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Pharmacognostical studies of leaves of *Machilus macrantha* Nees.

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ABSTRACT

The paper presents the pharmacognostical studies of leaves of *Machilus macrantha* Nees, a traditional medicinal plant of which organoleptic, microscopic and physicochemical properties have been studied. The present study will provide the information in respect of its identification.

Keywords: Fluorescence, *Machilus macrantha* Nees, microchemical, phytochemical.

INTRODUCTION

Several members of the genus *Machilus* are being used traditionally for wide variety of ethnopharmacological properties. *Machilus macrantha* is a commercial source of industrially important gum (JIGAT), used in the preparation of incense-sticks in South India.

The plant of *Machilus macrantha* (Lauraceae) commonly known as Gulmavu, is 25-30m in height and 2-3m in girth (1). It is reported in Indian states i.e. Bihar, Karnataka and Maharashtra, upto an altitude of 2100m. The leaves are used externally to treat ulcers (2).

MATERIALS AND METHODS

The fresh leaves of *Machilus macrantha* were collected in the month of April (2004) from Lonavala, Dist. Pune (MS), India. These were identified, confirmed and authenticated by the scientists of *Botanical Survey of India*, Pune, India. A voucher specimen of the leaf itself is deposited in department for future reference.

Collected fresh leaves were washed and used for study of organoleptic and microscopic characteristics. The powder of dried leaves were used for the determination ash values, extractive values and phytochemical investigations. All chemicals and reagents used for testing were analytical grade obtained from SD Fine Chemicals and Loba Chemicals, Mumbai (India).

Microscopy

Fresh leaves of *Machilus macrantha* were selected for the microscopical studies. Microscopic sections were cut on a microtome and by free hand sectioning. Numerous temporary and permanent mounts of the microscopical sections of the leaf specimen were made and examined microscopically. Histochemical reactions were applied with hydrochloric acid-phloroglucinol to reveal lignified elements, iodine-iodide for starch, Sudan IV for lipophilic substances, Dragendorff's reagent for alkaloidal substances, ruthenium red for mucilage and ferric chloride for phenolic compounds (3).

Photomicrographs of the microscopical sections were taken with the help of MOTIC photomicroscope provided with MOTIC IMAGES PLUS 2.0 software.

Powder characteristics

Preliminary examination, behavior of powder with different chemical reagents

and microscopical examination was carried out (4,5).

Leaf constants

The leaf constants for *Machilus macrantha* Nees. were determined by standard methods (6).

Micrometry

The measurements of different cells and cell contents were done with the help of calibrated ocular micrometer and MOTIC IMAGES PLUS 2.0 software.

Physico-chemical parameters

Percentage of total ash, acid-insoluble ash, water-soluble ash and sulphated ash were calculated as per the Indian Pharmacopoeia (7). The total ash of the powdered bark was tested for different inorganic constituents (8). Different extracts of the leaves were prepared for the study of extractive values (6). Fluorescence analysis of powdered bark was carried out by standard methods (9, 10).

Preliminary phytochemical analysis -

For the preliminary phytochemical analysis, 5 g powdered drug was extracted with petroleum ether

(60-80), chloroform, methanol and water successively. The extracts were dried and weighed. The presence or absence of different phytoconstituents viz. triterpenoids, steroids, alkaloids, sugars, tannins, glycosides and flavanoids, etc. were detected by usual prescribed methods (11, 12).

RESULTS

Organoleptic characters

Colour : Greenish on outer side and grayish underneath.

Size and shape : 9.0-20 X 3.5-8.0cm, elliptical and lanceolate. Apex of the leaf is variable, normally it is obtuse or acuminate at-times.

Taste : Mucilagenous.

Odour : Slightly aromatic, resembling to green mango leaves.

Touch : Smooth.

