Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 2, 2012

Full Proceeding Paper

PHARMACOGNOSTIC STANDARDS FOR DIAGNOSIS OF *PENTATROPIS CAPENSIS* (ASCLEPIADACEAE) A PLANT DRUG USED IN INDIAN SYSTEM OF MEDICINE

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Received: 30 July2010, Revised and Accepted: 05 Oct 2010

ABSTRACT

Asclepiadaceae includes predominantly climbers and straggling vines. The members of this taxon possess many bioactive constituents such as triterpenes, alkaloids, cyanogenetic glycoside, saponins, tannins and cyclitols. In the present work *Pentatropis capensis* (L.f) Bullock a herbaceous vine of the family called "uppilian kodi" in Tamil is studied from pharmacognostic standardization point of view.

Most of the genera of Asclepiadaceae exhibit striking similarities in growth form, floral organization and other external features, so, it is a prerequisite for a herbalist to emanate certain microscopic standards to establish the identity of the plants and to segregate the selected plant from its co-existing plants with simulating identity.

The present study deals with the pharmacognostic aspect of *Pentatropis capensis* to highlight its botanical parameters, particularly microscopic standards to establish the identity of the drug in its crude form.

Especially those features that are least affected by environmental stress are given accent in the present study. Cross sectional structure of the midrib and lamina, surface features of the epidermis and stomata, Venation pattern of the lamina, cross-sectional outline and vascular pattern of the petiole and structure of tissues in the stem and root are brought under the present preview. The validity of the result of the study in employing for the botanical identity of *Pentatropis capensis* is critically discussed. Preliminary phytochemical screening and fluorescence features were also studied for the crude drug and reported. Inorganic, organic and biochemical standards were also determined for the drug under study. These chemical and biochemical standards coupled with salient microscopic standards determined in the present work will certainly help in the unequivocal identity of the drug in the crude form.

Keywords: *Pentatropis capensis* (L.f) Bullock, Cortex, Calcium oxalate crystal, Palisade ratio, spongy parenchyma, Palisade parenchyma, Secondary phloem, Secondary xylem, Pith, Xylem vessel and stomata.

INTRODUCTION

Pentatropis capensis Bullock. belonging to family Asclepiadaceae is used in both the Indian systems of medicine, Ayurveda and Siddha. In Siddha system it is known as "Uppilankodi" and used as a pediatric medicine especially for children suffering from digestive problems, severe fever and also contains anti-astringent principles[1]. Siddhars used this plant in the preparation of gold and silver Parpams, unique herbo-metallic preparations which are useful in treating various ailments. In Ayurveda it is equivated as "Kakanasika" useful in upper respiratory infections and in controlling tumors. Chemically the source taxon is rich in compounds such as octacosanal, α-amyrin, friedelin, β-sitosterol and Salicylic acid.

Attempts are made in the present work to determine the pharmacognostic standards for this plant drug used in Indian Systems of Medicine. Such kind of studies will contribute towards the growth of herbal pharma industry by providing scientific parameters to assess the quality of raw materials as well as quality finished products. Pharmacognostic standards determined in the present work will help in the proper identification and quality check of this important drug used in Indian Systems of Medicine.

MATERIAL AND METHODS

The aerial parts of *Pentatropis Capensis* Bullock. were collected from in and around Thanjavur in the month of November 2011 and identified using the Flora of Presidency of Madras and authenticated with the help of specimen deposited at Rapinant Herbarium Department of Botany, St. Joseph's college, Trichy (RHT 13604). The collected materials were cleaned, shade dried and coarsely powdered. Free hand and microtome sections[2] of the fresh leaf, stem and root were taken and double stained with 0.25% Toluidine blue[3] and photo micrographs were taken as per standard procedures.

