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Pharmacognostical and Physicochemical Evaluation of Leaves of *Machilus macrantha Nees (Lauraceae)*.

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Abstract
Machilus macrantha (Lauraceae), is locally known as 'Gulmau' to have many uses in ethnomedicine. Establishment of pharmacognostic profile of the leaves will assist in standardization and identification of samples. The present study deals with pharmacognostic examination of morphological and microscopical characters of leaves of *Machilus macrantha* Nees. including leaf constant, ash values, extractive values, fluorescence analysis and phytochemical screening of the extracts revealed that the plant contains steroids, alkaloids, carbohydrates and phenolic compounds.

Key Words
Machilus macrantha Nees., pharmacognostical study, phytochemical screening.

Introduction
Pharmacognostical study is the preliminary step in the standardization of crude drugs. The detailed pharmacognostical evaluation gives valuable information regarding the morphology, microscopical and physical characteristics of the crude drugs. Pharmacognostic studies have been done on many important drugs, and the resulting observations have been incorporated in various pharmacopoeias[1]. There are a number of crude drugs where the plant source has not yet been scientifically identified. Hence pharmacognostic study gives the scientific information regarding the purity and quality of the plant drugs[2]. The genus *Persea* belongs to the family Lauraceae, comprising 200 species of trees, mostly distributed in the tropics of Asia and America. *Persea macrantha* (Nees) Kosterm. Other [Synonym: *Machilus macrantha* Nees] is evergreen tall tree grow up to 25-30 m. with 2-3 m. girth. It is known as 'Kulur maavu' or 'Gulmavu' in Kannada and 'Gulumb'in Marathi language in India[3]. It is distributed in the Peninsular regions of India and in Sri Lanka. Few decades ago, it was frequently noticed in several locations of the semi-evergreen and evergreen forests, in the Western Ghats of the Indian peninsula up to an altitude of 2100 m. The leaves are used externally to treat ulcer[4]. The pharmacognostical studies of leaves of this plant have not been reported.

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Therefore the present investigation was planned to study the pharmacognostical aspects of *Machilus macrantha* Nees leaf.

Materials and Methods
Plant material and its Identification
The fresh leaves of *Machilus macrantha* Nees. were collected in rainy season from Lonavala, Pune district, Maharashtra State, India. These were identified, confirmed and authenticated by the taxonomist of Botanical Survey of India, Pune, India. A voucher specimen of plant (Voucher no. 37) is deposited itself in Herbarium for future reference. Collected fresh leaves were washed and used for study of macroscopic and microscopic characteristics. The powder of dried leaves was used for the determination of ash values, extractive values and phytochemical investigations. All chemicals and reagents used for testing were analytical grade.

Macroscopy
The following macroscopic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste[5,6].

Microscopy
Fresh leaves of *Machilus macrantha* Nees. selected for the microscopical studies. Microscopic sections were cut 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 phloroglucinol:HCL

(1:1) to reveal lignified elements, Weak Iodine solution for starch, Sudan III for lipophilic substances, Dragendorff's reagent for alkaloidal substances, ruthenium red for mucilage and ferric chloride for phenolic compounds[7].

Powder Characteristics

Preliminary examination, behaviour of powder with different chemical reagents and microscopical examination was carried out[8,9].

Leaf Constants

The leaf constants for *Machilus macrantha* Nees. were determined by standard methods[6].

Physico-chemical Parameters

Percentage of ash values including total ash, acid-insoluble ash, water soluble ash, sulphated ash , extractive values, moisture content, foreign matter, crude fiber content were calculated as per the Indian Pharmacopoeia[9,10]. Fluorescence analysis of powdered leaves was carried out by standard methods[11,12].

Preliminary Phytochemical Analysis

For the preliminary phytochemical analysis, 10 g powdered drug was extracted with petroleum ether (60-80), benzene, chloroform, methanol and water successively. The extracts were dried and weighed. The presence or absence of different phytoconstituents viz. terpenoids, steroids, alkaloids, sugars, tannins, glycosides, flavonoids, phenolic compounds etc. were detected by usual prescribed methods[13-15].

