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Original Article

Pharmacognostical Standardization of the Leaves of *Machilus macrantha* Nees.

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Abstract

Machilus macrantha Nees. (Lauraceae) (MM) is commonly known as Gulmavu, Golam, Pisara, Kurma and Chittutandrimara in India. Ethnomedicinally, the plant is used in the treatment of asthma, rheumatism, tuberculosis and as consumption. The present study is focused on macroscopical, microscopical and physic-chemical standards and preliminary phytochemical nature of the leaves of plant. These observations which would be of useful to identify and establish the authenticity of the plant, *Machilus macrantha* Nees.

Keywords: *Machilus macrantha* Nees. Lauraceae, Microscopical, Phytochemical.

1. Introduction

Man has been using herbs and plant products for combating diseases since ancient time. The Indian subcontinent is enriched by a variety of fauna and flora of aromatic and medicinal plants. This extensive flora has been greatly utilized as a source of many drugs in the Indian traditional system of medicine. The World Health Organization is now actively encouraging developed and developing countries to use herbal medicine which they have been traditionally used for centuries[1]. *Machilus macrantha* Nees. (MM) is a heighted shade bearer tree growing up to 27-30 m tall. It is commonly recognized as Golam, Pisara, Kurma (Marathi), Gulmavu, Chittutandrimara (Kannada), Kollarmavu, Anaikkuru (Tamil) and Uravu (Malayalam) in India[2]. It is distributed in Western Peninsula, Ceylon and India. In India, mainly found in Bihar, Assam and Western Ghats of Karnataka, Tamilnadu and Maharashtra up to an altitude of 2100 m. Traditionally, the bark of the plant is used in the treatment of asthma, rheumatism, tuberculosis and consumption[3] while leaves are used externally on ulcer[4]. Pharmacognostic study of leaves[5], barks[6] and roots[7] have been reported earlier. Phytochemical studies reported the presence of in roots[8] and lignans[9] in leaves. To ensure reproducible quality of herbal medicines, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication followed by generating numerical values of standards for comparison. Pharmacognostical parameters for easy identification like organoleptic features, microscopy, leaf constants & physico-chemical analyses are few of the basic tools for standardization of herbals[10]. Hence, in the present work the pharmacognostical evaluation of leaves of *Machilus macrantha* has been performed.

2. Materials and Methods

2.1. Plant material

The leaves of the plant *Machilus macrantha* Nees. (Lauraceae) was collected from the Ray wood park of Lonavala, Dist.- Pune, Maharashtra, India. It was identified and authenticated by Dr. P.G. Diwakar, Joint Director, Botanical Survey of India, Pune (Voucher Specimen No. Santosh-1). The shade dried leaves were further size reduced and stored until further use in an air tight container. Fresh plant material was obtained for the microscopical evaluation.

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2.2. Chemicals and instruments

Motic microscope with software 2 plus and all other common glasswares. All the chemicals and reagents used were of analytical grade.

2.3. Macroscopic and microscopic analysis

The fresh leaves of *Machilus macrantha* Nees. were used for macroscopical and microscopical study. For macroscopic analysis, different organoleptic features such as colour, odour, taste, size, shape, apex, surface, base, margin, and venation were observed[11,12]. For the microscopic analysis, the studies of transverse sections and powder characteristics were carried out[13].

2.3.1. Leaf constants

In this, leaf surface preparations were prepared separately by leaves boiled with saturated chloral hydrate solutions and then observed under microscope for presence and quantification of epidermal cells, stomata, vein islet number, and vein-let termination number. Stomatal index was calculated as per standard method[14].

2.3.2. Physico-chemical constant

Physico-chemical standards such as the percentage of total ash, acid-insoluble ash, water-soluble ash, sulphated ash, water and alcohol soluble extractives, crude fiber content and foreign matter were calculated as per the Indian Pharmacopoeia [15,16]. Fluorescence analysis of the various extracts was carried out as per standard method [17].

