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PHARMACOGNOSTICAL AND ANITMICROBIAL STUDIES ON *PYCREUS PUNCTICULATUS*, (VAHL) NEES., ROOT

*** [1]KP Jaiganesh, [2]G Arunachalam**

[1]Division of Pharmacognosy -R&D- Laboratory, Thanthai Roever college of Pharmacy, Perambalur, Tamil Nadu, India – 621 212.
[2]Department of Pharmacognosy, PGP College of Pharmaceutical Science and Research Institute, Namakkal, Tamil Nadu, India – 637 207.

ABSTRACT

Pycreus puncticulatus, (Vahl) Nees., of Cyperaceae family, is a common weed, found in marshy places throughout India, Srilanka, Peninsula, Cochin and China, which has long been used traditionally for the treatment of various diseases including asthma, hepatitis and respiratory problems. The present investigation is carried out to establish the pharmacognostical standards, which ensures the proper identification and authentication of the plant. This present paper highlights the geomorphology, histo morphology of root, physico-chemical standards, preliminary phytochemical nature and antimicrobial potential of the plant. These observations would be of immense value in the botanical identification and standardization of the drug in crude form and also help to distinguish the drug from its other species.

Key words: Asthma, *Pycreus puncticulatus*, Pharmacognostical standards, Phytoconstituents, Antimicrobial.

INTRODUCTION

For centuries, plants and plant products have been used to treat various diseases. The standardization of natural products is a complex task, due to their heterogeneous composition, which is true for, either the whole plant or any plant part or extracts obtained thereof. To ensure reproducible quality of herbal products, authentication of the starting material is essential. According to WHO (Anonymous, 1988), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken.

Pycreus puncticulatus, (Vahl) Nees., (Family: Cyperaceae), is commonly known as "Korai", is a weed found in the marshy places and jungles, grown along with other cultivated plants and distributed throughout India, Srilanka, Peninsula, Cochin and China. The plant is used in folklore medicine to treat asthma, hepatitis and respiratory disorders and also reported for its laxative and purgative activities (Ambasta, 1986).

Thus, as there was ample scope to work on this plant for various pharmacognostic parameters, we, in this present study, have tried to provide comprehensive information on macroscopical and microscopical characters of root, physico-chemical standards, preliminary phytochemical nature and antimicrobial potential of *Pycreus puncticulatus*, (Vahl) Nees., will serve as an important tool to fix the standards for the future identification of this plant.

Corresponding Author

Jaiganesh KP
E-mail: kpjaiyaganesh@gmail.com

MATERIALS AND METHODS

Pharmacognostic studies

Plant materials

The plant materials for the proposed study were collected from the marshy areas of Thiruchirappalli, Tamil Nadu, India. Then, the specimens were identified and authenticated by The Taxonomist, Raphinat Herbarium (RHT 26276), St.Joseph's College

(Autonomous), Thiruchirappalli, Tamil Nadu, India. A voucher specimen (TRCP.14/06/2005) has been procured at The Division of Pharmacognosy -R & D- Laboratory, Thanthai Roever College of Pharmacy, Perambalur (District), Tamil Nadu, India, for future reference.

Plant description

Pycreus puncticulatus, (Vahl) Nees., is a dense, muddy, small weed, widely found in rice fields, marshy grounds, swamps and muddy pools and bears fruits from the month of September to February.

Collection of specimens

The plant material for the proposed study were collected and extreme care was taken to select healthy plant and for normal organs. The roots were separated out from the plant and fixed in FAA (Formalin 5ml + Acetic acid 5ml +70% Ethyl alcohol 90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol (TBA) as per the schedule (Sass, 1940). Infiltration of the specimens were carried out by gradual addition of paraffin wax (melting point 58°-60°c), until this barbituric acid solution attained super saturation. Then, the specimens were castled out into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of a rotary microtome, 10-12µm thickness of the sections was made. However, dewaxing of the sections was done by using customary procedure (Johansen, 1940). The sections were later stained with toluidine blue (O' Brine *et al*., 1964). Since, toluidine blue is a polychromatic stain, the staining was remarkably good and yielded varied cytochemical reactions. The dye rendered pink colour to the cellulose walls, blue to lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc., wherever necessary, sections were also stained with safranin, fast-green and iodine-potassium iodide for starch. Cleared sections were then mounted in glycerin for microscopical observation.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photomicrographs of different magnifications were taken with Nikon Labphot 2 microscopic unit. For normal observations, a bright field microscope was used and for the study of crystals, starch grains and lignified cells, a polarized light was employed. However, these structures have bire fringent property, under polarized light they tend to appear bright against the dark background. Descriptive terms of the anatomical features are given as per the standard anatomy books (Esau, 1964).