Chromatographic Finger Printing

10g of powdered material was defatted with petroleum ether and then extracted with methanol (50 ml x 3) on a water bath at 600C for 30 min, concentrated. A stock solution (10 mg/ml) was prepared in methanol. Suitably diluted stock solutions were spotted on precoated silica gel G60 F254 TLC plates (Merck) with the help of CAMAG Linomat V applicator. Plates were developed in solvent systems of different polarities to resolve polar and non polar components of the extract. The developed plates were scanned using TLC Scanner 3 (CAMAG). The photographs were made with the help of Reprostar 3 (CAMAG) digital camera. The polar components (phenolic compounds) in the extract were separated using Toluene: ethyl acetate: formic acid: methanol (6:6:1.6:0.4), and the developed plate was derivatized using ferric chloride reagent, characteristic peaks of the detected compounds were recorded at 540 nm[14,15].

Results and Discussion

Standardization of herbal drugs is mandatory which may help in understanding and solving some of the controversies with regard to their therapeutically active ingredients and action. The studies provide information in respect of their identification, chemical constituents and physicochemical characters which may be useful for pharmacognostical study and standardization of herbal drugs of folk medicinal practice of present era and enrichment of Ayurvedic Pharmacopoeia. The leaves of the *Machilus macrantha* Nees. are studied for pharmacognostical and phytochemical screening.

Macroscopy

Colour: Greenish on outer side and grayish green underneath.

Odour: Characteristics.

Taste: Mucilagenous

Size and Shape: 9.0x 22 x 3.8 x 9 cm, elliptical and lanceolate with acuminate or obtuse apex.

Touch: Smooth

Extra features: The leaf shows entire margin, asymmetrical bases, reticulate venation, glandular and non-glandular hairy trichomes. Petioles are medium sized or as long as the lamina, 10-20 cm long, thick, fistular, glandular and often yellowish green or purple in colour (Figure 1).

Microscopy

Transverse section of leaf passing through midrib and lamina

It is a dorsiventral leaf. Following tissues are present in midrib and lamina (Figure 2).

Midrib

Section passing through midrib shows concavity and 5-6 layers of thick walled collenchymatous cells 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. Thick walled nonlignified phloem follows the xylem. The vascular bundle is encircled 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 has also exhibit the presence of secretary cavities.

Lamina

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 and contain glandular and non-glandular trichomes. The Glandular trichomes are sessile with radiating glandular head. The non-glandular trichomes are covering trichomes. Covering trichomes are uniseriate, multicellular, 3-6 celled, mostly straight having acute apex or terminal cell with secretary cavity. Stomata are numerous on lower epidermis but few on upper epidermis. Results of various histochemical reactions are given in Table 1. Different leaf constants and micrometric analysis are tabulated in Table 2 and 3.

Surface Preparation of Leaf

In the surface view of the leaf, the lower epidermal cells are wavier in outline than upper epidermis. It shows anomocytic type stomata, glandular trichomes, simple covering uniseriate multicellular trichomes and prisms of calcium oxalate are also present in the lamina.

Powder study of Leaf

Preliminary examination of powder

Colour: Green
Odour: Characteristic
Taste: Bitter
Texture: Smooth

After addition of small quantity of water, a mucilaginous mass was not formed which indicates presence 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. Behaviour of powder with different chemical reagents is shown in Table 4.

Microscopical examination of powder (Figure 3) Trichomes: Both glandular and non-glandular trichomes. The Glandular trichomes are short stalked with radiating glandular head. The nonglandular trichomes are covering trichomes. Covering trichomes are uniseriate, multicellular, 3-6 celled, mostly straight having acute apex or terminal cell with secretary cavity.

- Xylem vessels: xylem vessels are with spiral thickening.
- Fibres: Well developed, sclerenchymatous fibres from vascular bundles.
- Epidermal cells: Polygonal with well developed anomocytic stomata.
- Mesophyll: Fragments of leaf showing spongy parenchyma cells.
- Calcium oxalate crystals: Both prismatic and rod shaped crystals of calcium oxalate.

