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Research Article

PHARMACOGNOSTICAL STUDIES ON *PERSEA MACRANTHA* (NEES) KOSTERM. LEAF AND BARK

RENJUMOL. R[1*], SHIRMILA JOSE G[2] AND RADHAMANY P M[3]

[1*,2,3] **Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram- 695581, Kerala, India. Email: renjur88@gmail.com**

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ABSTRACT

Objective: To study the pharmacognostic characters of leaf and bark of *P. macrantha.*

Methods: Macroscopic and microscopic features, powder characteristics, physico -chemical analysis and quantitative analysis of leaf and bark of *P.macarantha* were done by adopting various standard methods for the identification and purification of herbal drug.

Results: The macroscopic study revealed that the leaves are simple, opposite, decussate and caduceus and bark showed large number of wrinkles and undulations. Transverse section revealed the presence of epidermis, spongy parenchyma, collateral vascular bundles, pericycle, collenchymas in leaf and etc. The powder analysis revealed the presence of tracheids, fibres, stone cells, cork cells, wood parenchyma and spiral thickening. Physico-chemical parameters like ash values, extractive values will help in the pharmacognostical evaluation of *P. macrantha.*

Conclusion: Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. So the present study concluded that the above parameters are used for the proper identification of *P. macrantha* for the future studies.

Keywords: *Persea macrantha,* Pharmacognostical studies, Physico-chemical parameters, Quantitative analysis, Organoleptic analysis, Fluorescent analysis, Ash values.

INTRODUCTION

Persea macrantha (Nees) Kosterm. Syn. *Machililus macrantha* Nees is a member of the family Lauraceae. It is distributed in western Peninsula, Sri Lanka and in India up to an altitude of 2100 m. It is commonly known as Kulamavu, Kulirmavu, Ooravu, Uramavu (Malayalam), Kolar maavu, and Kollamavu (Tamil) and is locally known as Golum, Pisara, Kurma, Gulmavu and Chittutandrimara in various regions[1] . The tree has many folk uses in various states of India. Its bark has antiinflammatory and antiarthritic properties and is used for treating asthma and rheumatism. It is used to prepare incense sticks because of its insecticidal property. The leaf is used externally for ulcer and is also used to cure asthma, cough, diarrhea, dysentery, edema and wounds. Machiline and macranthine are the major alkaloids isolated from the alcoholic extract of roots[1,2]. The isolated alkaloid machiline showed marked hypotensive effect in test animals[3]. Some lignins like machicediol, machicenonol and machicenin have been isolated from leaves. Fruit oil is used in aromatherapy and in the preparations of cosmetic products[4,5]. Aqueous extracts of *P. macrantha* has significant inhibitory activity against both human and plant pathogenic bacteria[1,6].

Due to the high medicinal value of *P. macrantha*, its pharmacognostical evaluation is inevitable to avoid the chances of adulteration and enable identification of the material. Till date, there is no report on the pharmacognostical evaluation of *P.macrantha* from Kerala. Present study focused on the pharmacognostic characters of leaf and bark of *P. macrantha* .

MATERIAL AND METHODS

Plant material collection

The fresh leaves and bark of *P. macrantha* were collected in the month of December, 2010 from the medicinal garden, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram and authenticated by Dr. G.Valsaladevi, Curator of the same Department. A voucher specimen (N0. KUBH. 5814) was deposited in the herbarium of the same department for future reference. Fresh materials were washed and used for the analysis of macroscopic and microscopic characters. The plant parts were subjected to shade drying for about 10 weeks and crushed to powder using mixture grinder. The powder was stored in air tight container for further analysis[7].

Macroscopic and microscopic analysis

The macroscopic and microscopic examinations of leaf and bark were done according to standared methods[8]. The macroscopic characters observed were verified by referring the flora of Madras Presidency[9], flora of Agasthyamala[10] and Medicinal plants of Aryavaidya sala[11].The microphotographs were taken by Bright field microscope with digital camera Sony DSC-W350. Anatomical characters of leaf and bark were studied as per procedure[12] and microphotographs at different magnifications were taken with an Image Analyser (Olympus-BX51TF, Japan). Leaf constants were examined according to the prescribed procedure [13,14].