Physico-chemical parameters

The coarse powder of root was subjected to physico-chemical analysis such as the determination of ash values, extractive values, loss on drying and crude fiber content as per the Indian Pharmacopoeia (Anonymous, 1996). For fluorescence analysis, powdered root was sieved through 60 mesh and observations were made by adopting the standard methods (Chase *et al*., 1949; Kokashi *et al*., 1958).

Preliminary phytochemical screening

Air dried and coarsely powdered root was successively extracted with petroleum ether, chloroform and ethanol in a soxhlet apparatus by continuous hot percolation method. Aqueous root extract was prepared by cold maceration method by using 0.25% v/v $CHcl_3$ in water (Kokate, 1994). Each extract was concentrated by distilling off the solvent, which was recovered subsequently. The root extracts were subjected to various qualitative tests for an identification of chemical constituents group present in this plant (Horborne, 1998).

Antimicrobial studies

50 mg/ml of petroleum ether, chloroform, ethanol and aqueous root extracts were subjected to antimicrobial screening by cup plate method against the various pathogens such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans and Aspergillus niger* by using standard antibiotics Amikacin and Nystatin 25µg/m1 (Tim Cushier *et al*., 2005).

RESULTS

Exomorphology

The plant is a dense, muddy, small weed (Fig. 1a). Leaves are flat or canaliculated, which consists of inflorescence, 5-7 primary rays, rachis is elongated and 3-5 secondary rays are found in cluster **(**Fig. lb). Spikes are elongated up to 3 cm; spikelets are oblong, purple brown in colour; rachilla is flexuous, persistent and winged. Glumes are ovate; apex and margin is hyaline, keel, strong and 3 nerved. Stamen consists of anthers up to 1mm height. Nut is obovoid or broadly ellipsoid, laterally compressed (Matthew, 1983).

Microscopical features

The roots are fibrous, soft, unbranched and hydromorphic. The transverse section of the root exhibits outer cortex, middle cortex, inner cortex and stele (Fig. 2a).

i) *Outer cortex* is made up of less distinct, epidermal layer. The outer cortical zone is 50mm wide, consists of compact, polygonal, thin walled parenchyma cells.

ii) *Middle cortex* is aerenchymatous, consists of wide, tangentially elongated, rectangular cells arranged in concentric circles; cells are thin and delicate (Fig. 2b).
iii) *Inner cortex* is ensheathing stellar part, which consists of two or three layers of circular, thick walled parenchyma cells, are compact with minute intercellular spaces.
iv) *Stele* is a central solid core of vascular tissues, consists of two layers of endodermis with thick walled, square or rectangular cells (Fig. 2c). Single layered pericycle is present inner to endodermis, comprises of radially elongated, thin walled parenchymatous cells. Vascular tissue consists of radially arranged xylem and phloem strands. Xylem has seven, wide metaxylem elements with small exarch protoxylem elements (Fig. 2d). The metaxylem elements are circular to elliptic and thin walled. Protoxylem elements are narrow, thick walled and angular in cross view. Phloem strand consists of two or three sieve elements.
v) *Pith* is wide, consists of thick walled, compact, small cells. In the peripheral part of the stele and inner to pericycle, parenchaymatous ground tissue is present.

Physico-chemical standards

The root powder was analyzed for various physico-chemical standards which includes ash values, extractive values, loss on drying and crude fiber content and the results were depicted in Table 1.

