

PHARMACOGNOSTIC, PRELIMINARY PHYTOCHEMICAL EVALUATION AND HPTLC PROFILE OF LEAF OF *PICCHILATARU- PERSEA MACRANTHA* (NEES) KOSTERM - AN EXTRAPHARMACOPOEIAL MEDICINAL PLANT OF AYURVEDA

Prabhu Niranjana^{1*}, Padigar Shrikanth², Sagri Ravikrishna³, Narayan Prabhu Suchitra⁴

^{1*}PG Scholar, ²Professor and Head, Dept. of Dravyaguna, ³Associate Professor – Dept. of Agada tantra, ⁴Research Officer, Dept. of Pharmaceutical chemistry and Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi- 574118, Karnataka, India

Email: niranjan.ayu@gmail.com

ABSTRACT

Objectives: Ayurveda always emphasizes on utility of local flora in treatment. It also advises to know about the plant completely with respect to its identity, property and various therapeutic benefits including safety before prescribing it to a patient. **Methods:** *Persea macrantha* (Nees) Kosterm. belonging to the family Lauraceae is such a drug which is extra-pharmacopoeial medicinal plant of Ayurveda, called *Gulimavu* in Kannada which is endemic to Western Ghats and used very frequently by the folklore practitioners to treat Asthma, Joint pain, Fracture and Ulcer, thus proper identification is very important. **Results:** The Pharmacognostical study will help to standardize its identification and some of the important diagnostic features of the leaf. Preliminary phytochemical studies revealed the presence of several phyto-constituents which helps in characterization of the drug. **Conclusion:** The study will help the practitioners to get the genuine drug and best outcome in their practice.

Keywords: Extrapharmacopoeial drug, Pharmacognostic study, Preliminary phytochemical investigations, Standardization

INTRODUCTION

Persea macrantha (Nees) Kosterm. is an *anukta dravya* of Ayurveda. A proper *nama* and *roopa jnana* is very essential to establish its proper identification which is then possible to utilize it in clinical practice in various diseases.

The foot prints of treating diseases by using locally available natural resources can be traced from the pre-vedic period itself. The art and science of medicine further developed in the vedic period, where in the names and usage of around 100 plants were mentioned. This knowledge found its highest growth in the Samhita period, where a codified and systematically documented medical knowledge called Ayurveda was flourished. Though the details of medicinal plants mentioned in Samhita granthas are limited compared to that of Nighantu kala, they believed every plant is medicinal and the protocol of adding a drug into Ayurveda materia medica is elaborated by quoting about collecting the information from *Ajapa*, *Avipa*, *Gopa* and other persons who are well versed with names as well as forms of the plant¹. As the time passed the knowledge was disseminated to the general population and more over the common people indigenously developed their own way of treating the disease using Flora in and around the vicinity of their practice.

There are around 57,000 plant species in Indian subcontinent and among them around 8000 are medicinal, hence there is literally no limit to study and use those plants as per the guidelines of Ayurveda, to add those plants into Ayurveda materia medica which is the need of the present era to avoid burden on the vulnerable/ endangered plant species mentioned in Ayurveda due to its high demand.

Persea macrantha (Nees) Kosterm. Syn. *Machilus macrantha* Nees, is one such therapeutically potent drug belonging to the family Lauraceae is an extra-pharmacopoeial medicinal plant of Ayurveda, meaning it is an Anukta dravya which is not mentioned in any of the samhithas or nighantus of Ayurveda, but the

newly coined Sanskrit name is Picchilataru² and called *Gulimavu* in *Kannada* which is endemic to Western Ghats, Traditionally its bark and leaves are used very frequently by the folklore practitioners to treat Asthma, Joint pain, Mental ailments, Fracture and Ulcer^{3,4,5}. In the recent times it is proved to be having Anti-histamine⁶, Anti-arthritis, Analgesic, Anti-bacterial⁷ as well as Hypotensive activities and hence the demand for the drug is increased.