Powder characteristics

Organoleptic analysis, microscopic examination, behavior of powder with different chemical reagents and fluorescence analysis (254nm, 356nm & visible light) of powdered leaf and bark were carried out based on standared procedures[1,15,16,17,18]. For fluorescent analysis, fine powder of this material was boiled with different solvents, according to their increasing polarity. The boiled powder with solvent was examined under short (254 nm), long (365 nm) UV light and visible light. The color produced during treatment with different chemicals was noted according to the color chart[19].

Physico- chemical analysis

Physico- chemical parameters like percentage of moisture content, total ash, acid- insoluble ash, water soluble ash and sulphated ash, foaming index of leaf and bark were analysed according to the standared methods[13,20-22]. Various extracts were prepared for the study of extractive values of leaf and bark[23].

Quantitative analysis

Quantitative analysis of crude fibre and mucilage content of leaf and bark powder were done according to the procedure[13, 24-28]

RESULTS

Morphology/Macroscopic features

Persea macrantha is a moderate to large sized evergreen tree (Fig.1) about 25-30m in height and 2-3 m in girth. Stem is extensively branched. Bark is externally brown in color and internally light reddish brown color. It occurs in curved or sometimes flat pieces

with size of 7-8x18-20 cm and thickness of about 1.5 – 3cm. Outer surface of bark has numerous lenticels. Large number of wrinkles and undulations are also seen around the bark (Fig.2&3), while inner surface shows presence of numerous striations.

Fig. 1: It shows habit of *P. macrantha*

Fig. 2&3: It shows bark of *P. macrantha*

Leaf is alternate, exstipulate, elliptical and lanceolate, acute or obtuse. Leaf margin is entire with unequal bases. Venation is finely reticulate and petiole is medium sized with glabrous surface. Upper surface is dark green and lower surface is light green in color. Length is about 15.33cm and breadth is about 6.79cm. Inflorescence is axillary cyme. Fruit is globose drupe of about 2cm diameter with dark green in color turns red when ripen.

Microscopy

Leaf

Transverse section of leaf (Fig.4) passing through the midrib represent concavity on upper side and a prominent protuberance on adaxial surface. Midrib shows 5-6 layers of collenchymas below the epidermis. It is also characterized by the presence of polygonal parenchymatous cells in the center. Vascular bundles are collateral and are encircled with pericycle layers. The pericycle composed of 4-6 layers of lignified and thick walled cells. It is covered with parenchymatous cells followed by presence of 2-3 layered collenchyma cells. Above lower epidemis, 2-3 layered collenchyma cells present which are followed by multilayered parenchyma cells.

The lamina of the leaf shows upper epidermis, mesophyll and lower epidermis. Upper epidermis is composed of flat single layer of rectangular cells. Mesophyll is differentiated into palisade tissue and spongy parenchyma. Palisade cells are single layered, elongated and compactly arranged while spongy parenchyma which is composed of polygonal cells irregularly arranged and fill the entire space of the lamina. Lower epidermis consists of single layer of rectangular cells, identical to the upper epidermis. Both layers of epidermis are covered with a thick cuticle.

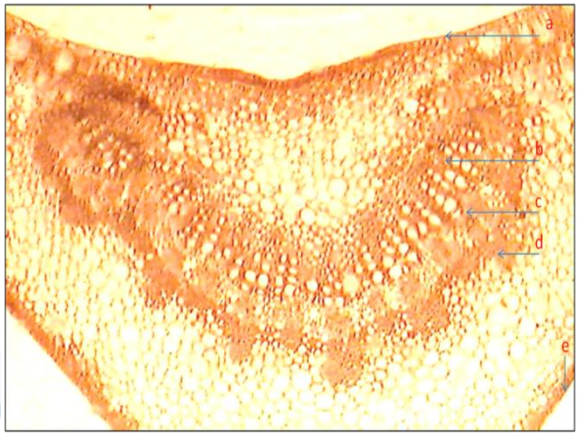


Fig. 4: It shows C.S of Leaf, a-Upper epidermis, b- Xylem, c-Phloem, d- Bundle cap, e- Lower epidermis

Vein-islet number is the average number of vein –islets per square millimeter of leaf surface which is determined as 18.2/ mm^2. Vein termination number is the number of veinlet termination per square mm of the leaf surface, midway between midrib of the leaf and its margin which is determined as 12.5/ mm^2.(Fig.5) .