Fluorescence analysis

The root powder was examined for its fluorescence properties by treating it with different reagents and the colour changes were observed in the day light and UV light (254nm). Similarly, petroleum ether, benzene, chloroform, ethyl acetate, ethanol and aqueous root extracts were also examined under day light and UV light (254nm) and the results were tabulated in Table 2 and 3.

Phytochemical screening

Qualitative tests of various root extracts show the presence of alkaloids, carbohydrates, phenolic compounds, tannins, phytosterol and lignin were tabulated in Table 4.

Antimicrobial studies

50 mg/ml of petroleum ether, chloroform, ethanol and aqueous root extracts of *Pycreus puncticulatus,* (Vehl) Nees., exhibits significant antimicrobial activity against the pathogens and the results were recorded in Table 5.

Table 1. Physico-chemical standards of *Pycreus puncticulatus,* (Vahl) Nees.,

Standards	% Yield
Total ash	6.42
Water soluble ash	3.07
Acid insoluble ash	2.66
Sulphated ash	1.15
Loss on drying	5.25
Crude fiber content	8.72
Ether soluble extractive value	2.89
Alcohol soluble extractive value	7.08
Water soluble extractive value	3.27

Table 2. Fluorescence analysis of *Pycreus puncticulatus,* (Vahl) Nees., root powder

Reagents	Day light	UV light(254 nm)
Root powder	Light brown	Reddish brown
Powder + 1N NaoH(Aq)	Brown	Brownish yellow
Powder + 1N NaoH(Alc)	Brown	Reddish brown
Powder + 1N Hydrochloric acid	Dark brown	Reddish brown
Powder + 50% Nitric acid	Dark brown	Reddish brown
Powder + 50% Sulphuric acid	Yellowish brown	Reddish brown
Powder + 5% Ferric chloride	Yellow	Brown
Powder + N/50 Iodine solution	Dark blue	Bluish black
Powder + Water	Pale yellow	Pale yellow

Table 3. Fluorescence analysis of *Pycreus puncticulatus,* (Vahl) Nees., root extracts

Extracts	Day light	UV light(254 nm)
Petroleum ether	Pale yellow	Colourless
Benzene	Pale brown	Pale brown
Chloroform	Light yellow	Yellowish green
Ethyl acetate	Pale yellow	Pale brown
Ethanol	Yellow	Yellowish brown
Aqueous	Pale yellow	Pale yellow

Table 4. Preliminary phytochemical screening of *Pycreus puncticulatus,* (Vahl) Nees., root

Phytoconstituents	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous extract	Powder
Carbohydrates	+	+	+	+	+
Glycosides	-	-	-	-	-
Fixed oils and fats	-	-	-	-	-
Saponins	-	-	-	-	-
Phenolic compounds	+	+	+	+	+
Tannins	+	+	+	+	+
Proteins and amino acids	-	-	-	-	-
Gums and mucilages	-	-	-	-	-
Lignins	+	+	+	+	+
Phytosterol	+	+	+	+	+

(+) = Presence of phytoconstituents
(-) = Absence of phytoconstituents

Table 5. Antimicrobial activity of *Pycreus puncticulatus,* (Vahl) Nees., root extracts

Micro organisms	Zone of inhibition (mm)				
	Petroleum ether extract (50mg/ml)	Chloroform extract (50mg/ml)	Ethanol extract (50mg/ml)	Aqueous extract (50mg/ml)	Standard (25µg/ml)
Escherichia coli	07	10	15	11	18
Pseudomonas aeruginosa	08	12	14	10	16
Staphylococcus aureus	10	13	15	12	17
Klebsiella pneumoniae	15	18	21	14	21
Candida albicans	04	06	13	04	18
Aspergillus niger	02	03	05	03	15