Coarsely powdered material was extracted with ethanol and water using cold extraction method. Preliminary qualitative analysis[4], fluorescence analysis[5] and the quantitative analysis[6] were carried out for the extracts as per standard textual procedures.[7]

Protein was estimated employing lowery's method[8]. Carbohydrates as per Yemm and Willis[9] and fats as per Osbome *et al*.[10]

Description of the Source Taxon

Pentatropis capensis is a straggler twining on shrubs and trees. It occurs in semi arid thorny forests and thickest. It contains watery latex. The leaves are elliptic measuring 4 X 3 cm. The lamina is chartaceous. Leaf base is subcordate and leaf apex is apiculate (Fig. 1). Petiole is 1cm long. The inflorescence is an axillary umbel; the flowers are pentamerous with 5 greenish sepals and 5 purplish corolla. Pollinia-pendulous, pollinial bags elliptical. Corona - staminal, single, with upward basal spur. Fruit – double follicle. Seeds – ovate, flattened winged. Coma - silky, white.

Fig. 1: *Pentadropis capensis* – flowering twig

Micromorphological Studies

Leaf

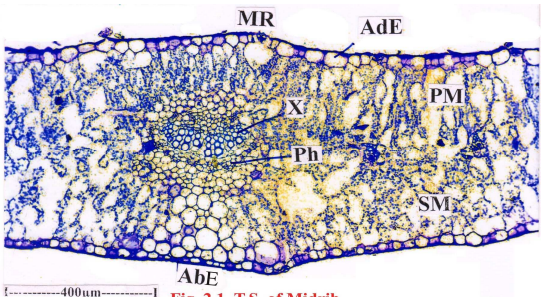

Fig. 2.1: T.S. of Midrib

AbE: Abaxial Epidermis; AdE: Adaxial Epidermis; MR: Midrib; PM: Palisade Meosophyll; SM: Spongy Mesophyll; Ph: Pholem; X: Xylem

The leaf is dorsiventral with less prominent midrib and thick lamina having mesomorphic features. The **Midrib** is slightly thicker than the lamina measuring 520µm thick. The adaxial part of the midrib is horizontally traversed by transcurrent layers of palisade cells (Fig. 2.1). The abaxial part of the midrib consists of vertical block of compact parenchyma cells supporting the vascular strand. The vascular bundle is small, collateral and is placed in the central part of the midrib (Fig.2.1). It consists of a few short parallel files of thick walled xylem and a thin arc of miniature nests of phloem. The vascular strand is 60 µm thick and 200 µm wide.

Lamina

The lamina is 350-400 µm thick. The abaxial and adaxial surfaces are smooth and even. It is amphistomatic, having stomata on both sides of the lamina. The mesophyll tissue is differentiated into adaxial zone of two or three layers of thin vertical cylinders of palisade cells. Abaxial region shows lobed and loosely arranged spongy parenchyma cell layers. The adaxial epidermis is slightly thicker, the cells being planocanvex in shape. The abaxial epidermis is thin and the cells are circular or spindle shaped (Fig. 2.2).

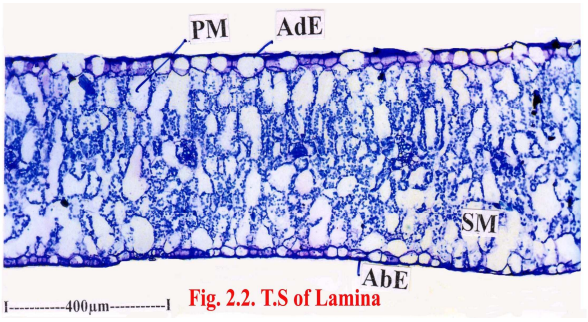

Fig. 2.2: T.S of Lamina

AbE: Abaxial Epidermis; AdE: Adaxial Epidermis; PM: Palisade Meosophyll; SM: Spongy mesophyll

Epidermal cells and stomata

The adaxial and abaxial epidermal cells are polygonal in surface view with fairly thick straight anticlinal walls. The periclinal walls are smooth with faint cuticular markings. The stomata on the adaxial epidermis are less frequent than on the abaxial epidermis (Fig.3 & 4). The stomata **are paracytic** type. A stoma has two **subsidiary** cells, one on either side, lying parallel to the guard cells. Some of the stomata are **amphi paracytic** having two parallel **subsidiaries** on either side (Fig. 3). The guard cells are broadly elliptical measuring 20 X 25 µm in size.