Karnataka is having rich source of medicinal plants and the western ghats of coastal region of Karnataka is famous among 4 hotspots of Biodiversity in India. A large number of traditional healers are dependent on this rich source and are making use of the plants for treating various ailments successfully. As the knowledge of these traditions was running through inheritance later on became secretive. A resurgence of interest in local health tradition and rural medicine is seen worldwide and the knowledge is in public domain. Hence trying to make it scientifically validated the Pharmacognostic as well as Phytochemical analysis is carried out.

The leaf of *Picchilataru* can be easily adulterated with low grade material (substandard) if the supply of crude drug is inadequate; hence Pharmacognostic study is the initial step to confirm the identity and to assess the quality and purity of crude drug. The adulteration of the crude drug can be prevented by means of its evaluation like macro-microscopic study. Microscopy is an indispensable tool for authentication of crude drug. Setting up a standard of pharmacognostic, morphological and microscopical characters of leaf *Picchilataru* will boost standardization, which can promise quality, purity and identity of samples⁶. Preliminary phytochemical studies helps in characteri-

zation of the drug which in future will help the practitioners to get the genuine drug and best outcome in their practice. The present study will provide the precise information in respect of identification as well as its characterization.

MATERIALS AND METHODS

Sample collection

The plant source was identified in the field at Veda vana (medicinal herb garden) of Shri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi with the help of regional flora (Flora of South Kanara) and a voucher specimen is deposited in Dept. of Pharmaceutical chemistry and Pharmacognosy, SDMCRAAS with Sample number as 17061201. The fresh leaf were collected and used for macro-microscopy, dried and powdered form was used for powder microscopy.

Macroscopic evaluation

The external features of the test samples were documented using Canon IXUS digital camera. The macroscopic features were compared to local flora for confirmation.

Microscopic evaluation

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections

were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

Powder microscopy

Pinch of leaf powder previously sieved is put on the slide and mounted in glycerine and powder characters are observed under the Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light.

Physico-chemical analysis

Organoleptic examination, macro, microscopy, and physicochemical studies, viz., total ash, water-soluble ash, acid-insoluble ash, water and alcohol soluble extractive, loss on drying at 105⁰C as per standardized methods⁸.

Preliminary phytochemical analysis

Tests for alkaloids, carbohydrates, steroids, saponins, tannins, flavonoids, phenol, coumarins, triterpenoids, carboxylic acid, resin and quinine⁹.

High Performance Thin Layered Chromatography

1g of *Persea macrantha* leaf powder was extracted with 10 ml of alcohol. 3, 6 and 9 μ l of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (7.0: 1.0). The developed plates were visualized in short UV, long UV, and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm and 620nm. R_f, colour of the spots and densitometric scan were recorded.

OBSERVATION AND RESULTS

Vernacular names

Sanskrit	<i>Picchilataru</i> ¹
Kannada	<i>Gulimavu/ Gulmavu/ Gulamavu/ Seeme maavu/ Kurma</i>
English	Ladder tree/ Machilus/ Large flowered bay tree
Telugu	<i>Naara</i>
Tamil	<i>Kollamavu</i>
Tulu	<i>Nirkkukku</i>
Marathi	<i>Golum</i>
Malayalam	<i>Kulamavu / Kulirmavu</i>
Canarese	<i>Chittutantre</i>
Coorg	<i>Kruramavu</i>

Taxonomical position

Kingdom	Plantae
Sub-kingdom	Viridiaeplantae
Phylum	Tracheophyta
Sub-phylum	Spermatophytina
Infra-phylum	Angiospermae
Class	Magnoliopsida
Sub-class	Magnoliidae
Super-order	Lauranae
Order	Lurales
Sub-order	Laurineae
Family	Lauraceae
Genus	<i>Persea</i>
Species	<i>P. macrantha</i> (Nees) Kosterm.