Microscopic evaluation is an indispensable tool for identification of medicinal herbs and is one of the essential parameter in modern monograph. In this regard the important microscopic features of the leaves of the plant have been documented such as very long, uniseriate, multicellular, 3-6 celled covering trichomes having terminal cell with secretary cavity and glandular trichomes. Ridged midribs showing the presence of patches of collenchyma, below upper & above the lower epidermis and lamina showing single layer palisade cells are the characteristic features of the leaf microscopy. Surface preparation of leaf showed presence of anomocytic stomata, long covering trichomes and crystals of calcium oxalate. The powder study of leaf showed presence of xylem vessels with spiral thickening, covering and glandular trichomes, anomocytic stomata with epidermal cells, tracheids and crystals of calcium oxalate.

Physico-chemical Parameters

The percentage of ash values, extractive values, moisture content, foreign matter and crude fiber content , fluorescence analysis of powdered leaves were determined as are tabulated in Table 5, 6 and 7.

Preliminary Phytochemical Analysis

Preliminary phytochemical screening indicated presence of steroids in petroleum ether and methanol extract. Methanol, ethyl acetate and aqueous extract indicated presence of tannins, phenolic compounds.

Aqueous extract gave positive tests for carbohydrates (Table 8).

Figure 4: HPTLC Finger print profile of methanol extract of *M. macrantha* Nees. leaves

(a) HPTLC profile under UV 366 nm; (b) fluorescent nature of the compounds under UV 254 nm; (c) after derivatization with alcoholic FeCl3 under white light; (d) TLC chromatogram after densitometric scan under UV 366 nm; (e) TLC chromatogram after densitometric scan under UV 254 nm.

Chromatographic Finger Print Profile

HPTLC finger print profile of the extract for both polar and non polar compounds has been developed (Figure 3). Rf values of the separated compounds are recorded (Table 9). Microscopy alone may be inadequate to identify the true plant material. Also microscopy does not reveal much about the deterioration of the crude drug, therefore many of the modern herbal pharmacopoeias and other regulatory agencies like WHO included TLC as a powerful and most economical tool for true identification of the plant material, especially in terms of its chemical constituents. The HPTLC finger print profile of the major chemical constituents in the methanolic extract along with their R*f* values were recorded which would serve as a quality control parameter to identify the raw material, specifically in cases where the identity of bioactive compounds is not known or marker compound is not available for analysis.

Discussion

As a part of standardization study, the macroscopical examination of *Machilus macrantha* Nees. leaves were studied. Macroscopical evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of drugs. The macroscopical characters of the leaves of *Machilus macrantha* Nees. can serve as diagnostic parameters. The ash value, extractive value, moisture content, foreign matter, crude fiber content and fluorescent analysis of powdered leaves extracts have been carried out. The results showed greater extractive values in hot extraction, indicating the effect of elevated temperature on extraction. Percentages of the extractive values were calculated with reference to air-dried drug. 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 powdered drug from the PT in various solvents was performed under normal and UV light. All the whole plant extracts are examined in short UV (254nm) and long UV (366 nm) to detect the fluorescent compounds.

Conclusion

Pharmacognostical studies and phytochemical screening can serve as a basis for proper identification, collection and investigation of leaves of the plant. These parameters, which are being reported for the first time, could be useful in the preparation of the herbal monograph for its proper identification.

Acknowledgement

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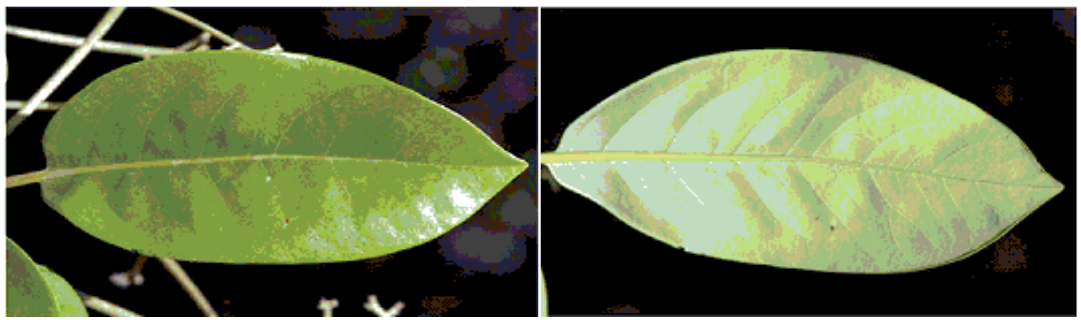

Figure 1: Leaf (Front and back side) of *Machilus macrantha* Nees.