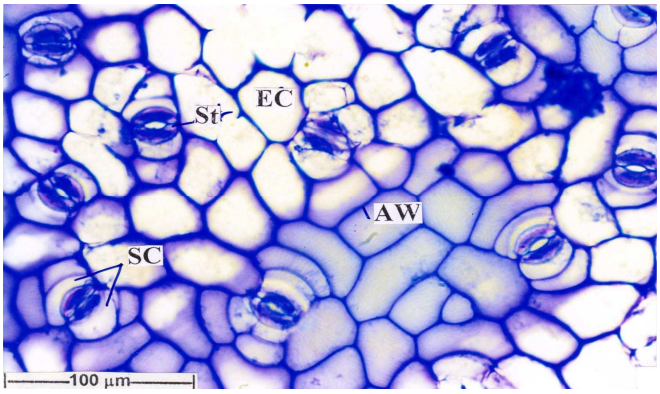

Fig. 3: Adaxial epidermal cells and stomata

AW: Anticlinal walls; Ec: Epidermal cells; St: Stomata; Sc: Subsidiary Cells

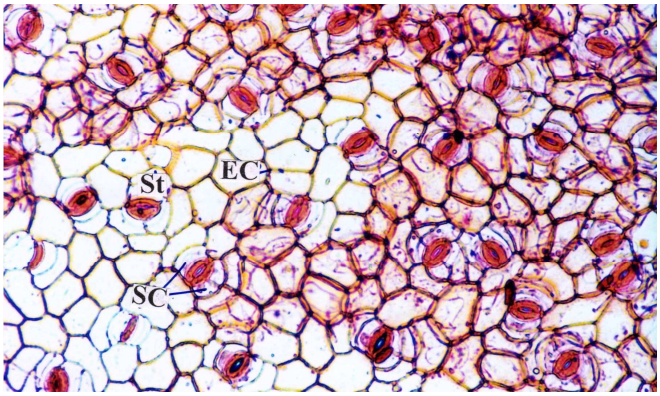

Fig. 4: Abaxial epidermis

EC: Epidermis Cells; SC: Subsidiary Cells; St: Stomata

Venation Pattern

The venation pattern of the lamina was studied both from the **paradermal** sections and from **cleared**-leaf. The venation system is reticulate with distinct vein-islets and vein-terminations. The islets are variable in shape and size; they are bounded by thin and straight veins. **Vein-terminations** are seen in almost all islets. The terminations are mostly unbranched, long and wavy. When the terminating veins are branched, the branches are limited in number and are of pinnate type (Fig.5.1, 2).