Morphology

Persea macrantha (Nees) Kosterm. Syn. *Machilus macrantha* Nees belonging to the family Lauraceae is a large evergreen tree, growing up to 30m in height, 3m in girth. Leaves are Simple, Alternate arrangement, clustered at the ends of branches, coriaceous measuring 9-18 X 2.8-6.3cm, entire margin, variable in shape from oblong and rounded at both the ends to elliptic-lanceolate and Acute at both the ends, glabrous above and glaucous beneath, finely reticulate venation, petioles 2-3.2cm long. Bark is 20-25mm thick, surface pale brown, mottled with dark blotches, scurfy and thinly scaly, rough,

exfoliations small, brittle. Flowers are Numerous, small, hermaphrodites, in axillary panicles, greenish-yellow coloured, perianth-tube short or obsolete, segments 6, sub-equal, persistent, reflexed in fruit, stamens 9 perfect, those of the 2 outer rows with glandular hairy filaments and 4-celled anthers. Ovary sessile, narrowed into the style, stigma is discoid. Fruits are globose or ovoid berry, seated on the persistent perianth measuring 1.3-2cm in diameter, smooth, dark green coloured dotted with white, ultimately becoming dark. Seeds are Single, small, round seeded, within the testa.

Organoleptic characters

<u>Leaf characters</u> -	<u>Leaf Powder characters</u> -
Colour: Greenish, glabrous above and glaucous beneath	Colour: Brown
Taste: Mucilaginous, Slightly Bitter	Taste: Slightly bitter
Odour: Aromatic, resembling fresh mango leaves	Odour: Aromatic, resembling mango leaves
Touch: Smooth	Touch: Smooth

Macroscopic characters

The leaves are dark greenish with a smooth texture, glabrous above and glaucous beneath, entire margin, finely reticulate venation, acute apex, asymmetrical bases, variable in shape from oblong to elliptic-lanceolate and acute at both the ends and base is broader on the lower side. (Fig.1)

Microscopic characters

T.S of leaf is divided into 3 parts namely lamina, midrib and vascular bundle region. (Fig. 2a)

Midrib

The upper and lower epidermis of lamina is continuous over the midrib. A 2-4 layers of collenchyma can be clearly seen below the upper and above the lower epidermis. Collateral type of vascular bundles with absence of cambium. Ground tissue is mostly parenchymatous, pericycle fibres covers outer side of the phloem. The rest of the midrib is occupied by cortical parenchyma with vascular bundle embedded in the middle. Vascular bundle is arc shaped collateral with xylem towards upper epidermis and phloem towards lower epidermis.

It covers upper layer under epidermis, 2-3 layers of collenchyma. (Fig. 2b)

Lamina

Epidermis has single layered upper epidermis covered with thick cuticle. Mesophyll is divided into two parts namely palisade and spongy parenchyma. Palisade is single layered, compact

with radially elongated cells having rosette crystals. Spongy parenchyma has six layers with no intracellular space and cells are closely arranged, in which some cells have cluster crystals. The lower epidermis is single layered. There are no trichomes observed either on upper or lower epidermis. (Fig. 2c)

Petiole

It has thick epidermis followed by collenchyma inner to which there are cortical parenchyma cells, centrally arranged vascular bundles which are arc shaped. Cortical parenchyma cells have starch cells with starch grains and vascular bundles are covered by pericyclic fibres. (Fig. 2d)

Powder microscopic characters

Powder microscopic characters showed the presence of parenchyma cells, epidermal cells in surface view, cortical parenchyma, mesophyll cell with bundle of fibres along with starch grains and spiral vessels. (Fig. 3)

Physico-chemical tests

Loss on drying of the leaf was found to be 10.52%, Total ash 8.0%, Acid insoluble ash 0.69%, water soluble ash 2.69%, alcohol soluble extractive value 9.83% and water soluble extractive value 6.48%.

Preliminary phytochemical tests

Phytochemical tests carried out showed the presence of alkaloid, steroid, carbohydrate, tan-

nins, flavonoids, saponins, terpenoid, phenols and carboxylic acid.

