



## 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<sup>1</sup>. There has been rapid increase in the standardization of selected medicinal plants of potential therapeutic significance<sup>2</sup>. 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<sup>3</sup>, 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<sup>4</sup>. 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<sup>5</sup>. 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<sup>6</sup>. The sections were later stained with toluidine blue<sup>7</sup>. 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<sup>8</sup>.

### 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<sup>9</sup>. For fluorescence analysis, powdered root was sieved through 60 mesh and observations were made by adopting the standard methods<sup>10, 11</sup>.

### 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<sub>3</sub> in water<sup>12</sup>. 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<sup>13</sup>.

#### **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<sup>14</sup>.

## **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 involucre 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 ensheathed by 1-3 layers of fibres.

#### **Endodermoid zone**

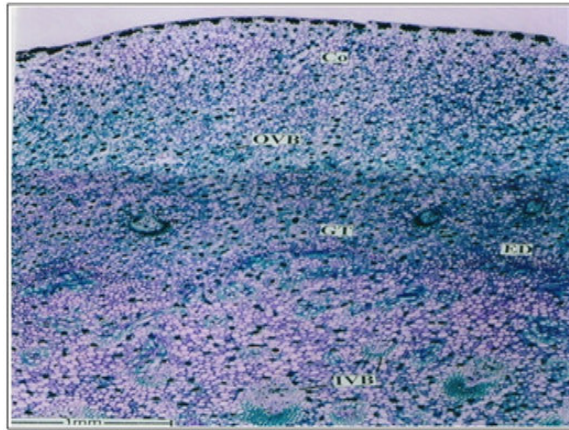
This is the inner boundary of the cortex and separates the stele from the cortex. It consists of 3-4 layers of tubular cells; the endodermoid layer is wavy in outline.

#### **Stele**

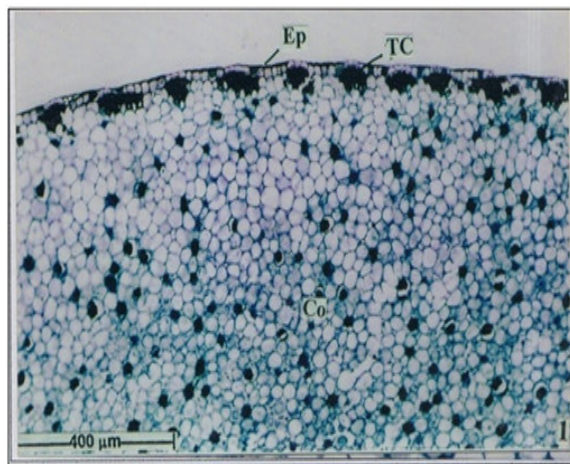
It is the central core of rhizome comprising of several discrete, scattered vascular bundles in the ground tissue. This type of stele is called atactostele. The vascular strands of the atactostele are of two types. Some bundles are circular and are of amphivasal type, vascular bundle has a central phloem, surrounded by xylem, and the bundle also has a thick sheath 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**

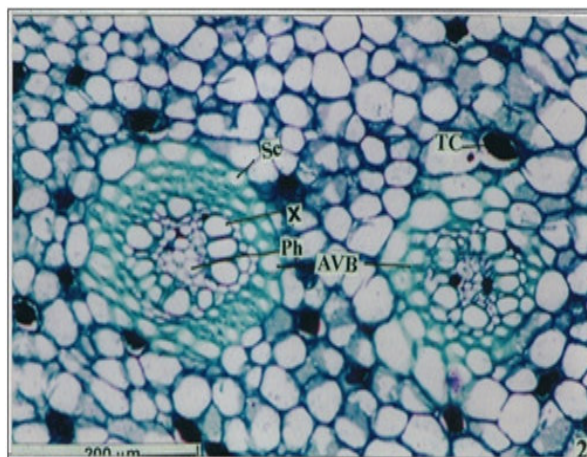
Two types of cell inclusions are seen in the ground parenchyma (Figure 6 - 8).



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

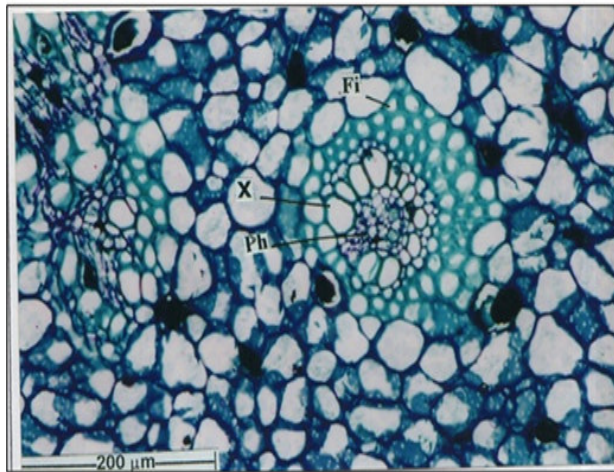


**Figure 3**  
*Cortex showing the distribution of tanniferous cells – Co – Cortex; Ep – Epidermis; TC – Tanniferous cells*