2.3.3. Preparation of extracts

The extracts of leaves of *Machilus macrantha* were prepared by successive extraction by soxhlation with various solvents of increasing polarity. The shade dried leaf powder was packed in thimble kept in the soxhlet apparatus and extraction was allowed to run successively using the solvents, petroleum ether (40-60°C), chloroform and ethanol. Finally, the marc was dried and macerated with chloroform-water for 24 hours to obtain the aqueous extract. Each extract was concentrated by rotary vacuum evaporator and finally on the water-bath and the obtained extracts were weighed. The physical characteristics and percentage yield of various extracts were tabulated[18].

2.3.4. Preliminary phytochemical analysis

In this, all the extracts were subjected to preliminary phytochemical screening for the detection of various chemical constituents. The presence or absence of different phytoconstituents viz. triterpenoid, steroids, alkaloids, carbohydrates, tannins, glycosides and flavonoids, etc. were detected by usual prescribed methods[19].

Results and Discussion

Macroscopic analysis of leaves

Morphological characters were determined on the basis of sensory profile of leaf of MM. The details are given in Table 1.

Table 1: Morphological characters.

Sr. No.	Particular	Observations
1	Colour	Green
2	Odour	Characteristic
3	Taste	Bitter
4	Size	10-22 cm length,
5	Shape	Elliptical and lanceolate
6	Margin	Entire
7	Apex	Acute
8	Base	Asymmetrical
9	Surface	Smooth
10	Vein	Reticulate
11	Petioles	2.5-3 mm, Pubescent

Microscopic analysis

Transverse section (T.S.) of leaf

It is obtained by free hand sectioning, in which blade passed through midrib and lamina of leaf. T.S. of the leaf showed dorsiventral nature. It has showed three regions viz. epidermis, mesophyll & vascular bundles. Epidermis was found on either side of the leaf i.e. Upper epidermis and Lower epidermis. Below upper epidermis and above lower epidermis, few layers of collenchymatous cells were observed. Upper epidermis was single layered rectangular epidermal cells having glandular and non-glandular trichomes. Mesophyll was differentiated into palisade and spongy parenchyma. Palisade cells were elongated arranged compactly in single layer

and were discontinued over midrib. Parenchyma consists of loosely arranged 4-5 layers of parenchymatous cells. Lower epidermis was single layered with rectangular cells. Vascular bundles were found to be collateral type; these were surrounded by sclerenchymatous bundle sheath. It consist of dorsal xylem and ventral phloem tissues. Lower portion consists of ground tissue It shows presence of group of calcium oxalate crystals (Fig.1 and 2).

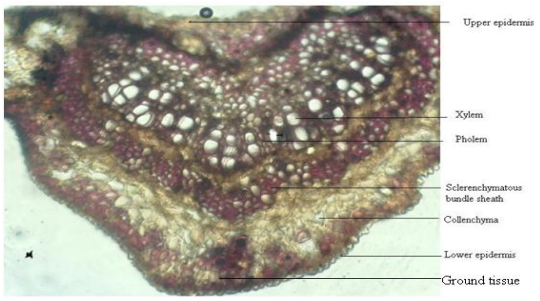

Fig.1. T.S. of leaf of *Machilus macrantha* passing through midrib.

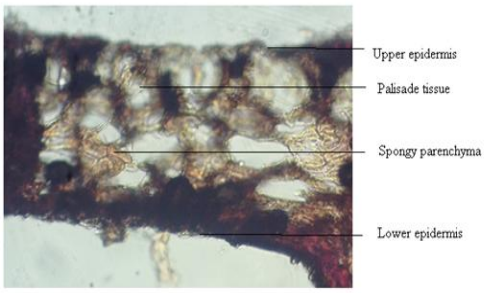

Fig.2. T.S. of *Machilus macrantha* passing through lamina.

Powder characteristic study

In this study, xylem vessels were found to be lignified, pitted walls and some of these having spiral arrangements. Cork cells were found to be thin walled & polygonally arranged. Xylem and phloem parenchymatic cells, spongy parenchyma, simple covering uniseriate multicellular, non-glandular trichomes and anomocytic stomata were observed. Cluster of calcium oxalate crystals were observed. Starch grains present were circular to oval in shape (Fig. 3).

