. No.	Parameters	Inference
1.	Stomatal index of upper epidermis	23
2.	Stomatal index of lower epidermis	37
3.	Palisade ratio	4
4.	Vein-islet number	3.2
5.	Vein-let termination number	5.4

V.Powder characteristics of Leaves:

The organoleptic evaluation of leaf powders shows brownish green colour, characteristics leafy odour as shown in table III. Powder characteristics show fibers, epidermal cells with stomata and oil globules, rosette crystal and multicellular warty trichromes, Spiral and annular vessel.

Table III: Organoleptic examination of powder

Sr. No.	Organoleptic parameter	Inference
1.	Colour	Brownish green
2.	Odour	Characteristic leafy
3.	Taste	sweet, bitter and mucilaginous
4.	Texture	Coarse fiber

VI. Histochemical Tests:

Histochemical test confirmed that presence of lignin, starch grains, ca-Oxalate crystals, tannin and mucilage as shown in table- IV.

Table -IV: Histochemical tests for Leaf powder

Sr. No.	Reagents	Observation	Characteristics
1.	Phloroglucinol+Conc. Hcl	Red	Lignified cells
2.	Iodine	Blue	Starch grains
3.	Phloroglucinol+Conc. Hcl	Dissolved	Calcium oxalate crystals
4.	$Fecl_3$ solution	Dark blue to black	Tannin cells
5.	Ruthenium red	Red	Mucilage

VI. Preliminary phytochemical screening:

Methanol and aqueous extract were used for phytochemical screening of the powder sample. The obtained results are described in following table V:

Table V: Preliminary phytochemical screening of different extracts

No. Parameters Result	Methanol Extract	Water Extract
I. Carbohydrate		
a. General	**-ve**	**+ve**
b. Reducing sugars	**+ve**	**+ve**
c. Inulin sugars	**+ve**	**+ve**
d. Hexose sugars	**+ve**	**+ve**
e. Pentose sugars	**+ve**	**+ve**
II. Proteins	**+ve**	**-ve**
III. Amino acids	**+ve**	**-ve**
IV. Fats & Oils	**-ve**	**-ve**
V. Steroids & Terpenoid	**+ve**	**-ve**
VI. Volatile Oils	**-ve**	**-ve**
VII. Glycosides		
a) Cardiac Glycosides	**+ve**	**+ve**
b)Anthraquinone glycosides	**-ve**	**-ve**
c) Saponin Glycosides	**-ve**	**+ve**
d) Cyanogenic Glycosides	**+ve**	**+ve**
Flavonoids	**+ve**	**-ve**
Alkaloids	**-ve**	**-ve**
Tannin(Phenolic Compounds)	**+ve**	**+ve**
Diterpenes	**+ve**	**-ve**
Naphthaquinones	**-ve**	**-ve**

DISCUSSION

Pharmacognostical standardization including chemical screening is meant for identification, authentication, and detection of adulteration and also compilation of quality control standards of crude drugs. Since, the plant *Pentatropis capensis* comes under controversial names of 'Kakanasa' and as an extra pharmacopoeial plant with enormous therapeutic uses, it is important to standardize it for use as a drug raw material. During this study it was found that leaf was fleshy, succulent, zerophytic with thick cuticle. The mesophyll is well developed as compared to midrib, veins and veins-let. The transverse section shows that the vascular bundle through midrib is not well developed as compared with other mesophytic leaf transverse section. The predominant localization of tannic deposits in the periphery of the leaf is in agreement with comments that secondary metabolites tend to be more concentrating in peripheral plant tissues. The midrib parenchyma of the studied leaves contain cells with massive tannic deposition for providing chemical barrier to the vascular bundles which are rich sources of organic nutrients and also a target of many organisms. The Pharmacognostical constant for this plant, the quantitative microscopy are clearly explained for its identification and authentification.

CONCLUSION

It is for the first time reported about detailed pharmacognostical study for identifying the presence of tannin content of leaf and its powder .This research paper may help to develop vegetable tannin as herbal corrosion protector of iron and steel. It is an initial step and further requires a long term study for localizing tannin in other vegetative organs and estimation of percentage of tannin content.