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

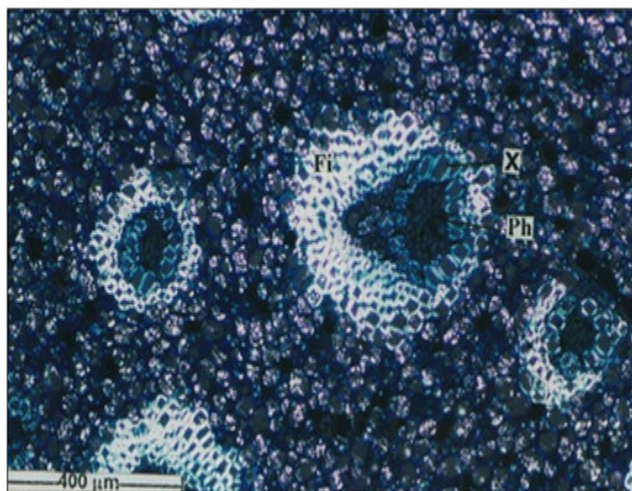




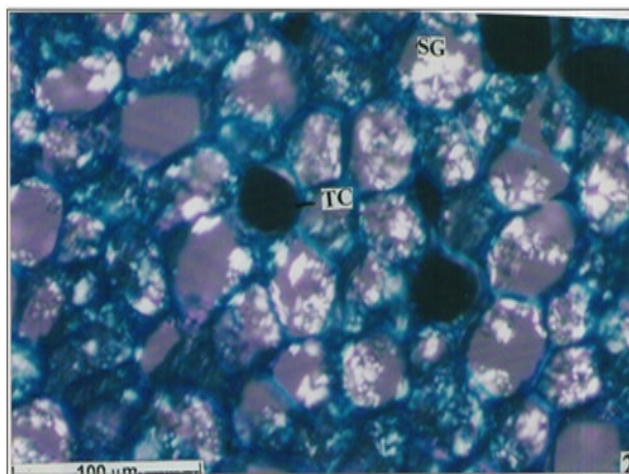
**Figure 5**  
*Amphivasal type of bundles – Fi –Fibres; Mx – Metaxylem;  
Px – Protoxylem; X – Xylem*



**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 – Tanniferous cells**

### **Tannins**

Tannin content is seen filled densely in the central stele parenchyma cells. Clusters of tanniferous 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 tanniferous cells. The starch grains were identified with an addition of I<sub>2</sub>-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<sup>15</sup>. 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<sup>16</sup>. 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 <sub>3</sub>	Dark brown	Reddish brown
Powder + 50% H <sub>2</sub> SO <sub>4</sub>	Reddish brown	Reddish brown
Powder + 5% FeCl <sub>3</sub>	Reddish brown	Reddish brown
Powder + N/50 I <sub>2</sub> 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 <sub>3</sub> 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.

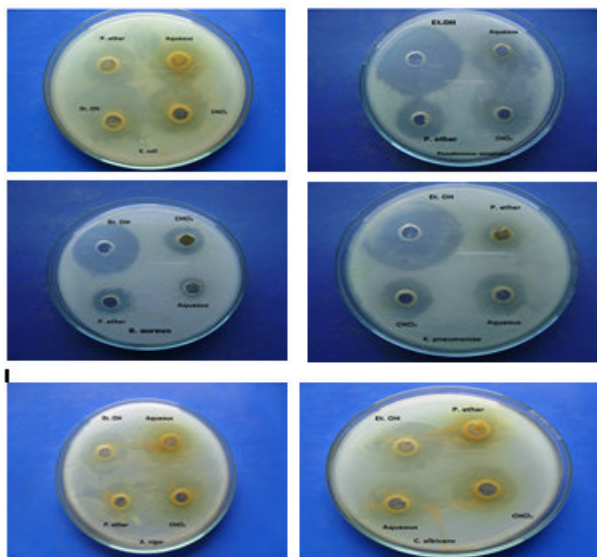
**Table 4**  
**phytochemical screening of *Pycreus puncticulatus* (Vahl.) Nees., rhizome**

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	+	+	+	+	+

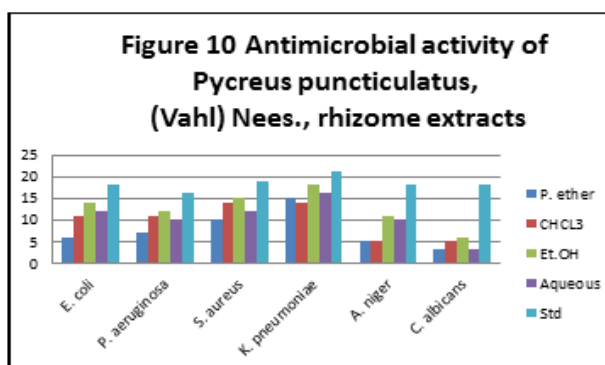
(+) = Presence of phytoconstituents; (-) = Absence of phytoconstituents

#### Antimicrobial studies (Figure 9, 10)

50 mg/ml of petroleum ether, chloroform, ethanol and aqueous rhizome extracts of *Pycreus puncticulatus*, (Vahl) 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)
<i>Escherichia coli</i>	06	11	14	12	18
<i>Pseudomonas aeruginosa</i>	07	11	12	10	16
<i>Staphylococcus aureus</i>	10	14	15	12	19
<i>Klebsiella pneumonia</i>	15	14	18	16	21
<i>Candida albicans</i>	05	05	11	10	18
<i>Aspergillus niger</i>	03	05	06	03	18

Antimicrobial properties of several plant extracts have been attributed due to the secondary metabolites<sup>17-19</sup>. 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 peptidoglycane layer which is not an effective permeability barrier<sup>20</sup>. Phenolic content of plant extracts possess antimicrobial activity<sup>21, 22</sup> and highly oxidized phenols are more inhibitory effect because of phenolic toxicity to microorganisms<sup>23</sup>. In

addition, leaf extracts also possess antimicrobial potential against all pathogens which may be due to the presence of steroids<sup>24</sup> and alkaloids<sup>25</sup>.

## 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.

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