Extra features : The leaf shows entire margin, unequal bases, finely reticulate venation and medium sized petiole. (Fig.1)

Microscopy

a] Transverse section of leaf (Fig.2)

It is a dorsiventral leaf. Following tissues are present in midrib and lamina:

Midrib (Fig 2a):

Section passing through midrib represents concavity on upper side and a prominent protuberance on abaxial surface. Midrib shows 5-6 layers of collenchyma below the upper epidermis. It is also characterised by presence of polygonal parenchymatous cells in the center. It shows collateral type of vascular bundles. Distinct phloem tissue can be seen on the ventral surface and well developed xylem tissue towards the dorsal surface of the midrib.

Xylem shows presence of tracheids, xylem parenchyma, protoxylem, and metaxylem towards lower periphery. Tracheids are tubular and elongated, while xylem vessels are reticulate. Thick walled nonlignified phloem follows the xylem. Phloem parenchyma is present in the form of a broad patch with scattered phloem fibres. The vascular bundle is encircled with pericyclic layer. The pericyclic layer is composed of 4-6 layers of lignified, thick walled cells. Pericycle is covered with parenchymatous cells followed by presence of 2-3 layered collenchyma above lower epidermis. Leaf shows presence of cluster of calcium oxalate crystals and starch grains. It also exhibits presence of secretory cavities.

Lamina (Fig 2b)

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 lamina. Lower epidermis consists of single layer of rectangular cells, identical to upper epidermis. Both layers of epidermis are covered with a thick cuticle. Stomata are present only on lower epidermis. Results of various histochemical reactions are given in Table 1. Different leaf constants and micrometric analysis are tabulated in Table 2 and 3.

b] Powder characteristics (Fig.3)

Preliminary examination of powder

Colour : Brown.

Odour : Characteristic.

Taste : Bitter.

Texture : Smooth.

After addition of small quantity of water, a mucilaginous mass was formed which indicates presence of considerable amount of mucilage. After pressing a little amount of powder between filter paper, no greasy stain was found, indicating absence of fatty oils. After shaking the powder with water in a test tube, no persistent foam was formed indicating absence of saponins. Behavior of powder with different chemical reagents is shown in Table 2.

Microscopical examination of powder

Trichomes : Nonglandular, multicellular, uniseriate, 156-170 μ in length and rarely observed (Fig.2a)

Fibres : Well developed, sclerenchymatous fibres from vascular bundles (Fig.2b).

Secretory glands : Large secretory glands entire or fragmented (Fig.2c).

Epidermal cells : Polygonal with well developed paracytic stomata (Fig.2d).

Mesophyll : Fragments of leaf showing spongy parenchyma cells.

Calcium oxalate crystals: Both prismatic and cluster crystals.

Starch grains : Simple.

Physico-chemical parameters

The percentage of total ash, acid-insoluble ash, water-soluble ash, sulphated ash and different extractives are tabulated in Table 4 and 5. The qualitative analysis of ash indicated presence of calcium, aluminium, potassium, chlorides and sulphates.

Table 1. Histochemical colour reactions.

Reagent	Constituent	Colour	Histological zone
Phloroglucinol+ hydrochloric acid	Lignin	Pink	Vascular bundles
Aniline sulphate + sulphuric acid	Lignin	Yellow	Vascular bundles
Weak Iodine solution	Starch	Blue	Vascular bundles, lamina
Sudan III Solution	Oil globules	Pink	Vascular bundles
Aqs. Ferric chloride	Tannins	Black	Lamina
Dragendorff's reagent	Alkaloids	Light orange	Lamina
Libermann-Burchardt reagent	Steroids	Greenish	Lamina
Millon's reagent	Proteins	-	-

Table 2. Leaf constants for M. macrantha.

Leaf constants	Value
Stomatal number	Lower surface : 450-500
	Upper surface : Nil
Stomatal index	Lower surface : 15.62
	Upper surface : Nil
Vein –islet number	05-07
Vein-termination number	03-04

Table 3. Measurements of cells.