Fig 1a. Entire plant of *Pycreus puncticulatus,* (Vahl) Nees.,

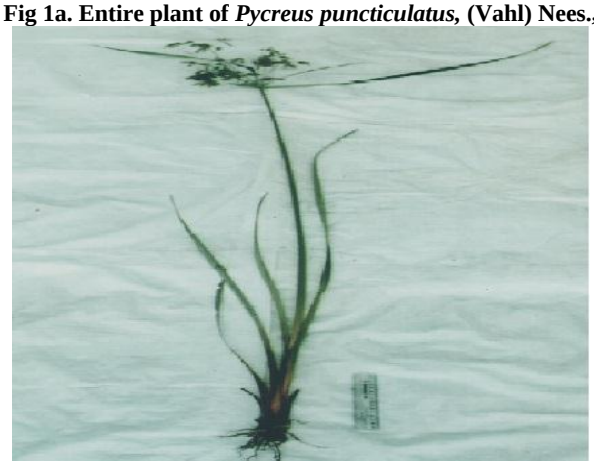


Fig 1b. Root portion of *Pycreus puncticulatus,* (Vahl) Nees.,

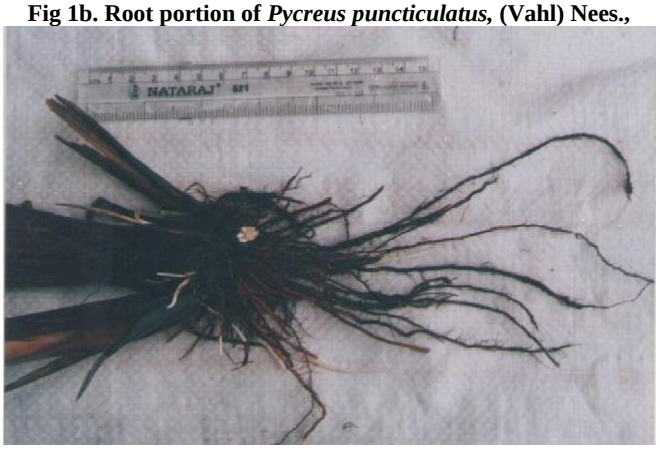


Fig 2a. T.S of Root: En, Endodermis; **IC,** Innercortex; **MC,** Middle cortex ; **OC,** Outer cortex; **PC,** Pericycle; **Ph,** Phloem; **Pi,** Pith; **Px,** Protoxylem; **Stl,** Stele

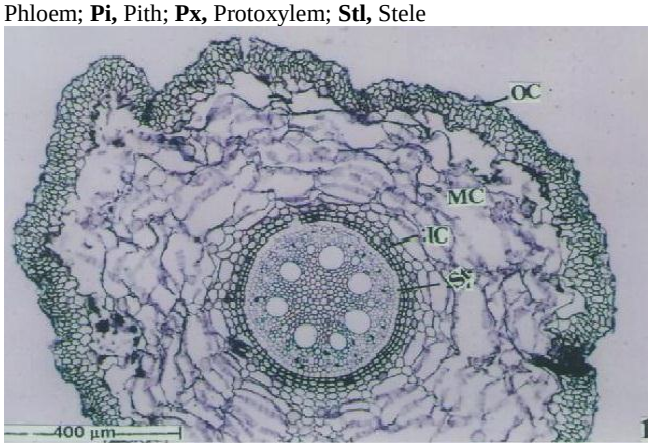


Fig 2b. T.S of Root (enlarged): En, Endodermis; **IC,** Innercortex; **MC,** Middle cortex ; **OC,** Outer cortex; **PC,** Pericycle; **Ph,** Phloem; **Pi,** Pith; **PX,** Protoxylem; **Stl,** Stele

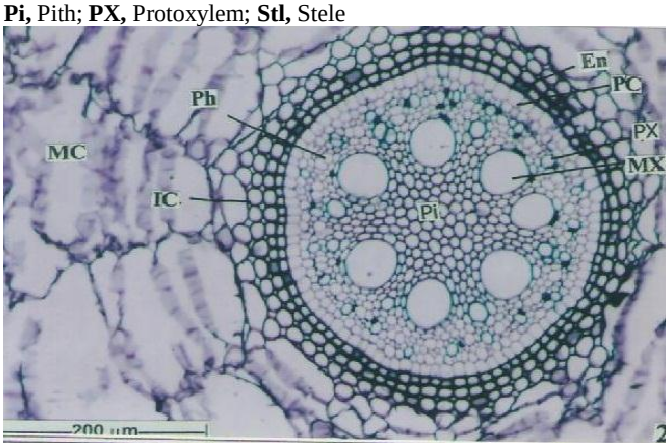


Fig 2c: Entire stele (enlarged): En, Endodermis; **IC,** Innercortex; **PC,** Pericycle; **Ph,** Phloem; **Pi,** Pith; **PX,** Protoxylem;**MX,** Metaxylem