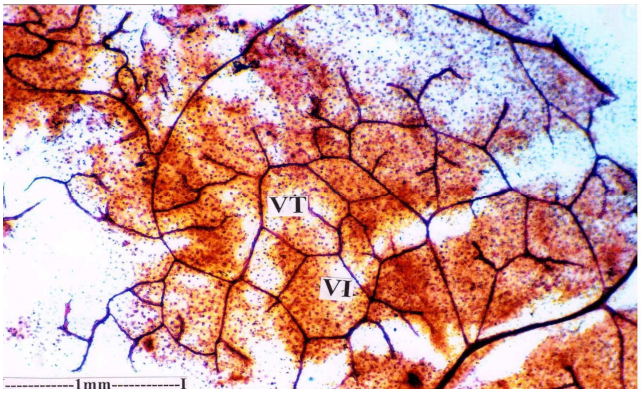

Fig. 5.1: Venation Pattern

VI:Vein Islet; VT: Vein Termination

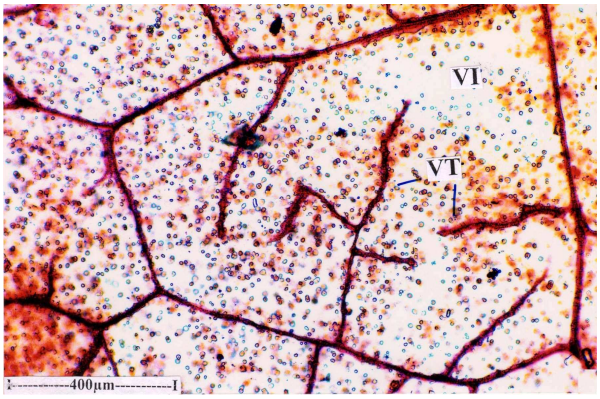

Fig. 5.2: Enlarged view of vein islet

VT: Vein Termination; VI: Vein Islet

Petiole

In cross sectional view, the petiole is circular with shallow wide adaxial concavity (Fig.6.1). The petiole is 1.2 mm thick. It consists of a thin layer of thick walled spindle shaped epidermal cells and circular or angular thin walled compact parenchymatous ground tissue. The cells are smaller in the peripheral zone and are larger in the interior portion. There is a wide and thick arc-shaped main vascular strand which is 200 µm of thickness and 450 µm of width. Thin parallel lines of xylem elements observed along with small discrete nests of phloem on the outer and inner sides of the xylem strand (Fig. 6.2). The xylem elements are angular and thick-walled. The sieve elements are narrow and are aggregated into small groups.

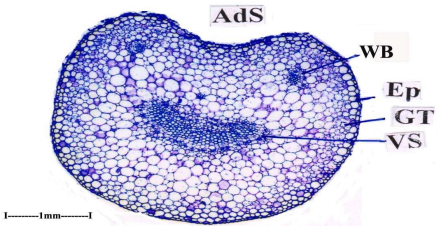

Fig. 6.1 T.S. of petiole-entire view

Ads: adaxial side; Wing Bundle; VS: Vascular strand; Ep: Epidermis; GT: Ground Tissue

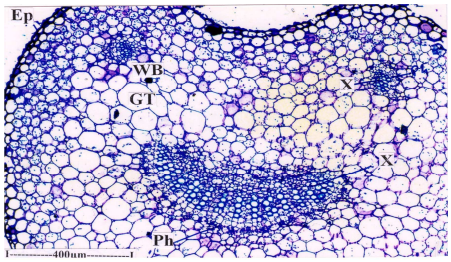

Fig. 6.2: T.S. of Petiole-A sector

Ep: Epidermis; GT Ground tissue; Ph: Phloem; WB: Wing Bundle; X: Xylem

Stem

Young stem

The young stem is circular with smooth and even surface. The epidermis is a thin layer of small, spindle shaped thick walled cells. There is a hypodermal layer of slightly larger hyaline cells. The cortex is 60 µm thick and it includes thin walled parenchyma cells. Along the inner boundary of the cortex occurs a circle of small masses of fibres. The fibres are less lignified.

The vascular cylinder is hollow and thick. It consists of numerous short radial rows of primary xylem. On the periphery of the xylem cylinder there are masses of circular vessels which are the initial cells of the secondary xylem (Fig.7).

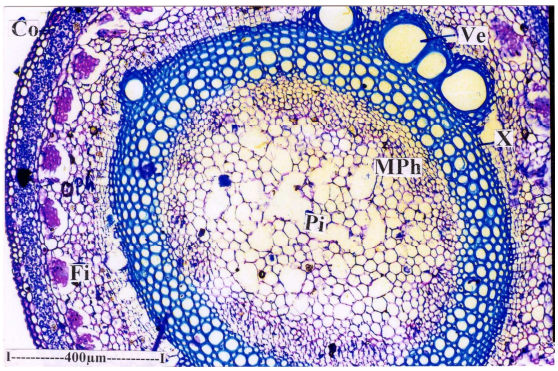

Fig. 7: T.S. of Youg Stem

Co: Cortex; Fi: Fissure; MPh: Medullary Phloem; OPh: Outer Phloem;Pi: Pith; Ve: Vessel; X: Xylem

Phloem occurs both on the **outer** and **inner** portions of the xylem. The outer secondary phloem is the usual phloem of the stem. The inner phloem is situated along the outer boundary of the pith and it is called **medullary phloem** or **intraxylary phloem**. The occurrence of this internal (medullary) phloem is an unusual feature.

Old stem

The old phloem is 2.2mm thick. It is deeply fissured, the fissures being steeply v-shaped (Fig.8.1). The old stem consists of wide periderm, fairly wide cortex, outer and inner phloem and secondary xylem cylinder (Fig. 8).

