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

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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 rhizome, 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.

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INTRODUCTION

For centuries, plants and plant products have been used to treat various diseases. During the past decade, the indigenous or traditional system has gained importance in the field of medicine. In most of the developing countries, a large number of populations depend on the traditional practitioners, who are dependent on medicinal plants to meet their primary health care needs. Although, modern medicines are available, herbal medicine retained their image for historical and cultural reasons. Since the usage of these herbal medicines has increased, issues and moto regarding their quality, safety, and efficacy in industrialized and developing countries are cropped up[1]. There has been rapid increase in the standardization of selected medicinal plants of potential therapeutic significance[2]. 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[3], 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. An authentication and quality assessment of herbal material deals with the pharmacognosy, which is based on macroscopic and microscopic characters. A big quantum of research works in the authentication of the correct plant source has been undertaken to provide means of differentiation among the many available plant sources. *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[4]. 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.

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.

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 rhizomes 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[5]. 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[6]. The sections were later stained with toluidine blue[7]. Since, O - 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 units. 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 birefringent 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[8].

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[9]. For fluorescence analysis, powdered root was sieved through 60 mesh and observations were made by adopting the standard methods[10, 11].

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[12]. 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[13].

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* (MTCC 724), *Klebsiella pneumonia* (MTCC 109) *Pseudomonas aeruginosa* (MTCC 741), *Staphylococcus aureus* (MTCC 96), *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 1344) by using standard antibiotics Amikacin and Nystatin 25µg/ml[14].

RESULTS AND DISCUSSION

The quality control parameters for the crude drugs as raw materials were established with the help of several official determinations based on morphology, microscopy and physicochemical studies. These studies were aimed at ensuring standardization of herbal drug under investigation. Morphological examination of drugs refers to evaluation of drugs by colour, odour, taste, size, shape and special features, like touch, texture etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs.

Macroscopy

The plant is a dense muddy, small herb growing up to 1 m height and 7 mm wide. Leaves are flat or canaliculated, as long as stem, to 8 mm wide, consists of inflorescence, decompound which is about 6 – 20 cm. The involucral bracts are 5 – 7 in numbers, it also consists of 5 – 7 primary rays, and the rachis is elongated over 8 cm and 3- 5 secondary rays are found in a cluster. The rhizome (root-stock) is thick, soft and fleshy. It is 1-2 cm in diameter (Figure 1).

Figure 1
Entire view of Pycreus puncticulatus (Vahl.) Nees.

Microscopy

The transverse section of rhizome exhibited four distinct zones as follows (Figure 2 - 6),

Epidermis

A thin layer of epidermis is made up of radially arranged rectangular cells with thick walls and less prominent cuticle.

Cortex

It is nearly 2 mm wide and consists of homogenous circular, thin walled, less compact parenchyma cells with minute intercellular spaces. Many vascular bundles are distributed in circular manner, are smaller with periphery and more prominent towards the interior. The cortical bundles are amphivasal type. They have central core of phloem surrounded by wide, thick walled xylem elements. Each vascular bundle is again ensheathened by 1-3 layers of fibres.

Endodermoid zone

This is the inner boundary of the cortex and separates the stele from the cortex. It consists of 3-4 layers of tubular cells; the endodermoid layer is wavy in outline.

Stele

It is the central core of rhizome comprising of several discrete, scattered vascular bundles in the ground tissue. This type of stele is called atactostele. The vascular strands of the atactostele are of two types. Some bundles are circular and are of amphivasal type, vascular bundle has a central phloem, surrounded by xylem, and the bundle also has a thick sheats of fibres. The second type is collateral vascular bundle which has xylem and phloem, in a radial line and the vascular bundle has thick fibrous sheaths. The xylem elements are 20 mm wide. They are thick walled and angular in outline.

Cell inclusions

Two types of cell inclusions are seen in the ground parenchyma (Figure 6 - 8).