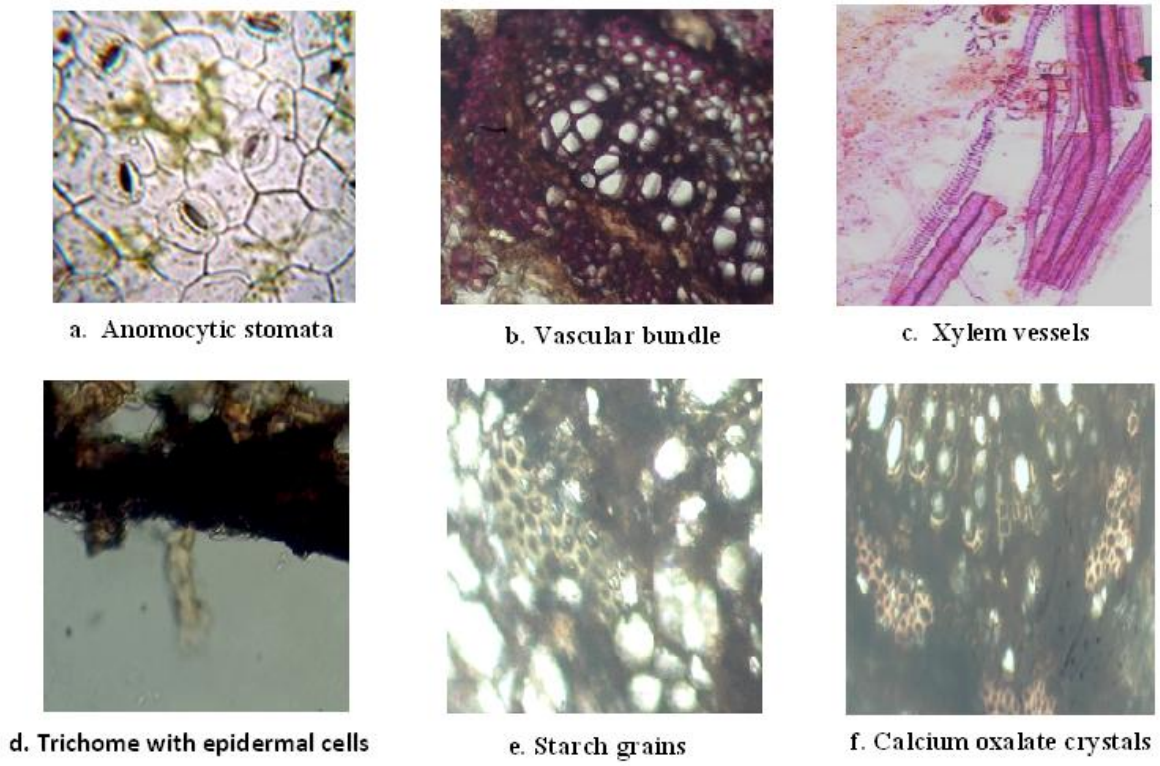

Fig. 3. Powder microscopy.

Leaf constants

It is determined as per standard method. It is useful for the identification and authentication of leafy crude drugs. Result is shown in Table 2.

Table 2: Leaf constants.

Leaf constants	Value
1. Stomatal number	
a. Upper surface	8-10
b. Lower surface	6-8
2. Stomatal index	
a. Upper surface	17.0-23.24
b. Lower surface	13.6-17.7
3.Vein islet number	8-13 (Avg. 10.5)
4. Vein termination number	10-16 (Avg. 13)
5. Palisade ratio	6-8

Preliminary Phytochemical Screening

After extraction with different solvents, the residues were dried and measured. The residue obtained was 3.0 %, 2.6%, 12.88 % and 13.72 %w/w for petroleum ether, chloroform, and methanol and water extract of leaves of MM, respectively. The yellowish green, greenish black, brownish black and brownish black residues were for petroleum ether, chloroform, ethanol and water extract of MM, respectively. The petroleum ether and chloroform extracts were sticky or greasy in nature. The ethanol and water extracts were amorphous in nature respectively. The preliminary phytochemical investigation of the petroleum ether, chloroform, methanol and water extracts of leaves of MM showed the presence of terpenoids, alkaloids, tannins, flavonoids etc (Table 3).