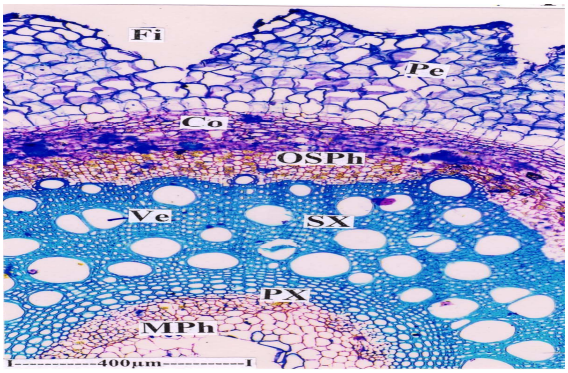

Fig. 8: T.S of Old Stem

Co: Cortex; Fi: Fissure; MPh; Medullary Phloem; OSPh: Outer Secondary Phloem; Pe: Periderm; Px: Primary Xylem; SX: Secondary Xylem; Ve: Vessel

Periderm is homogeneous and deeply fissured. It is 300 µm thick. The cells are random in alignment, excepting a narrow zone of innermost layers of cells which are radially aligned (Fig.8).

Cortical zone is about 170 µm thick and consists of elliptical compact cells.

Outer secondary phloem is 70-110 µm thick. It includes wide, angular fairly thick walled sieve-elements arranged in vertical rows (Fig.9).

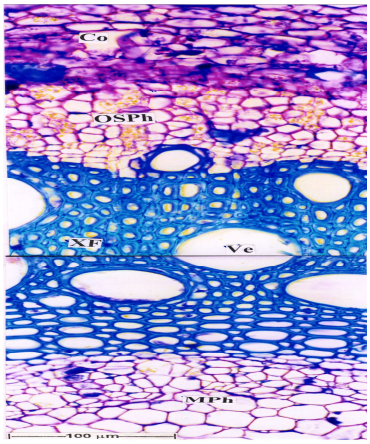

Fig. 9: T.S. of Stem- Vascular Tissue Enlarged

Inner secondary phloem (Intraxylary phloem)

It occurs in the area adjoining the primary xylem and along the periphery of the pith. The phloem is in large, circular masses situated in a circle around the pith.

Secondary xylem is dense and solid cylinder of circular, thick scattered vessels (Fig.8.2; 9). The vessels are 30-70 µm in diameter.

Crystals

Spherical spiny bodies of densely crowded crystals seen in the phloem (Fig.10.1, 2).

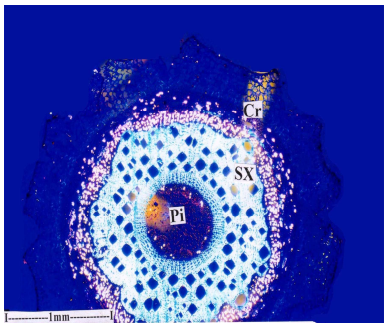

Fig. 10.1: T.S. of stem (under polarized light)

Cr: Crystals; Pi: Pith; SX: Secondary xylem

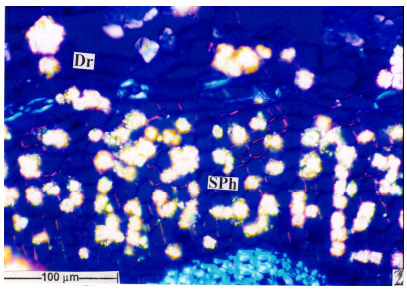

Fig. 10.2: T.S. of Stem under polarized light Calcium oxalate crystals in the cortex

Dr: Druses; SPh: Secondary Phloem

Root

Thick root measuring 2.1mm thick was studied. It consists of thick periderm which has shallow irregular fissures. The periderm is 150 µm thick (Fig.11.1). The periderm cells are radially oblong or rectangular, thin walled and homogeneous (Fig. 11.2). The cortex consists of about 8 layers of thin walled parenchyma cells. The cortical zone is 200 µm thick. The inner boundary of the cortex consists of thin broken layer of **sclereids**. The sclereids are **brachysclereid** type; they are circular with thick lignified walls and narrow lumen.