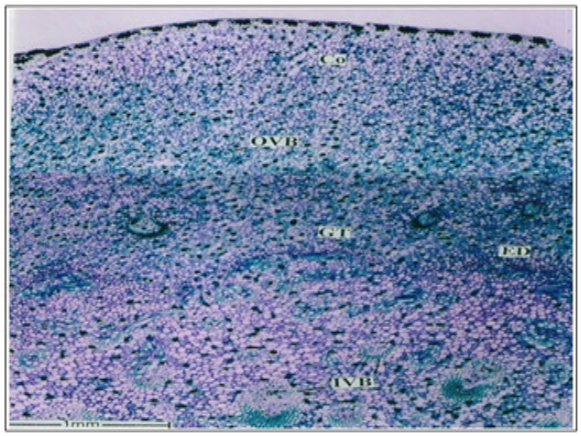

Figure 2
T. S of Rhizome (entire view); Co – Cortex; ED – Endodermoid; GT – Ground tissue; IVB – Inner vascular bundle; OVB – Outer vascular bundle

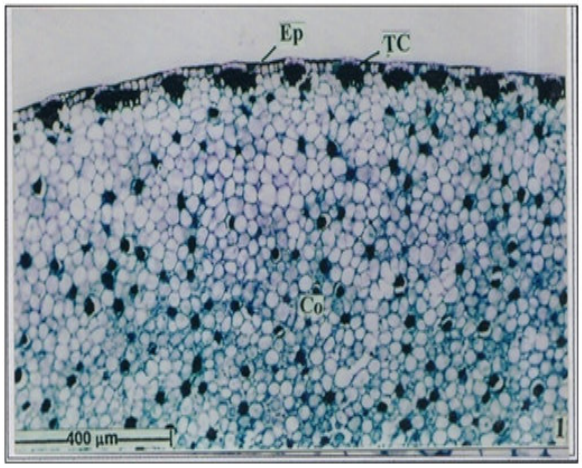

Figure 3
Cortex showing the distribution of tanniniferous cells – Co – Cortex; Ep – Epidermis; TC – Tanninifreous cells

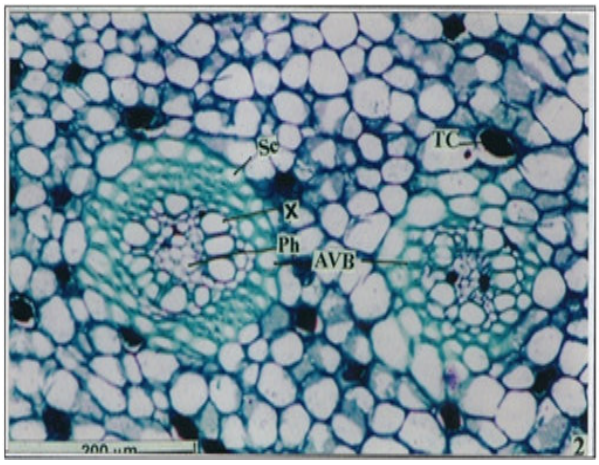

Figure 4
Amphivasal type of cortical bundles – AVB – Amphivasal cortical bundle; Ph – Phloem; Sc Sclerenchyma; TC – Tanniniferous cells; X – Xylem

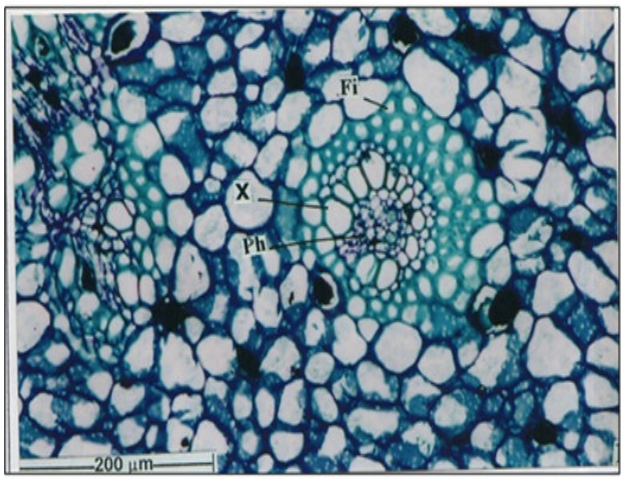

Figure 5
Amphivasal type of bundles – Fi –Fibres; Mx – Metaxylem; Px – Protoxylem; X – Xylem

