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Pharmacognostical Investigation and Preliminary Phytochemical Screening of Leaves of *Pamburus missionis* S.

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ABSTRACT

Traditional medicinal plants were ruling out the world in treatment of various diseases from many centuries and gaining their importance in health care system. *Pamburus missionis Swingle* is a small thorny shrub belongs to the family Rutaceae. It is used traditionally in the treatment of swellings, fractures, piles and fistula. Investigation of literature revealed that there is a lack of pharmacognostical and Phytochemical investigations of *Pamburus missionis Swingle*. The current study deals with the preliminary phytochemical screening and detailed pharmacognostical study of leaf of *Pamburus missionis Swingle*. The detailed study of pharmacognostical evaluation showed the presence of thick walled epidermal cells covered with thick cuticle, xylem and phloem elements, and secretory cavities in epidermal view. Preliminary Phytochemical examination revealed the presence of various phytoconstituents viz., alkaloids, glycosides, tannins, terpenoids, carbohydrates and flavonoids. The fluorescence analysis manifested the behavioral variation of the powdered drug.

Key words: Rutaceae, Microscopy, Phytochemical screening, Standardization.

INTRODUCTION

Pamburus missionis Swingle [1] is a small thorny shrub commonly called as kattunaranthi in tamil belonging to the family Rutaceae. The leaf of this plant is used traditionally for the treatment of fistula, joint swellings, rheumatism, fractures and piles. The anatomical and chemical investigations on *Pamburus* genus are very limited. *Pamburus missionis Swingle* is distributed in southern India. Earlier investigation was made on root and stem bark of *Pamburus missionis* S. and reported that it contains imperatorin, coumarins, diterpenes, flavones and xanthotoxis, isopimpinellin, scopoletin and luvangetin [2, 3].

MATERIAL AND METHODS:

Plant material:

Pamburus missionis Swingle leaves were procured from Talakona hills, Tirupati. It was identified and authenticated by Prof. K. Madhava Shetty, Department

of Botany, Sri Venkateswara University, Tirupati. Andhra Pradesh, India.

Description of the plant:

The plant is small short thorny shrubs. Leaf is elliptical to ovate, petiolate and measures about 6-10cm in length, 3-5 cm in width, narrow base and blunt apex. Margin is entire. It shows terminal inflorescence and flowers are 12-20mm in diameter, fragrant with small pointed sepals and petals are about 1cm long. Fruit when ripened orange colored and it is 4-5 celled containing 1 or 2 seeds are gummy.

Pharmacognostic evaluation:

Organoleptic evaluation:

The sensory parameters of leaf of *Pamburus missionis* S. such as size, shape, color, odour and taste were recorded.

Microscopic evaluation:

Preparation of sections:

The sections of leaf were made with the help of rotary microtome. Later the sections were made stained with toluidine blue, a polychromatic stain [4]. The cleared sections were then mounted with glycerin for microscopical observations.

Powder microscopy:

To a little quantity of powder in a watch glass, 1-2 drops of 0.1% phloroglucinol solution and Concentrated HCL was added in a ratio of 1:1. The stained powder was transferred onto a slide, mounted with glycerol and covered with cover slip [5]. The prepared slide observed under microscope with 10x10 magnifications. The presence of starch grains were detected by addition of 2-3 drops of 0.01m Iodine solution.

Determination of Stomatal Index:

Leaf fragments of about 5x5 mm in size were taken and it was cleared with chloral hydrate solution [6-7]. The cleared fragment of leaf was mounted with glycerol on microscopic slide. It is observed under microscope for quantification of Stomatal Number and Stomatal Index, vein islet number and Vein termination number. The slide was examined with 40x objective and 6x eye piece to which a Camera lucida was attached and recorded.

Physical evaluation:

Estimation of crude fiber:

2gm of powdered drug was taken and 50ml of 10 % v/v nitric acid was added. It was heated with constant stirring and strained. To the residue 50 ml of 2.5% v/v sodium hydroxide solution was added, heated and maintained at boiling point for 30 seconds [5]. After it was strained and the residue is weighed. The percentage of crude fibers was determined.

Moisture content:

10gm of accurately weighed fresh leaves of *Pamburus missionis* S. was placed in a tared porcelain dish and dried at 105°C for 5 hrs and weighed. Drying and weighing is continued at an interval of one hour until two successive weighing is constant.

Total ash:

Determination of total ash:

2gm of leaf powder of *Pamburus missionis* S. was taken in tared silica crucible and incinerated at a temperature not more than 450°C until free from carbon. The obtained ash was cooled and weighed. The percentage of ash was calculated with reference to the air dried drug [7].