HPTLC

HPTLC fingerprint was carried out using the alcoholic extract of leaf of *P. macrantha*. The photo documentation shows at short UV, 2 bands (green) were present corresponding to its R_f value of 0.38 and 0.56. At long UV, 8 bands were evident at different R_f of 0.08, 0.38, 0.46, 0.50, 0.56, 0.61, 0.75 and 0.84 (all fluorescent) with different intensities of purple, red and blue. After derivatisation with vanillin sulphuric acid under white light presence of 5 spots were seen corresponding to R_f of 0.18, 0.38, 0.50, 0.69, and 0.82 all are purple in colour. (Fig. 4a)

The densitometric scan at 254nm showed 9 peaks corresponding to different phyto constituents among which 0.41(14.46%), 0.65(16.20%), 0.73(13.46%) and 0.82(13.92%) are prominent (Fig. 4b). At 366nm 6 peaks were seen among which 0.70(23.12%), 0.67(18%), 0.53(21%), 0.46(15.80%) were prominent (Fig. 4c). At 620nm 8 peaks are seen among which 0.04(24.36%), 0.44% (15.64%), 0.79(32.81%) were evident (Fig. 4d).

DISCUSSION

The majority of people live in rural areas in India and they rely upon traditional medicines for their healthcare as well their cattle, this is due to strong belief on the traditional medicine and the healers. Lack of primary healthcare centers and transportation facilities also makes them to approach traditional healers. Besides, medicinal plants are easily available natural products, cost-effective with no or negligible side-effects¹⁰. The ethno-medico botanical study of *Persea macrantha* (Nees) Kosterm revealed that the drug is used extensively in treatment of various

ailments like joint pain, fracture, ulcer, mental ailments^{3,4,5}. The pharmacognostical study is one of the major criteria for identification of plant drugs. The preliminary phytochemical screening showed the presence of alkaloid, steroid, carbohydrate, tannins, flavonoids, saponins, terpenoids, phenols and carboxylic acid. Alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly evoke a bitter taste. Carbohydrates are building blocks they are nutritional elements required to serve energy. Alkaloids are quite diverse, they are narcotic used to relieve pain, and analgesic hence is used as muscle relaxants¹¹. Plants steroids are anti-inflammatory in nature due to this it is helpful in reducing the pain¹² also they act as a nutritional supplement; they block cholesterol absorption sites in the intestine helping to reduce cholesterol in humans. They are anabolic in nature thus promote bone density and muscle growth. Tannin substances are astringent, dry and pucker feeling in the mouth. Tannins are antiseptic on skin and mucus membrane, they are used as healing agents in inflammation¹³. The presence of flavonoids implies that it may have anti-inflammatory, antioxidant, anti-cancer activity. Saponins may enhance nutrient absorption and aid indigestion. Terpenoids are used extensively for their aromatic assets which are also believed to be as precursors of sterols and steroids. Presence of the above phytochemicals along with its safety data¹⁴ justifies its therapeutic value as to be a better wound healing, analgesic, anti-inflammatory, anti-arthritic, anti-bacterial and anti-oxidant drug as the action of the drug is directly dependent on its chemical composition.

CONCLUSION

The results of the pharmacognostical, physico-chemical analysis and HPTLC profiling helps in standardization with respect to its identity, purity and genuinity of the herbal material. Preliminary phytochemical screening showed the presence of alkaloid, steroid, carbohydrate, tannins, flavonoids, saponins, terpenoid, phenols and carboxylic acid. As the drug is safe for human consumption, with this sufficient data the drug can be taken up studying it as per guidelines of Ayurveda like assessment of its rasa panchaka and further for clinical studies to rationally include it into Ayurveda materia medica, thus providing a scientific and evidence based documentation of traditional knowledge as mentioned in Ayurveda.

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FIGURES

Fig.1: Macroscopy of Leaves of *Persea macrantha* (Nees) Kosterm.

