

PHARMACOGNOSTICAL AND PHYTO-PHYSCOCHEMICAL PROFILE OF THE LEAVES OF *MANGIFERA INDICA* L. VAR ALPHONSO (ANACARDIACEAE) - VALUABLE ASSESSMENT OF ITS QUALITY

K.BHUVANESWARI, K.PERIYANAYAGAM*

K. Periyamayagam, Asst Reader, Department of Pharmacognosy, College of pharmacy, Madurai Medical College, Madurai 625 020, Tamilnadu, India; Email: kpn1960@yahoo.com

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ABSTRACT

Objective To study in detail the micromorphology and physicochemical analysis of the leaves of *Mangifera indica* L. var Alphonso family Anacardiaceae.

Methods Macroscopy, microscopy, physicochemical analysis, preliminary phytochemical screening and other WHO recommended parameters for standardizations were performed.

Results Leaves are dark green and glabrous, oblong – lanceolate to elliptic, 6-16in long, entire margin and acute apex. Petiole is 1-4in long, and swollen at base. Microscopic evaluation revealed the presence of cyclocytic stomata in lower epidermis, secretory cells, glandular trichomes, xylem vessels, phloem, fibres. Vein islet numbers, vein termination numbers, stomatal number, stomatal index and other physico chemical tests like ash values, loss on drying, extractive values were determined. Preliminary phytochemical screening showed the presence of steroids, tannins, proteins and aminoacids, flavonoids, terpenoids, mucilage, volatile oil, saponin, carbohydrates and absence of alkaloids, fixed oil. Determination of inorganic minerals by Energy Dispersive Spectrum.

Conclusion The microscopic using histological identification, microscopic constants and other physico chemical examinations of the leaves of *Mangifera indica* L. var Alphonso can be used as a rapid, inexpensive botanical identification technique and is useful in standardization, hence would be of immense value in authentication of the leaf.

Keywords: *Mangifera indica*, Anacardiaceae, Microscopical evaluation, Physicochemical studies, Phytochemical studies, Standardization.

INTRODUCTION

Plants are one of the prime sources of medicines and recently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, around 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources. Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine. The world is now focusing towards the herbal medicine or phytomedicines that repair and strengthening bodily systems (especially the immune system that can properly fight foreign invaders) and help to destroy offending pathogens without toxic side effects.

Mango (*Mangifera indica* L.) is the most popular fruit crop in the orient particularly in India, where it is considered as the best among all indigenous fruits. It fill up relatively the same position as that enjoyed by apple in temperate America or Europe. It ranks first among all the fruits of India in area and production. Global production of mango is concentrated mainly in Asia and more precisely in India. India is one of largest producer and consumer of mango in the world. The country reportedly produces about 50 varieties of mango. The region wise popular varieties grown in different parts of the country comprise of 'Alphonso' and 'Kesar' in western India, 'Banganapalli', 'Totapuri' and 'Neelum' insouthern states, 'Fazli' in eastern states and 'Langra' and 'Chausa' in northern states^[1].

M. indica is commonly used in folk medicine for a wide variety of remedies. The root, bark, leaves, flowers, unripe and ripe fruit are acrid, cooling and astringent to the bowels and have been employed to cure "vata", "pitta", and "kapha". The parts of *M. indica* mentioned above have also been employed traditionally for treatment of leucorrhoea, bad blood; dysentery, piles, bronchitis, biliousness, urinary discharges, throat troubles, vaginal troubles, hiccough, ophthalmic, eruption, asthma and labouring under habitual constipation. It is also used as aphrodisiac, tonic, appetizer, beautifier of complexion, hiccough, laxative, diuretic, stomachic, antisyphilitic and for tanning purposes in various parts of the world^[2].

The widely available leaves of *M. indica* really do not have any match as a cheap natural and easily available from our backyard. It is traditionally known to be useful for the treatment of wide panel of disease like throat infection, burns, scalds^[3], antidiabetic^[4], antioxidant^[5], antimicrobial^[6], antiviral^[7] and antibacterial^[8,9].

Various phytoconstituent of *M. indica* include such as carbohydrate, tannin, protein, saponin, mucilage, terpenoid, flavonoid and glycoside. Mangiferin xanthone glycoside is a main constituent of *M. indica* present in the leaves, fruits, stem bark and root. Mangiferin has wide panel of pharmacological activity such as antipyretic^[10], antioxidant^[11], antitumour^[12], immunomodulatory^[13] and neuroprotective^[14].

In short, there is good level of traditional and experimental evidences to support various claims and advantages of this widely available plant. As mentioned earlier several reports have been published on the effects of the plant extract and chemical constituents on different biological activities *in vitro* and *in vivo*. An investigation to explore its pharmacognostic examination is inevitable. In Tamilnadu, (South India) *M. indica* L. var Alphonso is widely cultivated. Hence, in this work we report an attempt on microscopic evaluation, physicochemical determination and phytochemical screening for the standardization and quality assurance purposes of this cultivar.

MATERIALS AND METHODS

Chemicals

Formalin, acetic acid, ethyl alcohol, chloral hydrate, toluidine blue, phloroglucinol, glycerin, hydrochloric acid and all other chemicals used in this study were of analytical grade.