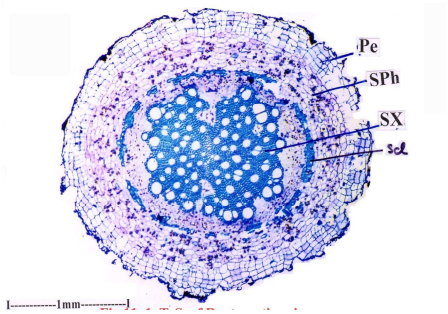

Fig. 11.1: T.S. of Root-entire view

PE: Periderm; Scl: Sclereids; SPh; Secondary Phloem; SX: Secondary Xylem

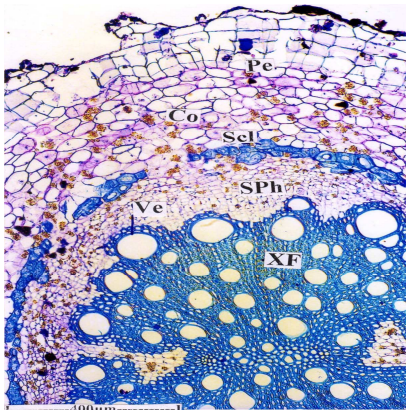

Fig. 11.2: T.S. of Root-A sector enlarged

Co: Cortex; Pe: Periderm; Scl: Sclereids; SPh: Secondary Phloem; Ve: Vessel; XF: Xylem Fibre

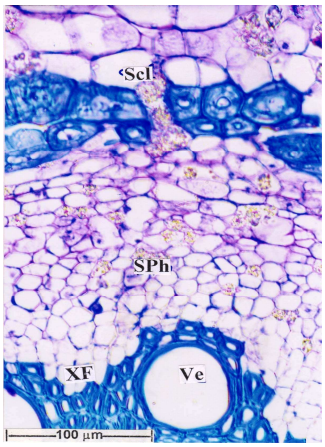

Fig.11.3: T.S. of Root-Secondary Phloem enlarged

Secondary phloem is wide and includes diffusely distributed sieve elements. Calcium oxalate crystals of **druses** are common in the phloem cells (Fig. 11.3).

Secondary xylem cylinder is uneven in outline with shallow and deep furrows (Fig.11.2). Secondary xylem includes xylem fibres and solitary, diffuse, wide circular vessels. Both narrow and wide vessels occur intermixed in the fibrous ground fibres. The vessels are 20-70 µm in diameter.

Chemical and Biochemical Studies

Extractive values determined for the test drug is presented in Table-1

Table 1: Extractive values as per IP

S.No.	Parameters	Values %
1.	Water	35.3
2.	Alcohol	11.8

Data of the Preliminary Phytochemical screening and fluorescence analysis are given in Table 2 and 3 respectively.

Table 2: Preliminary phytochemical screening of various extracts

S.No.	Test for	Hexane	Chloroform	Ethyl Acetate	Ethanol	Water
1.	Saponin	-	-	-	-	-
2.	Glycoside	-	+	+	+	+
3.	Tannin	-	-	-	-	-
4.	Sterol	-	-	-	-	-
5.	Terpenes	-	-	-	-	-
6.	Sugar	+	+	-	+	+
7.	Flavones	-	-	+	+	+
8.	Protein	-	-	-	-	-
9.	Quinone	-	-	-	+	-
10.	Phenolic compounds	+	+	-	+	+
11.	Alkaloid	-	+	-	+	+

Table 3: Fluorescence analysis of drug powder

S.No.	Treatment	After 24 hrs in day light	After 24 hrs in UV light
1.	Drug powder	Light green	Green
2.	Drug powder + 50% H^2SO^4	Dark green	Dark green
3.	Drug powder + aq. 1N NaOH	Greenish yellow	Bluish green
4.	Chloroform	Yellowish green	Fluorescent red
5.	Ethyl Acetate	Yellowish green	Light orange
6.	Alcohol	Fluorescent green	Orange
7.	Water extract	Pale yellow	Bluish green

Inorganic contents and major phytoconstituents estimated are given in Table-4 and 5 respectively.