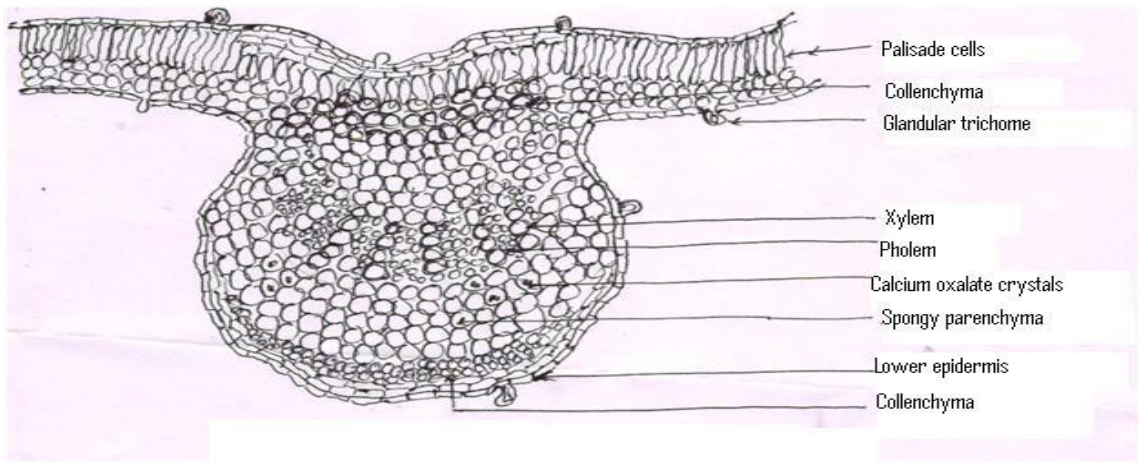

Figure 2: T.S. of *Machilus macrantha* Nees. leaf

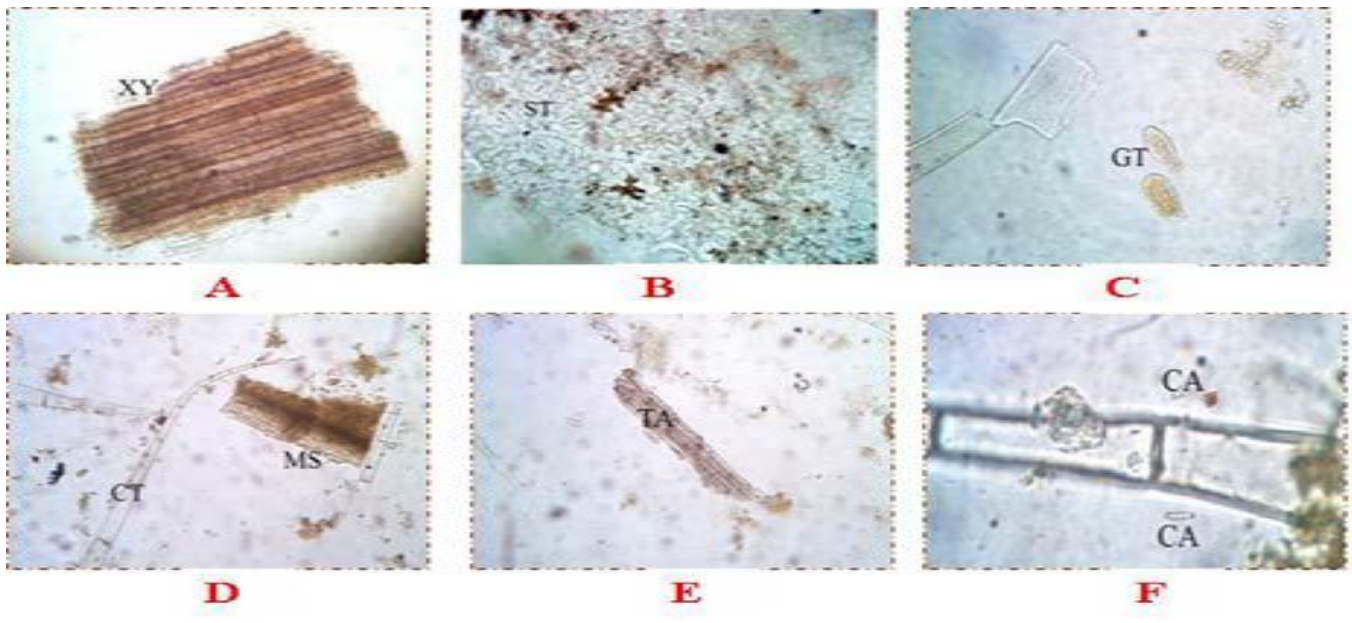

Figure 3: Powder characteristics of Leaves of *Machilus macrantha* Nees.
XY: Xylem vessels, ST: Stomata with epidermal cells, GT: Glandular trichome, CT: Covering trichome, MS: Mesophyll, TA: Tracheid, CA: Calcium oxalate crystal.