Acid-insoluble ash:

The total ash obtained from 2gm of leaf powder was boiled with 25 ml of dilute hydrochloric acid for 5 minutes and the insoluble mater was collected on an ash less filter paper. It was washed, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water soluble ash:

The total ash obtained from 2g of leaf powder was boiled with 25ml of water for 5 minutes and the insoluble matter was collected on an ashless filter paper. It was washed, ignited and weighed. The percentage of water soluble ash was calculated with reference to the air dried drug.

Determination of alcohol soluble extractive:

5g of accurately weighed leaves was taken and macerated with 100 ml of 95% alcohol for 24 hr. The contents were frequently shaken during the first 6hr and allowed to stand for 18hr. After 24 hr, 25ml of extract was filtered and evaporated. The extract was dried at 105°C to a constant weight.

Determination of water soluble extractive:

5g of accurately weighed leaves was taken and macerated with 100 ml of chloroform water for 24 hr. The contents were frequently shaken during the first 6hr and allowed to stand for 18hr. After 24 hr, 25ml of extract was filtered and evaporated. The extract was dried at 105°C to a constant weight.

Preparation of extract:

500g of dried coarsely powdered leaf of *Pamburus missionis* S. was packed in soxhlet apparatus and was defatted with petroleum ether (50-60°C). The marc left subsequently extracted with Petroleum ether, chloroform, ethanol and distilled water. The extracts obtained were concentrated using rotary evaporator and dried.

Preliminary Phytochemical Screening:

The extracts were subjected to preliminary Phytochemical screening for the detection of various plant constituents viz. carbohydrates, fixed oils, alkaloids, glycosides, terpenoids, flavonoids, tannins and phenols [9-13].

Fluorescence analysis of the powdered drug:

The fluorescence analysis of the powdered leaves was done by placing dry powdered leaves on a slide and observed by treating with several drops of different chemical reagents to detect the color changes under UV and Visible light.

RESULTS

Pharmacognostic evaluation

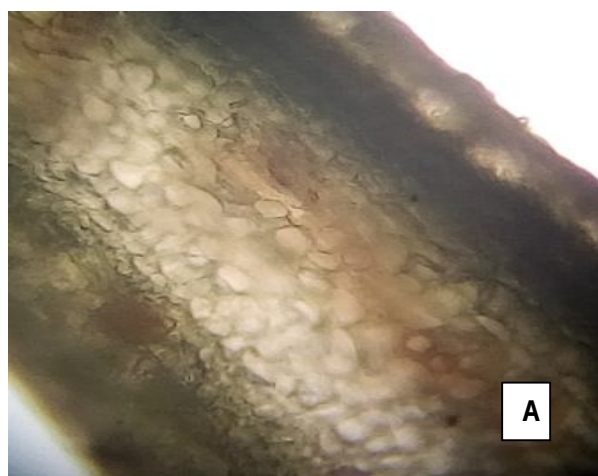
Organoleptic and Microscopic evaluation:

Macroscopically, the leaf of *Pamburus missionis* S. is simple, shape is oblong-obovate, petiolate, apex is acuminate and margin is entire. Fresh leaves are thick, dark green in color and measures about 6-10 cm in length and 3-5 cm in width. The leaf powder was grayish green in color with aromatic odor and aromatic taste (Figure 1).



Figure 1: Twig of *Pamburus missionis* S.

The microscopical examination of leaf was revealed the presence of thick walled epidermal cells covered with thick cuticle, xylem towards centre, phloem towards periphery, Secretary cavities were observed in surface view of epidermis and anomocytic type of stomata. (Figures 2, 3 & 4)



The powdered microscopical examination of leaf of *Pamburus missionis* S. revealed that the presence of lignified xylem and epidermal cells. (Figure 5)

The quantitative microscopical evaluation of fresh leaves and leaf powder was performed and the results were obtained. (Table 1)

Physical evaluation:

The various physical parameters of leaves and leaf powder viz., Crude fiber content, moisture content, Ash values and extractive values were determined. (Table 2)

The quantitative and qualitative pharmacognostical study helps in identification of the plant as these values are unique for each and every species of plant and they acquire an utmost important in standardization of crude drug.

Preliminary Phytochemical Screening:

The preliminary phytochemical screening for the extracts viz., petroleum ether, chloroform, ethanol and water was carried out and the results obtained. (Table 3)

Flourescene analysis:

The behavioral changes of powdered drug with different chemical reagents were determined at both UV and Visible light and it is reported. (Table 4)

DISCUSSION

In the present study, pharmacognostical parameters of leaf of *Pamburus missionis* S. were evaluated which serves as an establishment for the monograph of the crude drug.