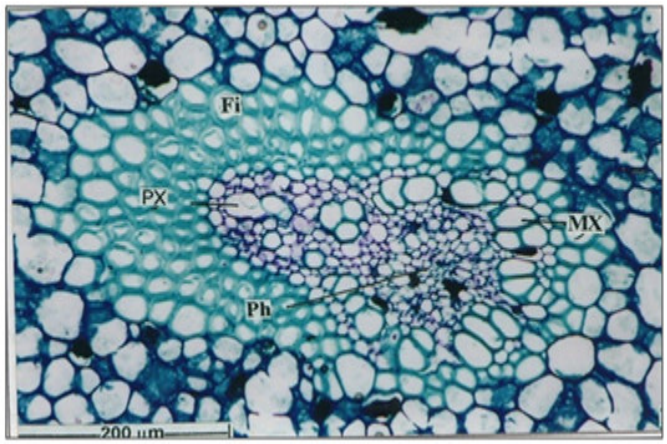

Figure 6
Collateral type of bundles – Fi – Fibres; Mx – Metaxylem; Ph – Phloem; Px – Protoxylem; X – Xylem

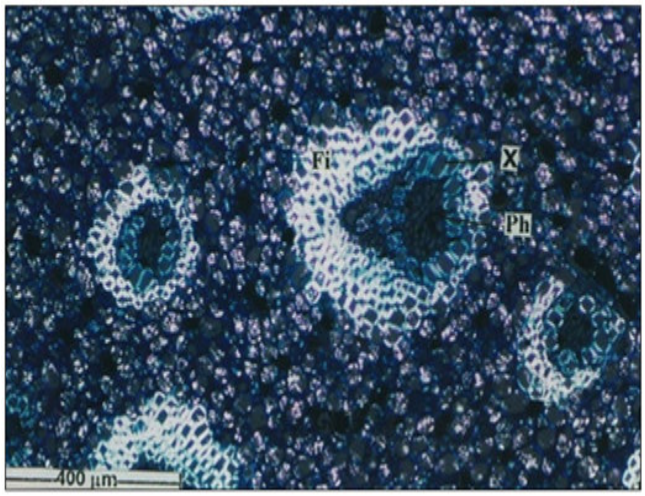

Figure 7
Vascular bundles – Fi – Fibres; Ph – Phloem; X - Xylem

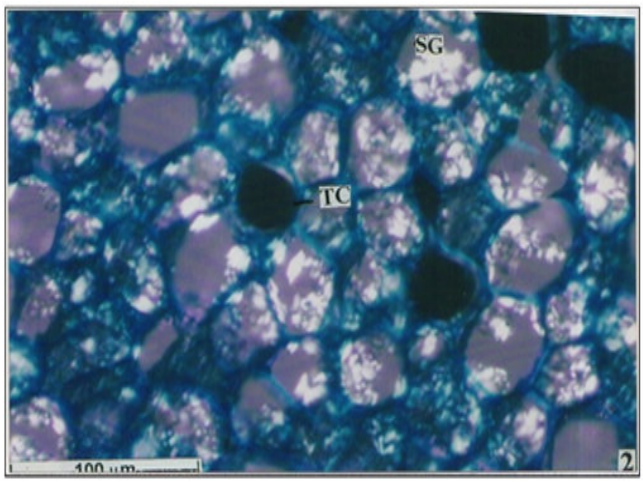

Figure 8
Starch grains - SG – Starch grains; TC – Tanniniferous cells

Tannins
Tannin content is seen filled densely in the central stele parenchyma cells. Clusters of tanniniferous cells are seen above the epidermal layer all around the rhizome. Scattered parenchymatous cells are filled with tannin, occurs throughout the cortex and in the central portion.

Starch grains
Abundant starch grains occur in all parenchyma cells except the tanniniferous cells. The starch grains were identified with an addition of I_2-KI solution; they appear bright white under polarized light. The starch grains are of various shape and size, ranging from circular to elliptic types. The present macroscopical and histo-anatomical observations of rhizomes thus provide useful information for quality control parameters for crude drug.