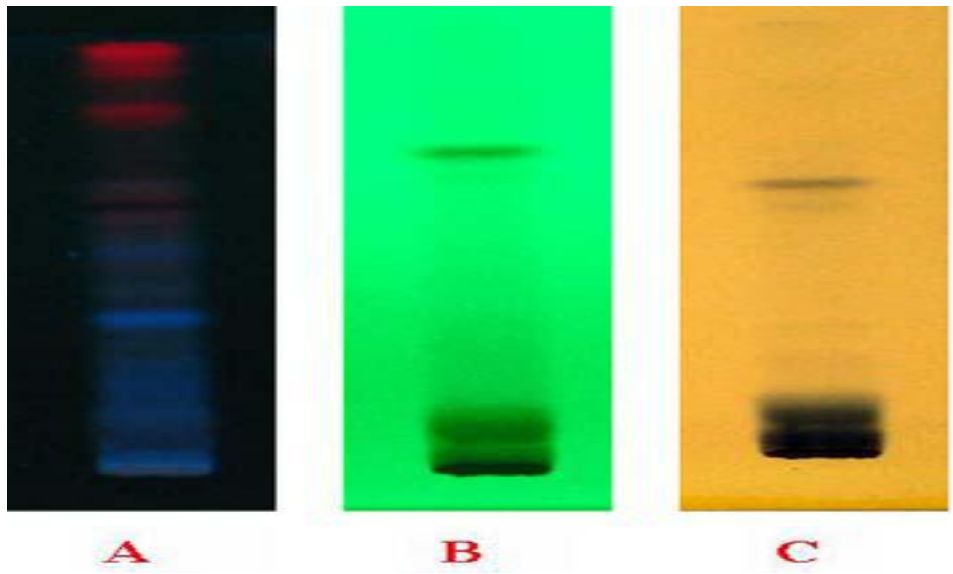

Figure 4: HPTLC Finger print profile of methanol extract of *M. macrantha* leaves: (a) HPTLC profile under UV 366 nm; (b) fluorescent nature of the compounds under UV 254 nm; (c) after derivatization with alcoholic FeCl3 under white light.