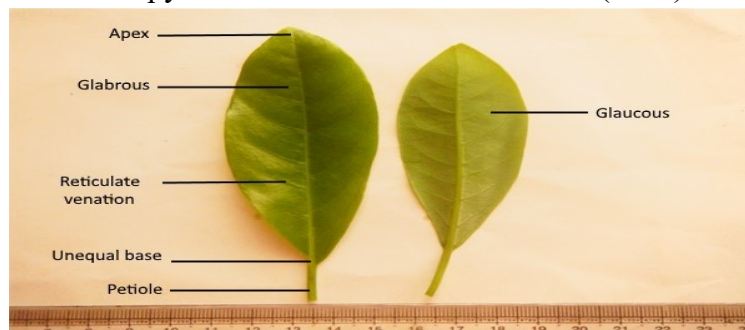


Fig. 2a: T.S of Leaf of *P. macrantha*

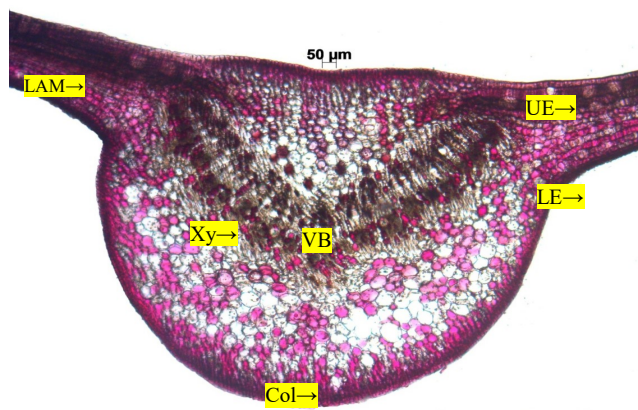
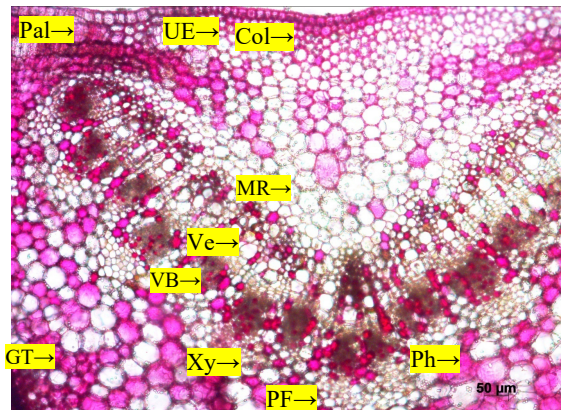
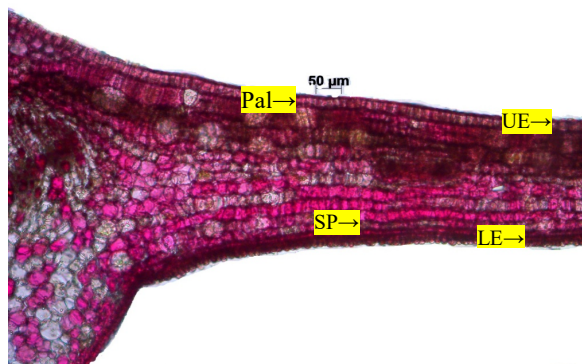


Fig. 2b: Upper region of Midrib enlarged



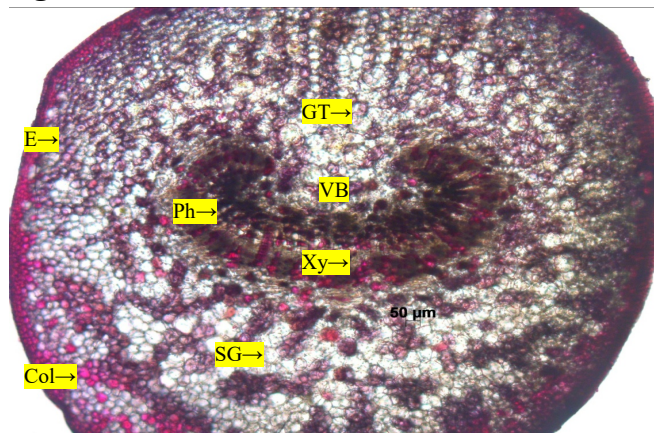
Col – collenchyma; UE – upper epidermis; LAM – lamina; LE – lower epidermis; Pal – palisade; SP – spongy parenchyma; VB–vascular bundle; Xy – xylem, GT – ground tissue; MR – midrib; PF – pericyclic fibres; Ph – phloem; UE – upper epidermis; Ve – Vessels

Fig. 2c: Lamina enlarged



Pal – palisade; SP – spongy parenchyma; UE – upper epidermis; LE – lower epidermis