Physico-chemical parameters
The rhizome powder was analyzed for various physico-chemical parameters which includes ash values, extractive values, loss on drying and crude fiber content and the results were depicted in Table 1. Estimation of ash value is also a significant parameter for the detection of nature of material, which is added to the drug the purpose of adulteration, impurities and determination of authenticity, quality and purity of test sample[15]. The ash values usually represent the inorganic salts present in the drug and is the residue remaining after incineration. Total ash values indicate the inorganic composition or earthy materials and other impurities present along with the plant material. The total ash value is relatively higher which may be due to high content of carbonates, phosphates, silicates and silica[16]. Extractive values were useful for the determination of exhausted drugs. The amount of an extract that drug yields in a solvent is an approximate measure of the amount of certain constituents that the drug contains. The water soluble extractive value indicating the presence of sugars, acids and inorganic compounds and the organic solvent extractive value exhibiting the presence of polar constituents like phenols, steroids and flavonoids.The higher or lower percentage of moisture content shows that the drug was resorted in humid, wet or dry climate.

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

Physico-chemical parameters	% Values
Total ash	9.52
Water soluble ash	6.37
Acid insoluble ash	2.42
Sulphated ash	4.41
Loss on drying	2.93
Ether soluble extractive value	3.85
Alcohol soluble extractive value	9.54
Water soluble extractive value	4.67
Crude fibre content	7.93

Fluorescence analysis
The fluorescence characteristics of rhizome extracts and powder after the treatment with different reagents emitted different colour radiations were observed in the day light and UV light (254nm) and the results were tabulated in Table 2 and 3.

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

Reagents	Day light	UV light
Rhizome powder	Pale brown	Brownish yellow
Powder + 1N NaoH(Aq)	Brown	Brown
Powder + 1N NaoH(Alc)	Brown	Reddish brown
Powder + 1N HCl	Dark brown	Reddish brown
Powder + 50% HNO_3	Dark brown	Reddish brown
Powder + 50% H_2SO_4	Reddish brown	Reddish brown
Powder + 5% $FeCl_3$	Reddish brown	Reddish brown
Powder + N/50 I_2 solution	Blue	Bluish black
Powder + Water	Yellow	Yellow

Table 3
Fluorescence analysis of *Pycreus puncticulatus* (Vahl.) Nees., rhizome extract

Extracts	Day light	UV light
Petroleum ether	Pale yellow	Pale yellow
Benzene	Pale brown	Brown
Chloroform	Light yellow	Yellow
Ethyl acetate	Pale yellow	Brown
Methanol	Light yellow	Yellowish brown
Ethanol	Pale yellow	Yellowish brown
Aqueous (0.25 % v/v of $CHCl_3$ in water)	Pale brown	Pale brown

Phytochemical screening
Qualitative tests of various rhizome extracts showed the presence of alkaloids, carbohydrates, phenolic compounds, tannins, phytosterol and lignin were tabulated in Table 4.

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

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

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

Antimicrobial studies (Figure 9, 10)
50 mg/ml of petroleum ether, chloroform, ethanol and aqueous rhizome extracts of *Pycreus puncticulatus,* (Vehl) Nees., exhibits significant antimicrobial activity against the pathogens and the results were recorded in Table 5.

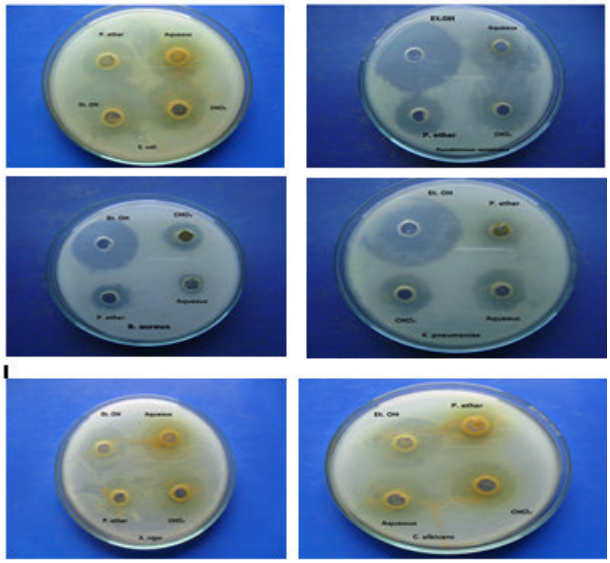

Figure 9
Antimicrobial activity of Pycreus puncticulatus(Vahl.) Nees., rhizome extract