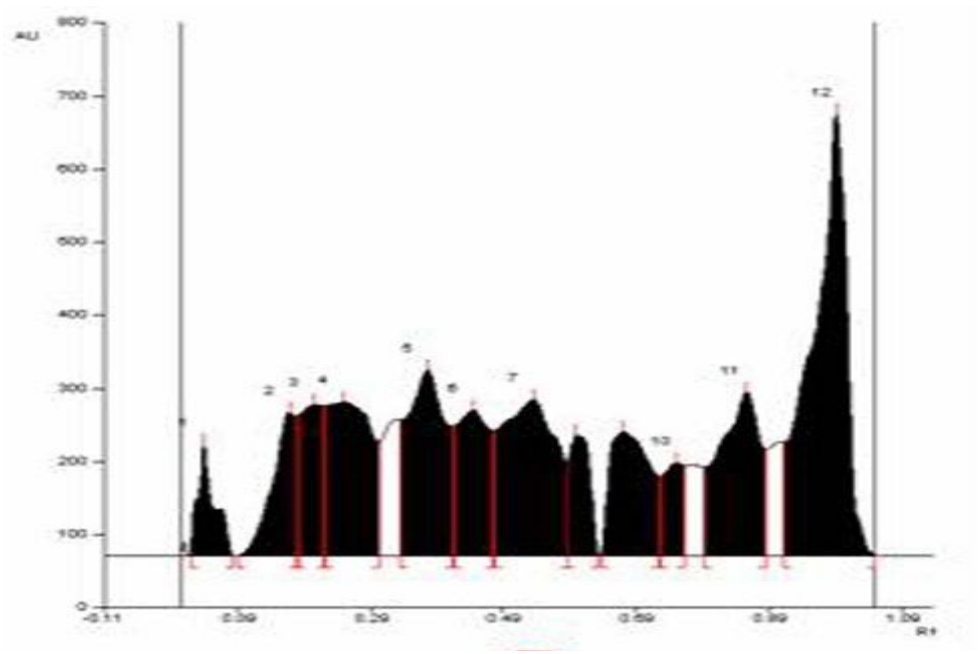

Figure 4(d): TLC chromatogram after densitometric scan under UV 366 nm.

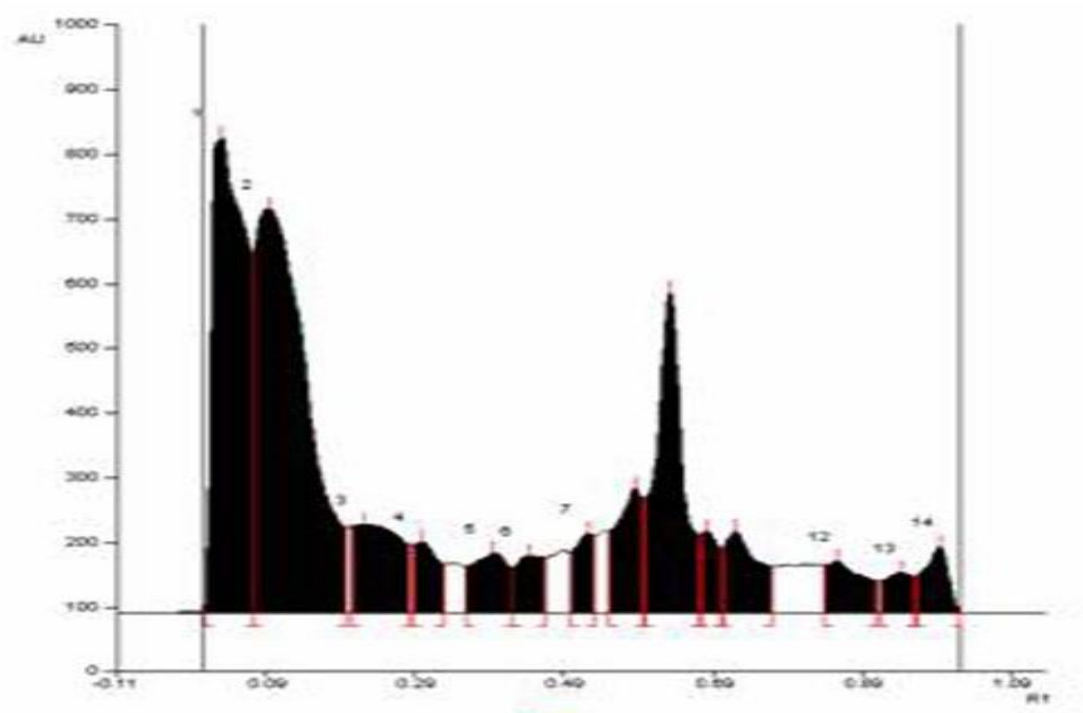

Figure 4(e): TLC chromatogram after densitometric scan under UV 254 nm.

Table1: Histochemical colour reactions.

Reagents	Constituents	Colour	Histological zone
Phloroglucinol + Conc. HCL	Lignin	Pink	Vascular bundles
Aniline sulphate	Lignin	Yellow	Vascular bundles
Weak Iodine solution	Starch	Blue	Lamina
Sudan III Solution	Oil globules	Pink	Vascular bundles
Aqs. Ferric chloride	Tannins	Black	Lamina
Dragendroff's reagent	Alkaloids	Light orange	Lamina
Libermann-Burchardt reagent	Steriods	Greenish	Lamina
Millon's reagent	Proteins	--	--

Table 2: Leaf constants.