Stomata are paracytic type (Fig.6) and are present only on lower epidermis. Stomatal frequency is 13-15/mm^2 and the stomatal index is 23% on the abaxial side. Leaf constants are tabulated in the table 1.

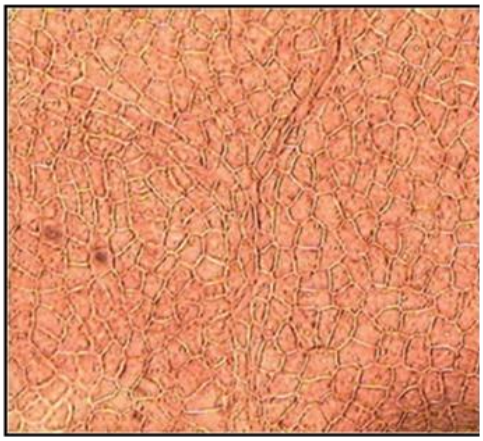


Fig. 5: It shows surface view of leaf with vein-islet and termination number

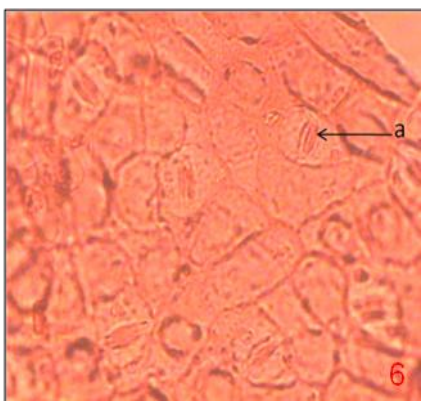


Fig. 6: It shows surface view of lower epidermis with paracytic stomata (a)

Table 1: It shows leaf constants of *Persea macrantha*

S. No.	Leaf constants	Value (Mean±Stdev)
1	Leaf	
	Length	15.33 ± 3.44
	Breadth	6.79 ± 1.46
2	Vein	
	Is-let number	18.2 ± 2.34
	Termination number	12.5 ± 0.97
3	Stomata	
	Frequency	13 ± 2

Stem

In transverse section of stem (Fig.7), epidermis is single layered with barrel shaped cells and possess thin cuticle striations. Outer and inner hypodermis is composed of 2-3 layers of collenchyma and chlorenchyma cells. Cortex is composed of parenchymatous tissue. Vascular bundles are conjoint, collateral, endarch and open. Bundles are arranged in the form of a brocken ring. In each bundle, phloem towards periphery and xylem towards the centre. Sclerenchymatous bundle cap is present. Below the phloem 2-3 layered cambium is observed. Xylem is pentagonal and protoxylem towards the centre and metaxylem towards the periphery. Pith is large, homogenous and composed of parenchymatous cells. Radially elongated parenchyma or medullary ray is present in between the bundles.

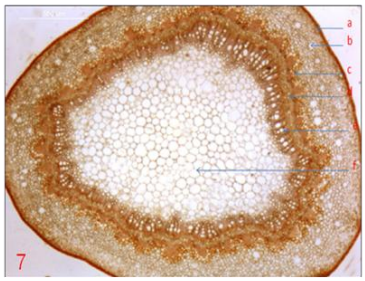


Fig. 7: It shows C.S of Bark a- Epidermis, b- Cortex c- Bundle cap, d- Phloem, e-Xylem, f- Pith

Powder analysis

Preliminary examination/ Organoleptic analysis of leaf and bark powder is given in the table 2 and results of powder analysis shows specific characteristic features (Figs.8-18). Behaviour of leaf and bark powder with different chemical reagents (Table 3) and the results of fluorescent analysis of leaf powder are shown separately (Table 3).