Table 4: Inorganic standards

S.No.	Name of the Minerals	Quantity
1.	Organic Carbon (%)	0.89
2.	Total Nitrogen (%)	0.53
3.	Total Phosphours (%)	0.15
4.	Total Potassium (%)	4.23
5.	Total Sodium (%)	0.12
6.	Total Calcium (%)	4.20
7.	Total Magnesium (%)	2.30
8.	Total Sulphur (%)	0.42
9.	Total Zinc (ppm)	0.26
10.	Total Copper (ppm)	0.01
11.	Total Iron (ppm)	52.30
12.	Total Manganese (ppm)	2.31
13.	Total Boron (ppm)	0.03
14.	Total Molybdenum (ppm)	0.04

Table 5: Major phytoconstituents

S.No.	Name of the Phytoconstituents	Quantity
1.	Total Alkaloids (mg kg^{-1})	1.52
2.	Total Flavonoids (mg kg^{-1})	2.09
3.	Tannin (mg kg^{-1})	0.04
4.	Lignin (mg kg^{-1})	0.02
5.	Glycosides (mg kg^{-1})	0.12
6.	Serpentines (mg kg^{-1})	0.13
7.	Terpenoids (mg kg^{-1})	0.08
8.	Saponins (mg kg^{-1})	0.06
9.	Phenols (mg kg^{-1})	0.24

Table-6 Depicts the amount of total fats, protein and carbohydrates present in the drug.

Table 6: Biochemical standards

S.No.	Name of the Biochemical standards	Quantity
1.	Total Carbohydrates (mg kg^{-1})	0.13
2.	Total Protein (mg kg^{-1})	0.46
3.	Total Fats (mg kg^{-1})	0.03

DISCUSSION

Pentatropis capensis Bullock belonging to family Asclepiadaceae is used in both the Indian systems of medicine, Ayurveda and Siddha. In Ayurveda the drug is equated as "Kakanasika" and in Siddha system it is known as "Uppilankodi" and used as a pediatric medicine. Chemically it yielded interesting compounds such as octacosanal, α-amyrin, friedelin, β-sitosterol and Salicylic acid.

In the present work, attempt is made to determine pharmacognostic parameters for the drug under study such as macroscopic and microscopic features, organic, inorganic and biochemical standards. These parameters will contribute in assessing the genuineness and quality of this ISM drug which in turn will help in preparing standard Ayurvedic and Siddha formulations for the health care of the human society.

Following are the standards determined for the drug under study as per Ayurvedic and Siddha Pharmacopoeia:

Macroscopic

Straggler stem, the leaves are elliptic measuring 4 X 3 cm. The lamina is chartaceous. Leaf basis is subcordate and leaf apex is apiculate (Fig. 1). Petiole is 1cm long. The inflorescence is an axillary umbel; the flowers are pentamerous with 5 greenish sepals and 5 lobes of corolla which are purple.

Organoleptic

No characteristic odour and taste-bitter

Microscopic

Midrib less distinct. Vascular system of the petiole single stranded. Deeply fissured periderm in the old stem, solitary, wide circular diffuse vessels in the stem and root are characteristic.

Test for Strength

Foreign organic matter – Not more than 1.1%

Extractive values

Water - Not less than 35%

Alcohol - Not less than 12%

Constituents

Alkaloids, reducing sugars, flavones and glycosides.

CONCLUSION

Attempts were made in the present work to determine Macroscopic, Microscopic, organic, inorganic and Biochemical standards for the plant drug.

Pentatropis capensis, is used in both Ayurveda and Siddha medicine to treat various ailments including cancer.

The standards evaluated in the present work can contribute in the identification of the drug under study if occurs in any form such as dry powder, leaf, stem and bark fragments. Such kind of studies will help in providing genuine and quality raw drugs to the Herbal industry.

ACKNOWLEDGEMENT

Authors wish to place on record their deep sense of gratitude to Hon'ble Vice Chancellor, SASTRA University for providing necessary infrastructure for carrying out this research successfully. We are indebted to Dean (Sponsored Research), SASTRA University for his constant encouragement and motivation. We place our sincere thanks to Prof. P.Jayaraman, Plant Anatomy Research Centre, West Tambaram, Chennai for his valuable suggestions.

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