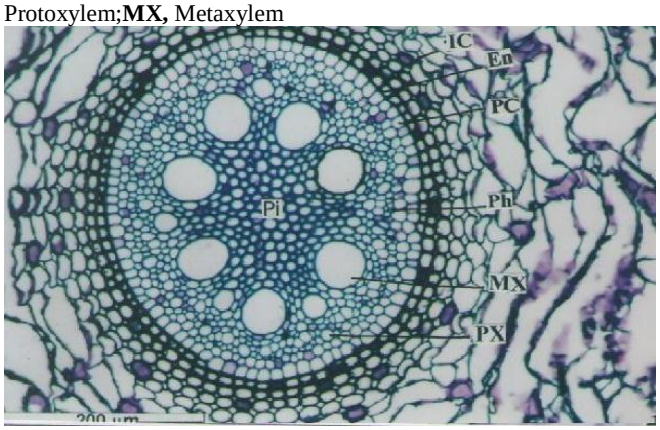
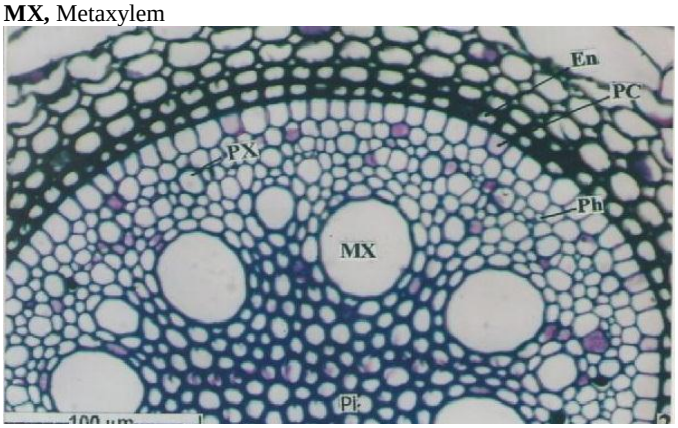


Fig 2d: A sector of stele (enlarged): En, Endodermis; **IC,** Innercortex; **PC,** Pericycle; **Ph,** Phloem; **Pi,** Pith; **PX,** Protoxylem; **MX,** Metaxylem



CONCLUSION

The present study reports the pharmacognostical characteristics of *Pycreus puncticulatus,* (Vehl) Nees., root will provide useful information for its correct identity. Histological studies on the root exhibits outer cortex, is made up of polygonal, parenchyma cells. Aerenchyma is made up of tangentially elongated cells and arranged in concentric circles in the middle cortex. Two or three layers of circular, thick walled parenchyma cells are present in the inner cortex. Stele consists of two layers of endodermis made up of rectangular cells. Vascular tissues are composed of radially arranged xylem and phloem as well as wide metaxylem and exarch protoxylem, is characteristic for this root.

Physico-chemical standards of the root powder is an important tool and are used to ascertain the quality of this plant material. Fluorescence analysis gives an idea about the presence of chromophores in this plant. Preliminary phytochemical screening reveals the phytochemical nature of this root. Antimicrobial potential of this root was confirmed, due to the presence of polyphenols, phytosterols and alkaloids.

In conclusion, the present study on pharmacognostical and antimicrobial screening of *Pycreus puncticulatus,* (Vahl) Nees., will be providing useful information with regard to its correct identity and help to differentiate from the closely related other species of Pycreus and may help the future workers in selecting the correct herbal specimen.

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