Fig. 2d: T.S of Petiole



Col – collenchyma; Ct – cortex; E – epidermis; GT–ground tissue;
 PF – pericyclic fibres; Ph – phloem; SG – starch grains; Ve – vessel;
 Xy – xylem; VB - vascular bundle

Fig. 3: Powder microscopy of leaf and petiole of *P. macrantha*

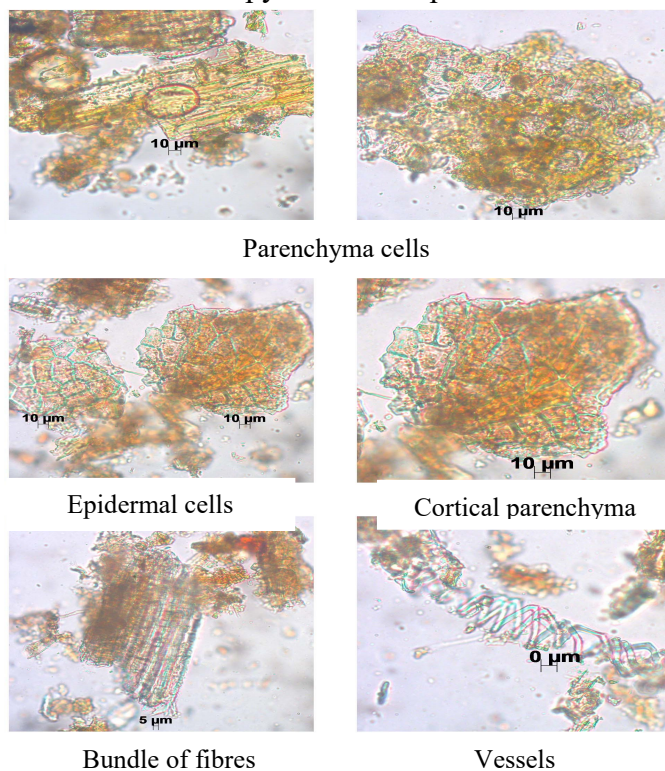
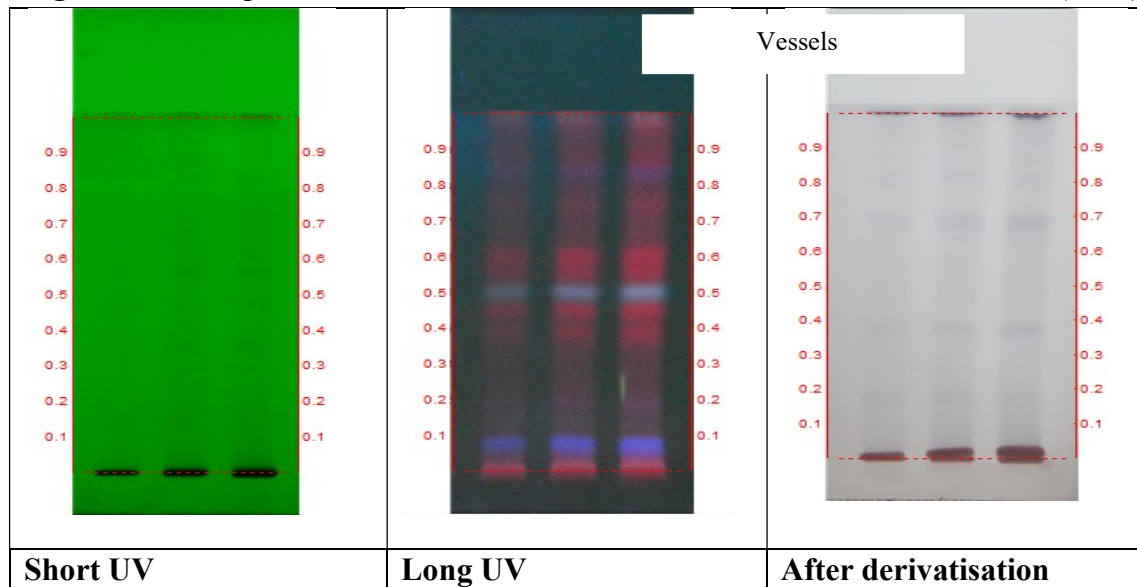


Fig. 4a: HPTLC photo documentation of ethanolic extract of *Persea macrantha* (Nees) Kosterm.