Type of cell	Size in micron (μ)
Upper epidermis	2.4 X 7.2
Collenchyma	7.2-14.2
Palisade cells	14.2 X 43.2
Parenchyma	19.7-33.6
Xylem vessels	21.6-38.4
Xylem parenchyma	9.1-9.8
Xylem fibres	14.2-14.5
Phloem parenchyma	7.0-7.3
Phloem fibres	7.2-7.5
Pericycle	12.0-24.0
Calcium oxalate crystals	7.2-14.4

Table 3. Behavior of leaf powder of M. macrantha with different chemical reagents.

Reagent	Colour / ppt.	Constituent
Conc.sulphuric acid	Reddish	Steroids present.
Aqueous Ferric chloride solution	Blackish	Tannins present.
Iodine solution	Blue	Starch present.
Picric acid solution	Yellowish	Alkaloids present.
Aqueous Mercuric chloride solution	Brownish	Alkaloids present.
Magnesium- hydrochloric acid	No change	Flavonoids absent.
Aqueous Silver nitrate solution	No ppt.	Proteins absent.
Ammonia solution	No change	Anthraquinone glycosides absent.
Aqueous Potassium hydroxide solution (5%)	No change	Anthraquinone glycosides absent.

Table 4. Ash values of M. macrantha leaf.

Types of ash values	% w/w (Mean [a]± SEM)
Total ash	6.04 ± 1.69
Acid insoluble ash	0.69 ± 0.06
Water soluble ash	2.52 ± 0.24
Sulphated ash	11.76 ± 0.79

[a] *Mean value of three readings.*

Table 5. Extractive values with different solvents.

Type of solvent	% Extractability (Mean [a]± SEM)
Petroleum ether (40-0)	2.43 ± 0.21
Benzene	3.05 ± 0.13
Chloroform	4.03 ± 0.18
Ethyl acetate	3.13 ± 0.14
Ethanol	12.02 ± 0.40
Methanol	14.03 ± 0.21
Water	19.20 ± 0.39

[a] *Mean value of three readings.*

Table 6.Consistency, colour & fluorescence analysis of different extracts of M.macrantha

| Extract	Consistency	Color in		
		Daylight	Short UV	Long UV
Petroleum ether	Sticky mass	Yellow	Green	Dark orange
Benzene	Sticky	Pale brown	Greenish brown	Dark brown
Chloroform	Sticky mass	Pale brown	Greenish brown	Dark brown
Ethyl acetate	Semisolid	Black	Greenish black	Dark brown
Ethanol	Solid	Greenish brown	Greenish black	Dark brown
Methanol	Solid	Greenish brown	Greenish black	Dark brown
Water	Semisolid	Reddish brown	Greenish black	Dark brown

Table 7. Fluorescence analysis of powdered leaves of M.macrantha

| Sample	Color in Daylight	Color in Short UV	Color in Long UV
Powder	Light green	Light green	Light green
Powder+Sodium hydroxide in methanol	Dark green	Green	Orange
Powder+Sodium hydroxide in water	Dark green	Dark green	Dark green
Powder+1N hydrochloric acid	Dark green	Dark green	Violet
Powder+50%nitric acid	Brown	Green	Blue
Powder+50%sulphuric acid	Dark green	Green	Green
Powder + nitrocellulose	Green	Green	Purple
Powder+Methanolic sodium hydroxide + nitrocellulose.	Light green	Green	Yellowish orange

Table 8. Qualitative phytochemical analysis of various extracts of leaves of M.macrantha