Table 3: Preliminary phytochemical screening.

Type of constituents	Petroleum ether (40-60^{O}C)	Chloroform	Methanol	Water
1. Alkaloids				
a.Dragendroff's reagent	--	+	+	--
b. Hager's reagent	--	+	+	--
c. Mayer's reagent	--	+	+	--
d. Wagner's reagent	--	+	+	--
2. Glycosides				
a. Killer-Killiani test	--	--	--	--
b. Legal's test	--	--	--	--
c. Borntrager test	--	--	--	--
3. Sterols				
a.Liebermann-Burchard's test	+	+	+	--
b.Salkowski test	+	+	+	--
3. Saponins				
a. Foam test	--	--	--	--
b. Haemolysis test	--	--	--	--
4. Flavonoids				
a.Shinoda test	--	--	+	+
5. Tannins				
a. Ferric chloride test	--	--	+	+
b. Lead acetate test	--	--	+	+
6. Carbohydrate				
a. Molisch test	--	--	+	+
7. Proteins and Amino acids				
Biuret test	--	--	--	--
Ninhydrin test	--	--	--	--

Where, (+ Positive test, -- Negative test).

Physico-chemical analysis

Air dried powdered leaves was used for quantitative determination of physico-chemical standards. Total ash, acid insoluble ash, water soluble ash, sulphated ash, water soluble and alcohol soluble extractive as well as moisture content, foreign organic matter and crude fibre content were determined as per WHO recommendations. Water soluble extractive value was found to be very high when compared to other extractable matter in the drug (Table 4). The fluorescence analysis is useful for the determination of chemical nature of phytoconstituents. Thus the fluorescence analysis of the extracts was carried out and data is shown in the Table 5. As a part of standardization study, the macroscopical and microscopical examinations of *Machilus macrantha* were done. Macroscopical evaluation is a technique of qualitative assessment based on the study of organoleptic features of the drug.

Microscopical study of leaf helps to know the internal arrangement of the tissues. It has shown three regions of tissues viz. epidermis, mesophyll and vascular bundles. Vascular bundle covered by lignified sclerenchymatous bundle sheath. Presence of cluster of calcium oxalate crystals and oval shaped starch grains are diagnostic tool of this leaf. Physico-chemical properties like extractive value, ash value, moisture content and fluorescent analysis of extracts have been carried out. The results showed greater extractive values in hot extraction, indicating the effect of elevated temperature on extraction. The percent extractives in different solvents indicate the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent. The fluorescence analysis of the extract in various solvents was performed under normal and UV light to detect the fluorescent constituents.

Table 4: Physico-chemical properties.

Sr. No.	Parameters	Result (% w/ w)
1	Total ash value	5.8
2	Acid insoluble ash value	0.72
3	Water soluble ash value	2.68
4	Sulphated ash value	10.12
5	Water soluble extractive value	13.7
6	Alcohol soluble extractive value	12.8
7	Moisture content	8.8
8	Foreign organic matter	3.8
9	Crude fibre content	36

Table 5: Fluorescence analysis of different extracts of leaf of *Machilus macrantha.*

Extract	Consistency	Daylight	Short UV (254 nm)	Long UV (366 nm)
Petroleum ether	Sticky mass	Yellowish green	Green	Brownish
Chloroform	Semisolid	Greenish black	Greenish brown	Dark brown
Methanol	Solid	Brownish black	Greenish black	Reddish brown
Water	Solid	Brownish black	Greenish black	Dark brownish

Conclusion

Pharmacognostical studies of leaves of *Machilus macrantha* could be useful for its identity and authenticity. The information produced in the present investigation is also helpful in the preparation of its herbal monograph in official herbal pharmacopoeias.

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