Pharmacognostical studies and preliminary phytochemical investigations on the bark of *Persea macrantha* (Nees) Kosterm (Lauraceae)

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*Persea macrantha* (Nees) Kosterm (Lauraceae) is an important traditional medicinal plant being used for treatment of asthma and rheumatism. Systematic pharmacognostical evaluation of bark of the plant has been carried out with respect to macroscopy, microscopy, physicochemical parameters and estimation of different standards. TLC profiles were developed for petroleum ether and chloroform extract. The preliminary phytochemical investigations indicated presence of alkaloids, tannins, carbohydrates and steroids. The results obtained from standardization of bark established the macro- and microscopical parameters, physicochemical parameters, TLC profiles that characterize the genuine plant drug (*P. macrantha*). These parameters can be utilized for quick identification of the drug and are particularly useful in the case of powdered materials.

**Keywords:** Lauraceae, *Machilus macrantha*, *Persea macrantha*, Pharmacognosy, Phytochemical.

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**Introduction**

*Persea macrantha* (Nees) Kosterm syn. *Machilus macrantha* Nees (Family—Lauraceae) is up to 27 m tall tree and 3 m in girth with cylindrical bole up to 7.5 m long (Plate 1). Leaves are oblong to elliptic-lanceolate, flowers are yellow in panicles in upper axils and fruits are ovoid, smooth, dark green\(^1\). It is found in Western Peninsula, Sri Lanka and in India up to an altitude of 2100 m. In India it is locally known as *Golum*, *Pisara*, *Kurma*, *Gulmavu* and *Chittutandrimara* in various regions.

The tree has many folk uses in various states of India; the bark is used in consumption, asthma and rheumatism\(^2\) while the leaves are used externally in ulcer\(^3\). Gaind & Baveja has reported detail pharmacognostical studies of root\(^4\). The alcoholic extract of the limed roots indicated presence of alkaloids, machiline and macranthine are the major alkaloids isolated from the roots\(^5,6\). The isolated alkaloid, machiline showed marked hypotensive effect in test animals\(^7\). Some lignans like machicendiol, machicenonol and machicenin have been isolated from leaves of the plant\(^8,9\).

Crude extracts of the bark have been studied for anti-inflammatory activity\(^10\) and antiarthritic activity\(^11\). Detail pharmacognostical studies of leaf of the plant have been reported by Kulkarni *et al*\(^12\).

Since there is no report of systematic pharmacognostical and phytochemical studies on bark, in order to fix some standards for its

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Plate 1 — *Persea macrantha* tree
identification, this study was planned to study detailed pharmacognosy and preliminary phytochemistry of the same.

Materials and Methods

Plant material

The fresh bark of *P. macrantha* was collected in the month of April from Lonavala, Dist. Pune (MS), India. It was identified and authenticated by the scientists of Botanical Survey of India, Pune, India and a voucher specimen (b12) of the bark (Plate 2) is deposited in department for future reference.

Macro- and Microscopy

Macroscopic examination of the bark was carried out according to standard procedures. Fresh bark was selected for the microscopical studies. Sections were cut on a microtome and by free hand sectioning. Numerous temporary and permanent mounts of the microscopical sections of the bark specimen were made and examined microscopically. Histochemical tests were carried out using 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. Photomicrographs of the microscopical sections were captured with the help of Motic photomicroscope (Canada) provided with Motic Images Plus 2.0 software.

Powder characteristics

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

Physicochemical parameters

Percentage of total ash, acid-insoluble ash, water-soluble ash, sulphated ash and loss on drying were calculated as per the Indian Pharmacopoeia. The total ash of the powdered bark was tested for different inorganic constituents. Various extracts were prepared for the study of extractive values of the bark. Fluorescence analysis of powdered bark was carried out by standard methods.

Preliminary phytochemical analysis

For the preliminary phytochemical analysis, 100 g of powdered bark of *P. macrantha* was extracted with petroleum ether (40-60°C), chloroform, ethyl acetate and methanol successively, using Soxhlet apparatus. The aqueous extract was prepared by cold maceration technique. The extracts were concentrated under vacuum using rotary vacuum evaporator, dried and weighed. Each extract was tested for presence of different phytoconstituents, viz. triterpenoids, steroids, alkaloids, sugars, tannins, glycosides, flavonoids, proteins and amino acids by usual prescribed methods. The TLC pattern of petroleum ether extract and chloroform extract was studied using pre-coated silica gel plates (Merck).

Plate 2 — Bark of *Persea macrantha*
Quantification of total phenolics

The concentration of phenolic compounds in powdered bark was determined by Folin-Ciocalteu colorimetric method\textsuperscript{25}. The absorbance was measured at 765 nm against a reagent blank, using Shimadzu UV-1601 spectrophotometer. The total polyphenolic content was calculated as gallic acid equivalents and expressed in % as gallic acid.

Quantification of total tannins and carbohydrates

Total tannins were estimated by using Folin-Denis method\textsuperscript{26}. The absorbance was measured at 760 nm using Shimadzu UV-1601 spectrophotometer. Analyses were carried out in triplicate and the quantification was calculated from a calibration curve obtained with tannic acid. Total tannins were expressed as g/100 g of tannic acid equivalent.

Total carbohydrates were estimated by using phenol-sulfuric acid method\textsuperscript{27}. The absorbance of the solution was measured at 490 nm using Shimadzu UV-1601 spectrophotometer. Glucose was used as a standard to obtain a calibration curve.