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

Table 3: Quantitative microscopy of Leaf.

Type of cells	Size in micron
Upper epidermis	2.6 x 6.8
Collenchyma	6.8 x 13.4
Palisade cells	13.4 x 42.0
Parenchyma	17.2-28.6
Xylem vessels	21.1-35.8
Xylem parenchyma	8.8 – 9.2
Xylem fibers	13.8-14.1
Pholem parenchyma	6.8 x 7.0
Pholem fibers	7.1 x 7.4
Pericycle	14.5 x 25.3
Calcium oxalate crystals	7.4 – 14.6

Table 4: Behaviour of leaf powder of *M. macrantha* with different chemical reagents.

Reagent	Colour	Inference
Conc.H2SO4	Reddish	Steroides present
Aqueous Fecl3	Bluish black	Tannins present
Iodine solution	Blue	Starch present
Picric acid	Yellowish	Alkaloids present
Aqueous Mercuric chloride solution	Orange	Alkaloids present
Magnesium-hydrochloric acid	No colour	Flavonoid absent
Aqueous silver nitrate solution	No ppt	Proteins absent
Ammonia solution	No change	Anthroquinone glycosides absent
Aqueous KOH solution	No change	Anthraquinone glycoside absent

Table 5: Ash values.

Types of ash values	Values obtained % w/w (Mean + SEM)
Total ash	5.8 + 1.48
Acid insoluble ash	0.72+ 0.06
Water soluble ash	2.68 + 0.26
Sulphated ash	11.72+ 0.82
Moisture content	8.8 + 1.4
Foreign matter	4.0 +1
Crude fiber content	42 + 5

Table 6: Extractive values.

Type of solvent	% Extractive value (Mean + SEM)
Petrleum ether (60-80)	2.1+ 0.18
Benzene	3.2+ 0.12
Chloroform	3.8+ 0.14
Ethyl acetate	3.2+ 0.14
Ethanol	11.02+ 0.56
Methanol	14.05+0.21
Water	18.6+ 0.42

Table 7: Florescence analysis of different extracts of leaf of *Machilus macrantha* Nees.

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

Table7a: Florescence analysis of powdered leaves of *Machilus macrantha* Nees.

Sample	Daylight	Short UV (254 nm)	Long UV (366 nm)
Powder + Methanolic NaOH	Yellowish green	Yellowish green	Yellowish green
Powder + NaOH Solution	Dark green	Green	Orange
Powder + 1N HCL	Dark green	Dark green	Violet
Powder + Conc. HNO3	Brown	Green	Blue
Powder + Conc. H2SO4	Dark green	Green	Green
Powder + Nitrocellulose	Green	Green	Purple
Powder + Methanolic NaOH + Nitrocellulose	Light green	Green	Pale Orange

Table 8: Preliminary phytochemical analysis of various extracts of leaves of *M. macrantha* Nees.

Type of constituents	Petroleum ether	Benzene	Chloroform	Ethyl acetate	Ethanol	Methanol	Water
Steroids	+	+	+	+	+	+	-
Carbohydrates	-	-	-	-	-	-	+
Alkaloids	-	-	-	-	+	+	+
Glycosides	-	-	-	-	+	+	+
Reducing sugars	-	-	-	-	-	-	+
Flavonoids	-	-	-	-	-	-	-
Tannins	-	-	-	+	+	+	+
Proteins	-	-	-	-	-	-	-
Amino acids	-	-	-	-	-	-	-

(+)- Present, (-)- Absent

Table 9: Rf values of the separated compounds.

	R*f* value		
Peak	Under UV 254	Under UV 366	White light after derivatization
1	0.03	0.04	0.03
2	0.08	0.17	0.10
3	0.21	0.20	0.22
4	0.30	0.24	0.30
5	0.39	0.38	0.58
6	0.44	0.44	0.62
7	0.52	0.53	0.68
8	0.58	0.60	0.72
9	0.62	0.67	0.85
10	0.67	0.75	0.99
11	0.72	0.86	
12	0.86	0.99	
13	0.93		
14	0.98		

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