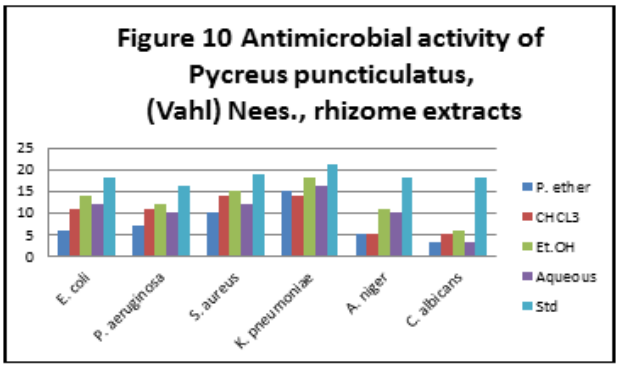

Table 5
Antimicrobial activity of *Pycreus puncticulatus,*
(Vahl) Nees., rhizome extracts

Microorganisms	Zone of inhibition in mm				
	Pet. ether extract (50 mg/ml)	Chloroform extract (50 mg/ml)	Ethanol extract (50 mg/ml)	Aqueous extract (50 mg/ml)	Standard (25 µg/ml)
Escherichia coli	06	11	14	12	18
Pseudomonas aeruginosa	07	11	12	10	16
Staphylococcus aureus	10	14	15	12	19
Klebsiella pneumonia	15	14	18	16	21
Candida albicans	05	05	11	10	18
Aspergillus niger	03	05	06	03	18

Antimicrobial properties of several plant extracts have been attributed due to the secondary metabolites[17 - 19]. The reason for the difference sensitivity between the gram-positive and gram-negative bacteria could be ascribed to the morphological differences between these microorganisms, gram-negative pathogens having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to hydrophilic solutes with an exclusion limit of about 600 Da. The gram positive bacteria should be more susceptible having only an outer peptido glycone layer which is not an effective permeability barrier[20]. Phenolic content of plant extracts possess antimicrobial activity[21, 22] and highly oxidized phenols are more inhibitory effect because of phenolic toxicity to microorganisms[23]. In addition, leaf extracts also possess antimicrobial potential against all pathogens which may be due to the presence of steroids[24] and alkaloids[25].

CONCLUSION

As there is no pharmacognostic/anatomical work record of this much valued traditional drug, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. In other words, the pharmacognostic features examined in the present study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations. Pharmacognostical studies can serve as a basis for proper identification, collection and investigation of the plant. These

parameters, which are being reported, could be useful in the preparation of the herbal monograph for its proper identification. The quality control parameters for the crude drugs as raw materials were established with the help of several official determinations based on morphology, microscopy and physicochemical studies. These studies were aimed at ensuring standardization of herbal drug under investigation. Morphological examination of drugs refers to evaluation of drugs by colour, odour, taste, size, shape and special features, like touch, texture etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. The millenarian use of these plants in folk medicine suggested that they represent an economic and safe alternative to treat infectious diseases. Finally, it is recommended that awareness of local communities should be enhanced incorporating the traditional knowledge with scientific findings.