Table 2: It shows preliminary examination/ organoleptic analysis of leaf and bark powder

S. No.		Leaf	Bark
1	Color	Dark green	Brown
2	Odour	Aromatic, resembling to green mango leaves.	Characteristics.
3	Taste	Bitter	Bitter
4	Texture	Smooth	Smooth

Result of Powder characteristics

Leaf

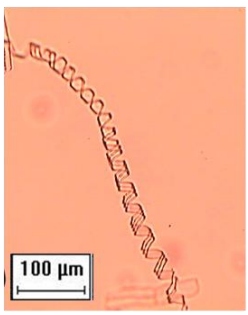


Fig. 8: It shows spiral thickening present in leaf powder

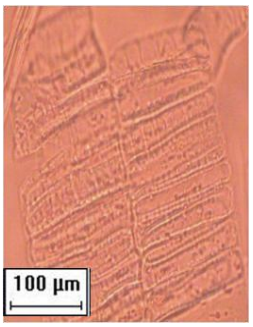


Fig.9: It shows tracheids present in leaf powder

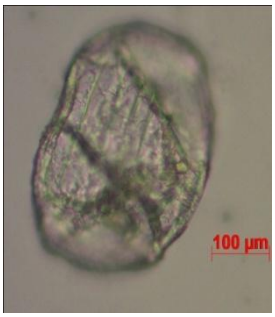


Fig. 10: It shows stone cell present in leaf powder

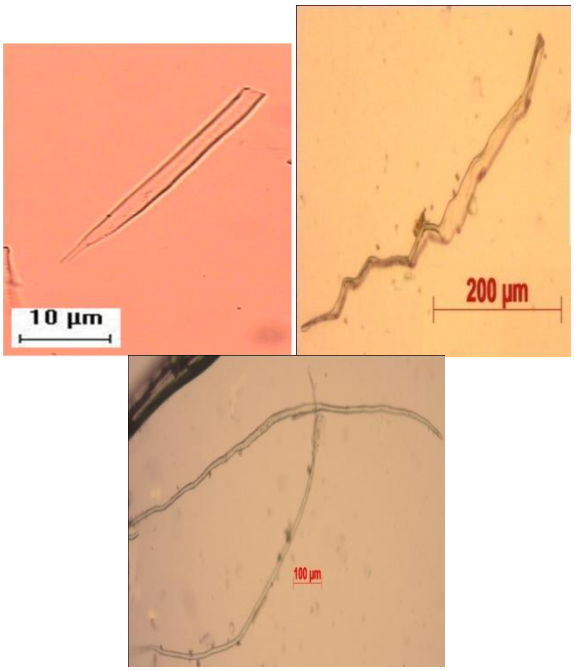


Fig. 11: 12 & 13: It shows fibres present in leaf powder

Bark

Fig. 14& 15: It shows stone cells present in bark powder

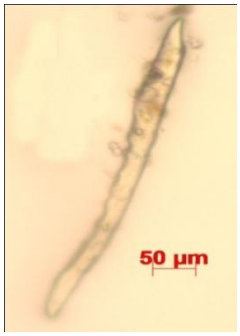


Fig. 16: It shows fibre present in bark powder

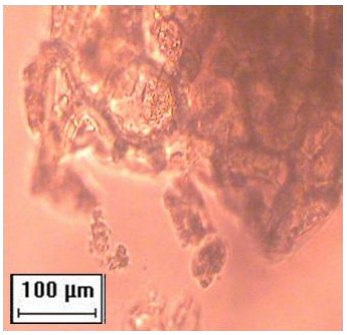


Fig. 17: It shows cork cells present bark powder

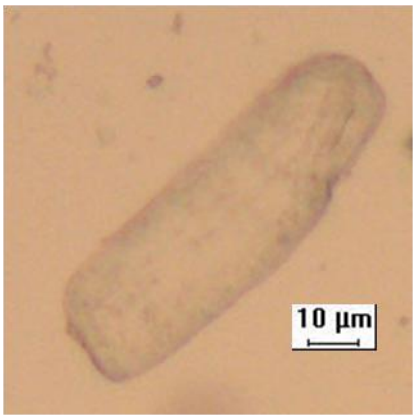


Fig. 18: It shows wood parenchyma cell present in bark powder

Table3: It shows behaviour of leaf and bark powder with different chemical reagent