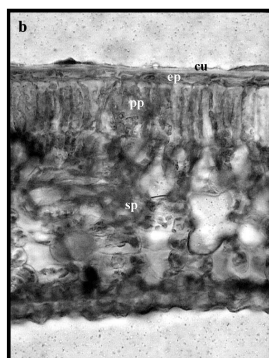| Type of constituent	Petroleum Ether	Benzene	Chloroform	Ethyl acetate	Ethanol	Methanol	Water
Steroids	+	+	+	+	+	+	+
Carbohydrates	-	-	-	-	-	-	+
Alkaloid	-	-	+	+	+	+	+
Glycosides	-	-	-	-	+	+	+
Reducing sugars	-	-	-	-	-	-	+
Flavonoids		-	-	-	-	-	-
Tannins and phenolics	-	-	-	+	+	+	+
	-	-	-	-	-	-	-
Proteins	-	-	-	-	-	-	-
Amino acids	-	-	-	-	-	-	-

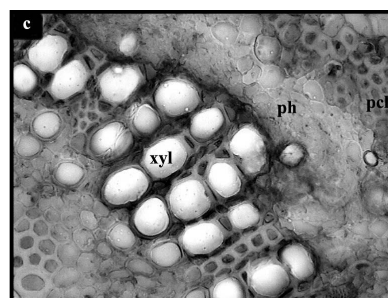*Note: (+) Indicates present; (-) Indicates absent.*
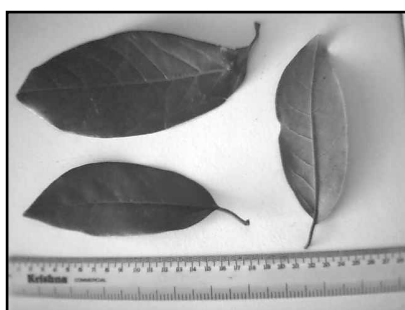
Fig.1. Leaves of M. macrantha
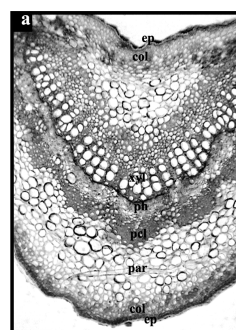
Fig.2. Leaf structure of M. macrantha.
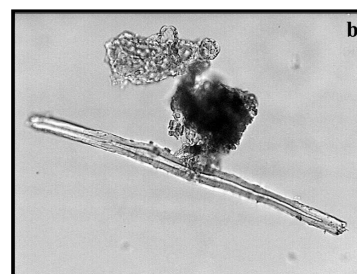***a) transverse section through midrib***

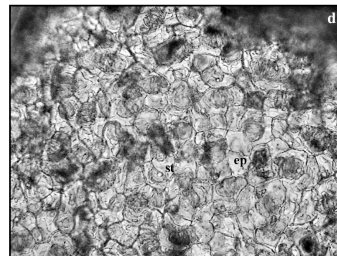*b) Transverse section through lamina*

c) Details of vascular bundle.

Fig.3. Powder characteristics of M. macrantha leaves a)
trichome

b) Fibre

c) Secretory cells

d) Epidermis with stomata

Abbreviations- epi: epidermis; col: collenchyma; xyl: xylem; ph: phloem; pcl: pericycle; par: parenchyma; sp: spongy parenchyma; cu: cuticle; pp: palisade parenchyma

Fluorescence analysis of the powdered bark or extract does not indicated presence of any fluorescent compound (Tables 6 and 7).

Preliminary phytochemical analysis

Preliminary phytochemical screening indicated presence of alkaloids in chloroform, methanol and aqueous extracts while petroleum ether extract showed presence of steroidal moiety. Aqueous extract also gave positive tests for carbohydrates. Methanol and aqueous extract indicated presence of tannins (Table 8).

Discussion

In the last two decades of the century the scientists are sincerely trying to evaluate many plant drugs used in traditional system of medicine. The pharmacognostical study is one of the major criteria for identification of plant drugs. The present study on pharmacognostical characteristics of *Machilus macrantha* leaf will provide useful information for its correct identity. The leaves are greenish with a smooth texture and possess odour similar to green mango leaves. The entire margin, unequal bases, dorsiventral arrangement, single layered palisade cells and vascular bundles encircled by pericyclic layer are few of the important characteristics of the leaves.

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