Estimation of total alkaloids, mucilage, unsaponifiable matter and crude fibre content

The total alkaloids and mucilage content was determined by the prescribed method\textsuperscript{28} and the unsaponifiable matter of the bark was determined by the standard procedure of Indian Pharmacopoeia\textsuperscript{17}. The total crude fibre content of the bark was determined by the procedure described by Raghuramulu et al\textsuperscript{29}.

Results and Discussion

Macroscopy

The bark is externally brownish and internally light reddish brown in colour. It occurs in curved or sometimes flat pieces with size of 7-8×18-20 cm and thickness of about 1.5-3 cm (Plate 2). It has mucilaginous taste which is followed by bitter sensation. The odour is characteristic, unpleasant and has slight astringent effect on throat. The bark shows fibrous fracture.

The bark shows number of masses of moss and fungal growth. Outer surface of bark has got numerous lenticels. Number of wrinkles and undulations are also seen on outer surface, while inner surface shows presence of numerous striations. The bark is smooth and has glistening appearance due to numerous shining calcium oxalate crystals in sunlight. Overall the bark is compact, hard and lighter in weight. Fresh bark detached from trunk of the tree is yellowish and turns to brown and then reddish brown on storage.

Microscopical characteristics

Transverse section of bark showed roughly four regions- periderm, cortex, a band of sclerenchyma and secondary phloem (Plate 3a). Periderm is composed of cork, phellogen and phelloderm (Plate 3b). Cork is stratified and consists of several layers of radially arranged rows of thin walled, elongated cells. Phellogen is made up of one to two layers of thin walled, rectangular, lignified cells. Phelloderm is one to two layered in thickness and exhibits thin walled, non-lignified, brown coloured and roughly rectangular cells.

Secondary cortex is composed of ten to thirteen layers of parenchymatous cells (Plate 3c), encircling either isolated or groups of scattered sclereids. Each sclereid is more or less rectangular in shape and pitted with thickened inner and radial walls. Some of cortical cells contain prismatic, microphenoideal calcium oxalate crystals and simple starch grains.

A continuous, well-developed layer of sclereids (sclerenchymatous band) separates the cortex and secondary phloem region. The inner and radial walls of the sclereids are thicker than the outer walls giving the appearance of the letter ‘U’. Groups of small pericyclic fibres are found on the outer side of sclerenchymatous band (Plate 3d).

Secondary phloem region contains phloem parenchyma, phloem fibres and medullary rays (Plate 3e). Phloem parenchyma consists of thin walled cells containing starch and calcium oxalate crystals. Numerous mucilage cells were seen in phloem parenchyma. Phloem fibres occur in group of 2-3, embedded in phloem parenchyma. These are mostly circular and lignified. Medullary rays which are generally 1-3 cells wide divide radially the phloem parenchyma. The results of histochemical tests are given in Table 1.

Powder characteristics

Macroscopic

The powder is brown in colour, smooth in texture, bitter and with characteristic odour. 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
Plate 3 — Microscopical structure of *Persea macrantha* bark: (a) Transverse section, (b) periderm, (c) cortex, (d) sclerenchyma, (e) secondary phloem. Abbreviations— pd-periderm, co-cortex, scl-sclerenchyma, ph-phloem, ck-cork, phl-phellogen, phd-phelloderm, mr-medullary rays, pp-phloem parenchyma pt-pericyclic fibers.
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 2.

**Microscopic**

The powder showed presence of fibres which are lignified, elongated, tubular, narrow, pointed and isolated phloem fibres with simple or branched pores (Plate 4).

The stone cells are found in groups or isolated. They are roughly rectangular or oval in shape, lignified with thick walls and pitted in nature. The cork cells are polygonal, appear brownish in colour. The powder showed abundant, microsphenoidal and prismatic calcium oxalate crystals (35-40 µ). The starch grains are simple, adequate having size of about 26-29µ.

**Physicochemical parameters**

The results of determination of total ash, acid-insoluble ash, water-soluble ash, sulphated ash, loss on drying and different extractives are tabulated in Table 3. The qualitative analysis of ash indicated presence of calcium, aluminium, potassium, chlorides and sulphates. Fluorescence analysis of the powdered bark does not indicate presence of any fluorescent compound.

**Preliminary phytochemical screening**

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,
ethyl acetate and aqueous extract indicated presence of phenolic compounds.

The TLC profile of petroleum ether extract indicated presence of seven compounds with the solvent system petroleum ether: ethyl acetate (8:2). Chloroform extract showed presence of four compounds with the solvent system chloroform: methanol (8:1) (Table 4).

The results of estimations of total phenolics, total tannins, total carbohydrates, total alkaloids, mucilage content, unsaponifiable matter and crude fibre content are presented in Table 5.

Evaluation of physicochemical parameters is an indispensable part in preparation of modern monograph. Thus, different ash values, extractive values, loss on drying and fluorescence characteristics can be used for standardizing the crude drug samples. The TLC developed profiles can be used for identification as well as guide for isolation of various compounds from the extracts.

**Conclusion**

The diagnostic features have been established to identify *P. macrantha* bark. Some of the important diagnostic features of the bark are its characteristic odour, presence of ‘U’ shaped sclereids and numerous mucilage cells. The bark has shown remarkable
amount of alkaloids and polyphenolics. An important observation from phytochemistry point of view is absence of flavonoids and glycosides in the bark. The present studies on the bark of *P. macrantha* will be useful for its identification and authentication.

References