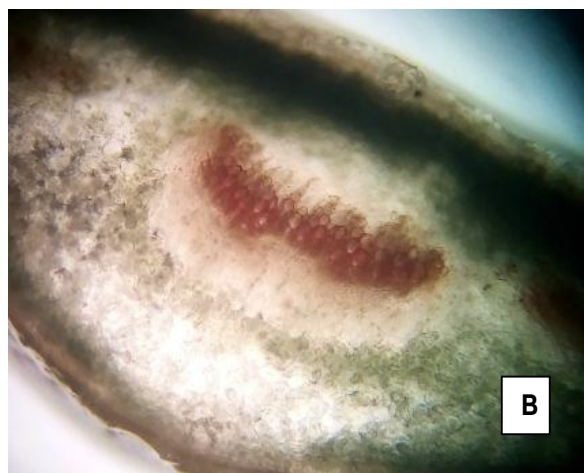


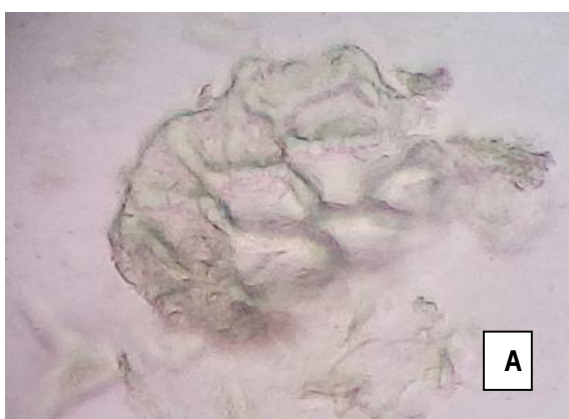
Figure 2: **A**-T.S. of leaf through midrib portion, AdS – Adaxial surface, La – Lamina, MR – Mid rib, GT – Ground Tissue. **B**-Vascular bundles portion through midrib of leaf, VB – Vascular bundles, Ep- Epidermis, X – Xylem and Ph – Phloem.



Figure 3: Stomata of leaf, GC- Guard cells and Sc- Subsidiary cells.



Figure 4: Secretory cavities – Leaf.



A



B

Figure 5: Powder microscopy of leaf, A- Epidermal cells, B- Lignified Xylem vessels.

Table 1: Quantitative Microscopy of *Pamburus missionis* S.

Parameters	Values (mean)
Stomatal number upper surface	141.89
Stomatal number lower surface	124.4
Vein Islet number	28.96
Vein Termination number	5.35

Table 2: Physicochemical parameters of leaf powder of *Pamburus missionis* S.

Parameters	Values %w/w
Crude fiber	26
Moisture content (Loss on drying)	9
Total ash	2.36
Acid insoluble ash	0.086
Water soluble ash	0.23
Alcohol soluble extractive value	61.86
Water soluble extractive value	37.60

Table 3: Phytochemical analysis of various extracts of *Pamburus missionis* S.

Phytoconstituents	Method	Aqueous Extract	Alcoholic Extract	Pet-ether Extract	Chloroform Extract
Flavonoids	Shinoda Test	+	+	-	-
	Zn. Hydrochloride test	+	+	-	-
	Lead acetate Test	+	+	-	-
Alkaloids	Wagner Test	+	+	-	+
	Hager's Test	+	+	-	+
Tannins & Phenols	FeCl ₃ Test	+	+	-	+
	Potassium dichromate test	+	+	-	+
Saponins	Foaming Test	-	-	-	-
Terpenoids	Salkowski test	+	+	-	-
Carbohydrates	Molish test	+	+	-	-
Glycoside	Keller-Killani Test	+	+	-	-

- Negative = Absent

+ Positive = Present

Table 4: Fluorescence analysis of *Pamburus missionis* S.

Solvent used	Visible light	UV light	
		254nm	366nm
Distilled water	Clear	Green	Green
1 N NaOH	Dark Green	Fluorescent green	Fluorescent green
1N Methanol	Pale Brown to Pale Green	Pale green	Pale Green
1N HCl			
Ethanol	Dark green	Fluorescent green	Fluorescent green
50% HNO ₃	Reddish brown	Bright green	Moderate green
FeCl ₃	Olive green	Olive green	Olive green
CHCl ₃	Pale green	Pale green	Pale green
Picric acid	Fluorescent green	Fluorescent green	Fluorescent green

The fluorescence analysis of the powdered leaf was examined and the various behavioral changes had observed with different reagents at both UV and Visible light. The phytochemical analysis of different solvent extracts viz., petroleum ether, chloroform, ethanol and water were examined and it revealed the presence of various secondary metabolites which gains an importance in medicine.

CONCLUSION

In the current investigation, the pharmacognostical investigation, physicochemical parameters and fluorescence analysis and preliminary phytochemical screening of *Pamburus missionis* S. was carried out. The results of current study help as a reference material to develop a monograph.

The preliminary phytochemical screening of leaves of *Pamburus missionis* S. had shown the presence of secondary metabolites which can play a vital role in medicine.

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