S. No.	Reagent	Leaf (Color/Precipitate)	Bark (Color/Precipitate)
1	5% $FeCl_3$	Ivy green	Ivy green
2	NH_4 Solution	Granet brown	Oxblood red
3	Glacial acetic acid	Parsely green	Burnt orange
4	Conc. HNO_3	Cardinal red	Current red
5	50% HNO_3	Burnt orange	Burnt orange
6	Conc. HCl	Ivy green	Current red
7	50%HCl	Parsely green	Burnt orange
8	Conc. H_2SO_4	Black	Purple Madder
9	50% H_2SO_4	Parsely green	Brick red
10	50%NaOH	Oxblood red	Oxblood red
11	50%KOH	Oxblood red	Oxblood red
12	50%Mercuric Iodide	Oxblood red	Signal red
13	$HgCl_2$	Parsely green	Burnt orange

Table 4: It shows results of fluorescent analysis of leaf powder.

S. No.	Particulate of the treatment	Colour under Visible light	Colour under Short UV (254nm)	Colour under LongUV (365nm)
1	Powder as such	Lettuce green	Sage green	Ivy green
2	Powder +50% NaCl	Sage green	Parsely green	Ivy Green
3	Powder + Ab. Alcohol	Scheeles green	Leek green	Black
4	Powder + n-Hexane	Sage green	Spinach green	Black
5	Powder +n-Butanol	Fern green	Ivy green	Black
6	Powder +1N NaOH	Ivy green	Ivy green	Black

Table 5: It shows results of fluorescent analysis of bark powder

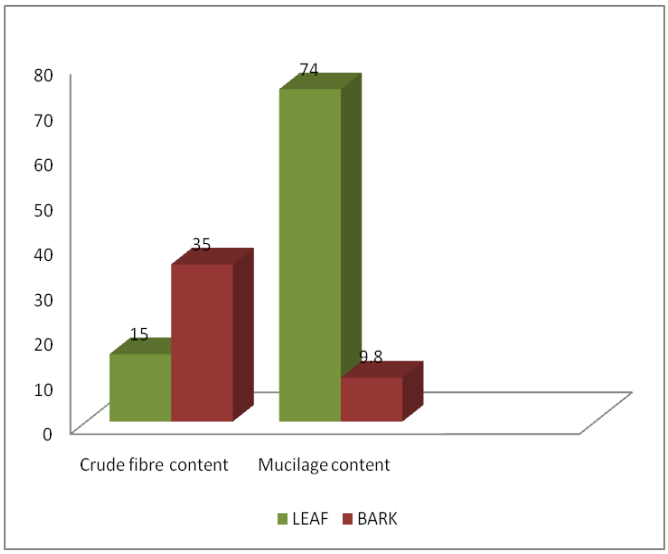
S. No.	Particulate of the treatment	Colour under Visible light	Colour under Short UV (254nm)	Colour under Long UV (365nm)
1	Powder as such	Brown	Dark brown	Blackish brown
2	Powder +50% NaCl	Brown	Greenish brown	Blackish brown
3	Powder + Ab. Alcohol	Brown	Greenish brown	Blackish brown
4	Powder + n-Hexane	Brown	Ivy green	Blackish brown
5	Powder +n-Butanol	Brown	Ivy green	Blackish brown
6	Powder +1N NaOH	Brown	Greenish brown	Blackish brown

Physico-chemical parameters

Extractive values mainly represent the percentage of organic constituents. These values are specific for each drug. Crude fibre is the residue of resistant tissues which can be obtained after giving treatment to the powder with dilute acid and alkali. Determination of the crude fibre is useful in distinguishing between similar drugs or in the detection of adulteration. It also helps to remove the more resistant parts of plant organs which can be used for microscopical examination. The process removes starch and other cell contents. It also destroys lignin present in the cell wall of lignified tissue. For *P.macrantha*, the percentage of crude fibre was found to be 15% and 35% respectively for leaf and bark. Result of physico-chemical characters are shown in the table 6 and graph1.