ACKNOWLEDGEMENT

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A

Natural habitat of plant

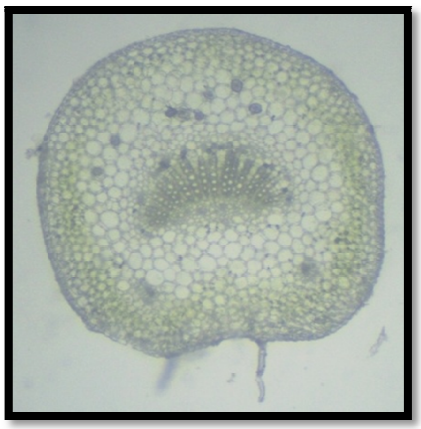
B

Flowering twig

C

Leaf measurement

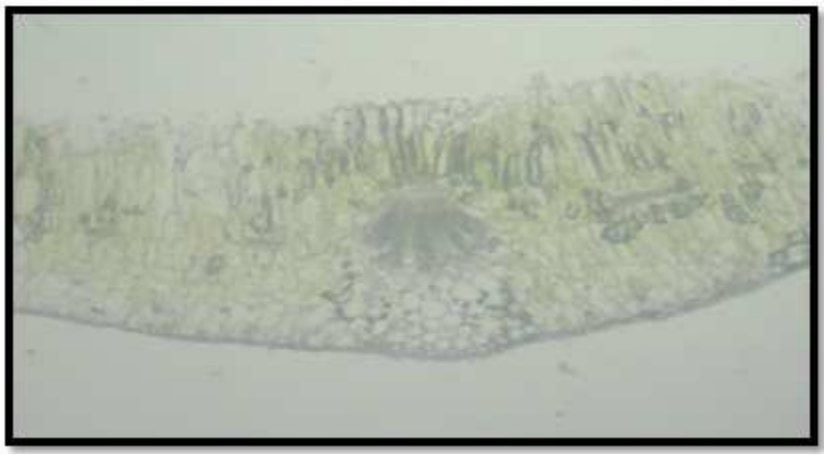
D



T.S. of petiole

E

T.S. of petiole stained

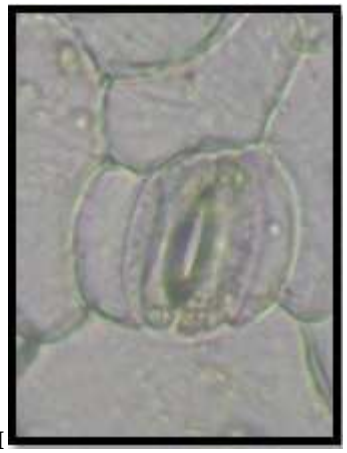


F

T.S. of leaf through midrib

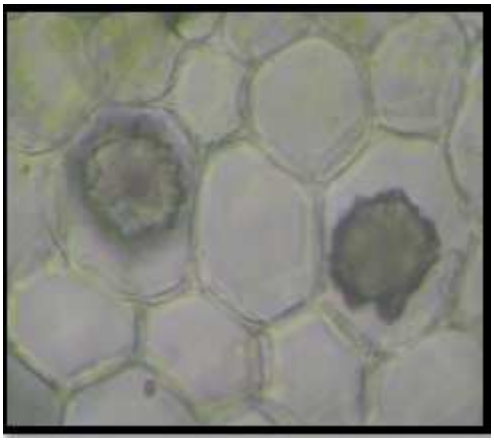
G

Epidermis with cicatrix cell



H

Paracytic stomata



I

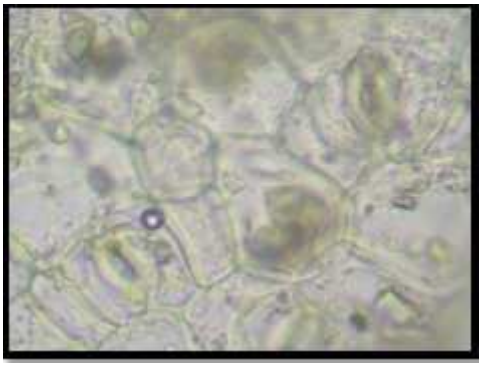
Rosette crystal of Ca-oxalate

J

Oil globules

K

Blunted warty trichome



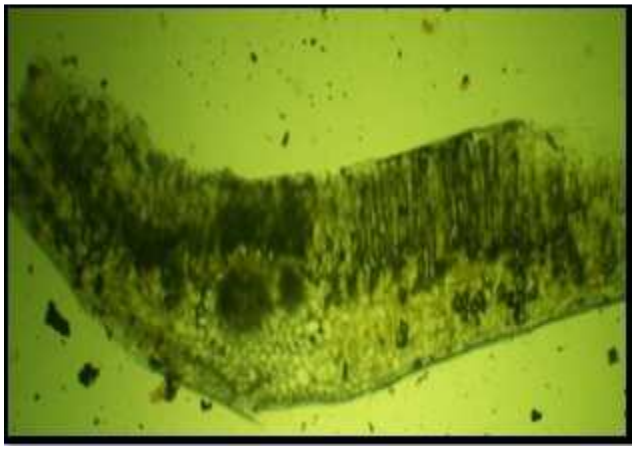
L

Tannin content

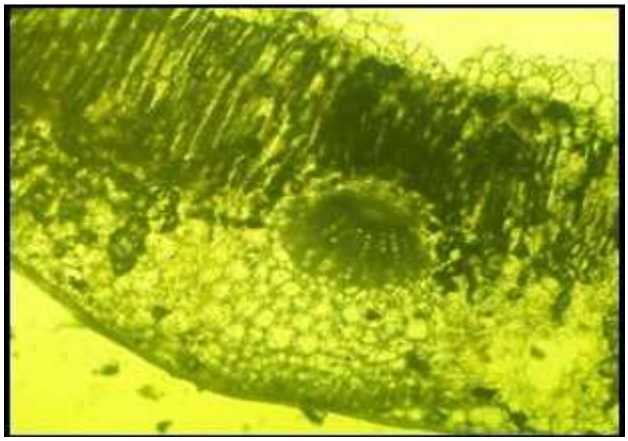
M Fiber

N Spiral vessel

O Surface view- Vein and veinislet



P T.S. of leaf- tannin localization



Q Tannin concentration- Palisade parenchyma

Figure 1. A,B :Natural habitat and flowering twig of *Pentatropis capensis*; C: Measurement of lamina with petiole; D: Transverse section of petiole shows multicellular warty thrichrome and wide and thick arc shaped main vascular strand; E: Transverse section of petiole after stain shows thin parallel lines of lignified xylem elements ; F: Transverse section of leaf through midrib shows small collateral vascular bundle at center; G: Epidermis with cicatrix cell; H: Epidermal surface shows paracytic stomata; I: Rosette crystal of ca- oxalate on epidermal cell; J: Oil globules with epidermal cell surface view; K: Bunted warty trichome on leaf; L: tannin content in leaf powder; M: Fragment of fiber in Leaf powder; N: Leaf powder shows spiral vessel; O: Surface view of lamina shows vein and veinislet; P: Transverse section of leaf shows location of tannin after treatment with $Fecl_3$; Q: Palisade cell of upper epidermis shows abundant tannin content.

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