Track 1- *P. macrantha* – 3µl Track 2- *P. macrantha* – 6µl Track 3- *P. macrantha* – 9µl
 Solvent system – Toluene: Ethyl Acetate (7:1)

Fig. 4b: Densitometric scan of *Persea macrantha* at 254nm

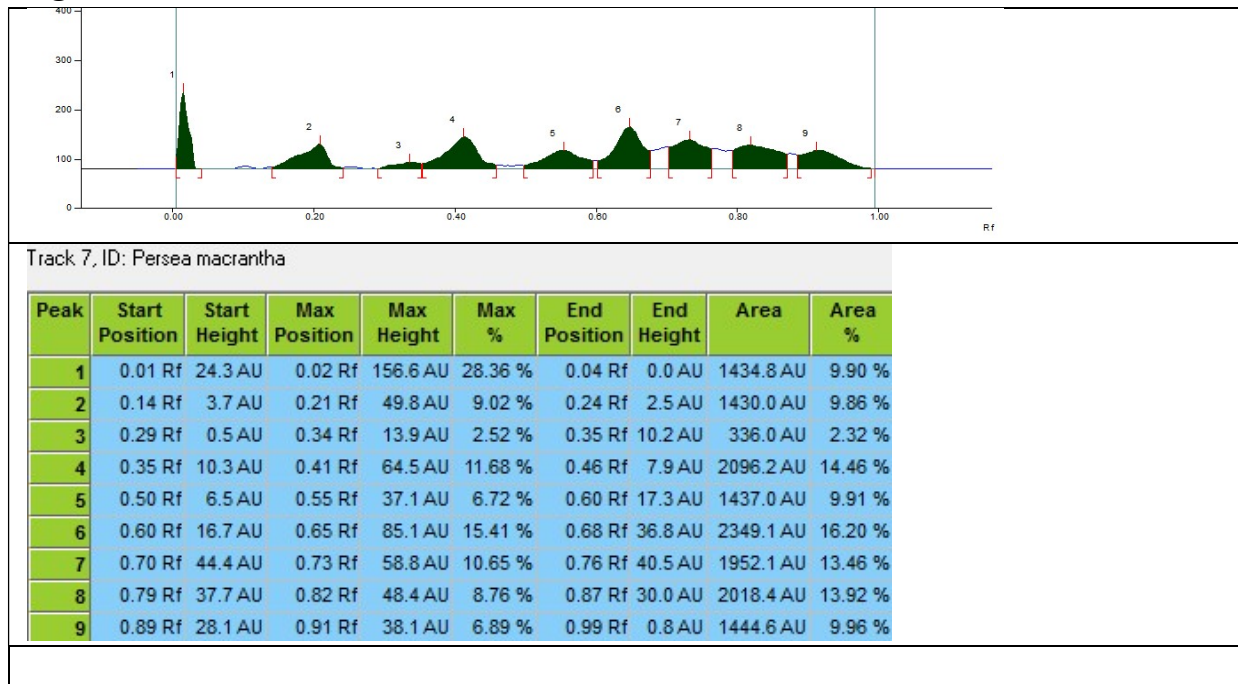


Fig. 4c: Densitometric scan of *Persea macrantha* at 366nm