Table 6: It shows result of physico - chemical analysis of leaf and bark

S. No.	Parameters	Results(%w/w, Mean ± Stdev)	
		Leaf	Bark
1	Moisture content	9.26 ± 0.0499	10.83 ± 0.005
2	Ash values		
	Total ash	4.05 ± 0.001	1.35 ± 0.001
	Acid insoluble ash	0.49 ± 0.0001	0.004 ± 0.0001
	Water soluble ash	1.63 ± 0.0001	0.84 ± 0.0001
	Sulphated ash	8.28 ± 0.020	1.84 ± 0.0101
3	Foaming index	< 100	250
4	Extractive values		
	Petroleum ether extractive	8 ± 0.02	2 ± 0
	Chloroform extractive	10 ± 0.2	3.2 ± 0.011
	Methanol extractive	26.6 ± 0.011	14.6 ± 0.011
	Distilled water extractive	7.2 ± 0.030	14 ± 0



Graph 1: It shows quantitative analysis of leaf and bark

DISCUSSION

New scientific strategies are required for the evaluation of natural products with specific biological activities which requires large screening process. The macroscopic studies of *Persea macrantha* revealed a number of distinguishing morphological characters helpful for the identification are fibrous fracture, numerous lenticels, large number of wrinkles and undulations in th e bark. Leaf is characterized by its smell which is similar to the mango leaves and also the leaf is alternate, exstipulate and finely reticulate. Inflorescence is axillary cyme. Fruit is globose drupe. The microscopic studies were also found to be helpful in providing certain distinguishing features like single layered palisade cells and vascular bundles encircled by pericyclic layers in leaf and presence of pentagonal xylem, radially elongated parenchyma, large pith and 'U' shaped sclereids. The stomatal type is identified to be paracytic type, which is present only on abaxial side. The macro and micro morphological features of the leaf and bark described are distinguishes it from other plants. Organoleptic analysis of crude drug powder helps to the identification of the powder from the other adulterant powder because taste, texture, odor and color are specific to each plant(28). Behaviour of powder with different chemical reagents establishes certain botanical and chemical standards which would help in identification as well as in checking adulteration and it also greatly helps in quality assurance of finished products of herbal drugs[29]. Fluorescent analysis of the leaf and bark powder showed various colors in visible, short and long UV. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Some constituents show fluorescence in the visible range but many natural products which do not visibly flourescent in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation[7].

The physical constant evaluation of this drug is an important parameter in detecting adulteration or improper handling of drug. Physico-chemical parameters of the powdered drug such as ash values, moisture content and extractive values are serving as a standard to determine the quality of the plant[29, 30]. Determination of ash value gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Deterioration time of the plant material depends upon the

amount of water present in plant material. If the water content is high, the plant can be easily deteriorated due to contamination by fungal colonies. The total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. Extractive values were also determined which are primarily useful for the determination of exhausted or adulterated drugs. Thus, the leaves and bark of *Persea macrantha* may serve as a potential source of bioactive compounds having various medicinal values.

CONCLUSION

In the present study, the plant was subjected to pharmacognostic evaluation. Pharmacognostical study is the preliminary step in the standardization of crude drugs. The detailed pharmacognostical evaluation gives valuable information regarding the morphology, microscopical and physico - chemical characteristics of the crude drugs. It also gives the scientific information regarding the purity and quality of plant drug. During this investigation, various standardized parameters such as macroscopic, microscopic, powder sharacteristics, physico-chemical and quantitative analysis were carried out and which could be helpful in proper identification of ***Persea macrantha*** and ensure the purity of samples.

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