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Kumar D, Bhat ZA, Singh P, Shah MY, Bhujbal SS. *Ailanthus excelsa* Roxb. is really a Plant of Heaven. Int J Pharmacol. 2010 May; 6 (5): 535-50.
2. Prabhu KS, Lobo R, Kumar CD, Rajendran K, Shirwailkar A. Pharmacognostical evaluation of *Sphaeranthus indicus* (Linn.). Nat. Prod. Sci. 2006; 12 (2): 85-8.
3. Indian Pharmacopoeia, 3rd Edn, Vol. 1, Government of India, Ministry of Health, Controller of Publications, New Delhi, India, 947-50, (1996).
4. Ambasta SP. The Useful Plants of India. 1st Edn, Council of Scientific and Industrial Research (CSIR), New Delhi, 705, (1992).
5. Jaiganesh KP, Arunachalam G. Pharmacognostical and antimicrobial studies on the leaf of *Pleiospermum alatum*, Swingle. Pharma Science Monitor, 2012 Oct; 3(3.1): 1884 – 93.
6. Sandeep Singh, Singh DP, Dilip K.Singh, Pankaj Maurya, Ankit Saini. Pharmacognostic study of *Moringa concanensis*, Nimmo., bark. Int J Res Development in Pharmacy and Life Sci. 2014 July; 2(4): 538 – 4.
7. Senthamarai R, Vasuki K. Pharmacognostical studies of *Biophytum sensitivum*, Dc., stem. Intl J Biol and Pharm Res. 2013 Oct; 4 (12): 1289 - 92.
8. Sonje SB, Bhuktar AS. Anatomical studies of *Crotalaria juncea*, L., J of Global Sci. 2016 Aug; 5(5): 4071 - 4.
9. Anonymous, Indian Pharmacopoeia, 3rd Edn, Vol.1, Government of India, Ministry of Health, Controller of Publications, New Delhi, India, 947-950, (1996).
10. Chase CR, Pratt RJ. Fluorescence of powdered vegetable drugs with particular reference to the development of a system of identification. J Am Pharm Assoc. 1949 Jun; 38 (6): 324-31.
11. Mohamed Rafiq Khan, Vijitha V. Pharmacognostic profile and phytochemical investigation on *Syzyium cumini*, bark extracts. Int J Pharma and Bio Sci. 2015 July; 6(3): 359 – 65.
12. Kokate CK. Practical Pharmacognosy, 4th Edn, Vallabh Prakashan, New Delhi, 108-121, (1994).
13. Horborne JB. Phytochemical Methods, 3rd Edn, Chapmann and Hall, London, 16-30, (1998).
14. Perez C, Paul M, Bazerque P. Antibiotic assay by agar-well diffusion method. Acta Biol Med Exp. 1990, 15: 113 - 5.
15. Wallis TE. Practical Pharmacognosy, 5th Edn, JA Churchil Ltd., London, (1984).
16. Ansari SH. Essentials of Pharmacognosy, 1st Edn, Birla Publications, New Delhi, (2006).
17. Sharma DK. Pharmacological properties of flavonoids including flavonolignans -integration of Petro crops with drug development from plants. J Sci Ind Res. 2006 Jun; 65 (6): 477-84.
18. Kosalec I, Pepeljnjak S, Bakmaz M, Vladmir-Knezevic S. Flavonoid analysis and antimicrobial activity of commercially available Propolis products. Acta Pharm. 2005 Dec; 55 (4): 423-30.
19. Tim Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids, Int J Antimicro Ag. 2005 Nov; 26 (5): 343-56.
20. Nikaido H, Vaara M. Molecular Basis of Bacterial Outer Membrane Permeability. Microbiol Rev. 1985 Mar; 49 (1): 1-32.
21. Acar G, Dogan NM, Duru ME, Kivrak I. Phenolic profiles, antimicrobial and antioxidant activity of the various extracts of Crocus species in Anatolia. Afr J Microbiol Res. 2010 Jun, 4 (11): 1154 – 61.
22. Rama Prabha M, Vasantha K. Phytochemical and antibacterial activity of *Calotropis procera* (Ait.) R. Br. Flowers. Int J Pharma and Bio Sci. 2012 Jan-Mar; 3(1): P1-P6.
23. Ciocan ID, Bara II. Plant products as antimicrobial agents, Genetică și Biologie Moleculară, 2007; 8(1): 151-6.
24. Cowan MM. Plants products as antimicrobial agents, Clin Microbiol Rev. 1999 Oct; 12(4): 564-82.
25. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origins: Current Methods and Future Trends. Afr J Bio Tech. 2008 Jun; 7 (12): 1797- 06.