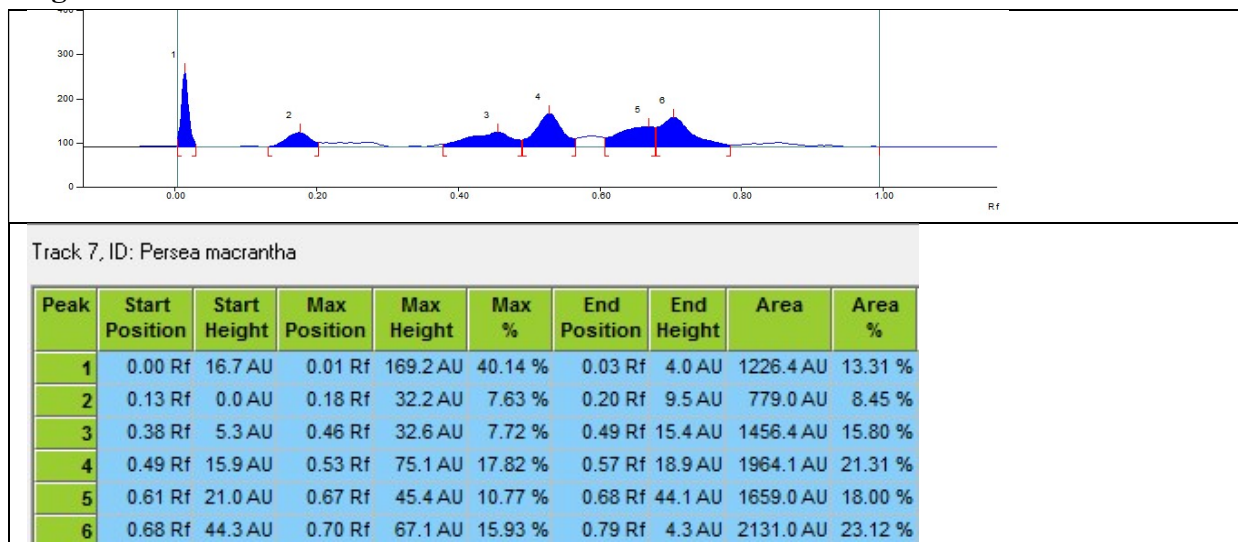
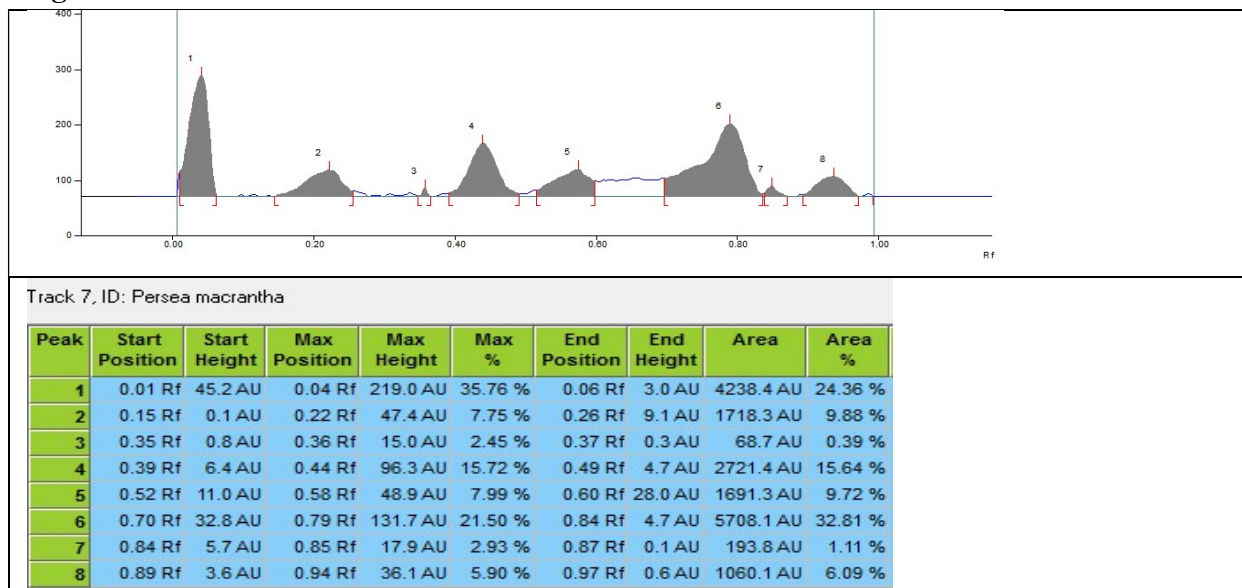


Fig. 4d: Densitometric scan of *Persea macrantha* after derivatization at 620nm



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Conflict Of Interest: None Declared

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