Plant collection and authentication

The leaves of the plant *Mangifera indica* Linn. var Alphonso selected for our study was collected from AC and RI, Tamil Nadu Agricultural University, Madurai District and was authenticated by Dr.P. Jayaraman, Director of Plant Anatomy Research Institute, Tambaram, Chennai, TamilNadu, India, and Dr. T. Arumugam Professor – Horticulture, AC and RI, TamilNadu Agricultural University, Madurai- 625 104, TamilNadu, India.

Macroscopic analysis

Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste etc was noted^[15].

Microscopic analysis:

Transverse section midrib region of fresh leaf pieces were cut and fixed in FAA and then dehydrated by employing graded series of ethyl alcohol and tertiary butyl alcohol^[16]. Sections were taken using microtome. Permanent mount was prepared using saffranin fast green double staining technique^[17]. In order to supplement the descriptive part the photomicrographs in different magnifications of all necessary cells and tissues were taken with NIKON Coolpix 8400 digital camera and Labphot2 microscopic unit.

Physicochemical analysis:

Total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying, extractive values and leaf constants such as vein islet numbers, vein terminal number, stomatal number and stomatal index, palisade ratio were determined^[18,19].

Preliminary phytochemical screening:

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure^[20].

Determination of trace elements by Energy Dispersive Spectrometer (EDS):

The SEM allows the observation of materials in macro and submicron ranges. SEM is capable of generating 3-D images for analysis of topographic features. When SEM is used along with EDS the analyst can perform an elemental analysis on specimens of microscopic sections or contaminants that may be present^[21].

RESULTS

Macroscopy:

Mangifera indica tree is simple, belonging to the family Anacardiaceae.(Fig 1). The shape of the leaves are lanceolate to elliptic 6-16in long and alternate. It is dark green, glabrous, entire and acute apex. Petiole is 1-4in long and swollen at base. Inflorescences usually have primary, secondary, and tertiary pubescent, cymose branches pale green to pink or red and bear hundreds of flowers. Fruits are drupe, round to ovate to oblong and long. Fruits can weigh from less than 50 g (0.35 lb) to over 2 kg (4.4 lb).



Figure 1: habit of *M. Indica* l.var anacardiaceae

Microscopy of the leaf:

Shape: Midrib consist of wide and thick adaxial hump and broad semicircular abaxial part. Thickness: 1.3mm thick, 1.4mm broad.

Tissue arrangement: Both adaxial and abaxial end possess outer thin, sclerenchyma line. (Fig 2)

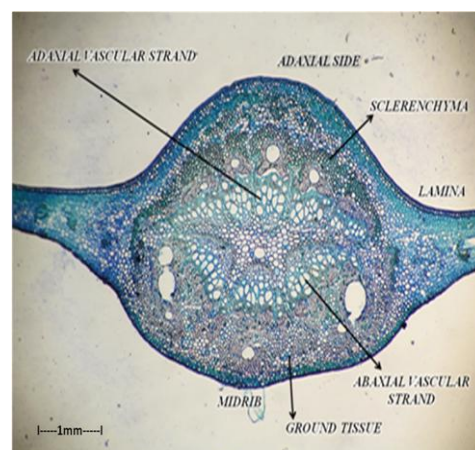


Figure 2: t.s of midrib

Epidermis: It has polygonal, thick, straight anticlinal walls.

Trichomes: Glandular trichomes are seen on both epidermis occasionally. Glands are spherical and multicellular, consist of two rows of vertically elongated cells forming globular body(Fig 3). It is seated in shallow cavity of epidermis and about 60µm thickness.

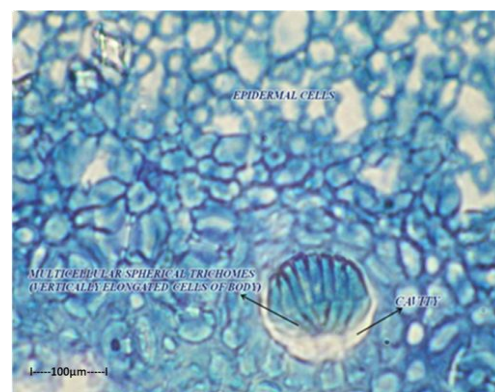


Figure 3: glandular trichomes

Stomata: The stomata are deep seated below the level abaxial epidermis, with two bulged guard cells surrounded by mostly two subcells of narrow subsidiary cells. So it is cyclocytic having circular rings of subsidiary cells (Fig 4).

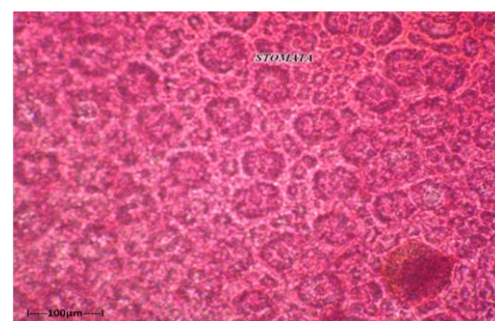


Figure 4: epidermis- surface view

Vascular system: Multistrandard includes 3 abaxial strand (a central small block and two lateral conical block) and a flat spindle shape adaxial strand (segmented in to 6 or 7 units by narrow gaps) collateral with elliptical, angular, wide, thin walled clustered or radial rows of xylem elements and wide circular secretory canals seated in the phloem tissues of the vascular segments. Deep arc of sclerenchyma caps surrounds the vascular segments.

Lamina: 190 µm thick with narrow thick walled squarish cells of adaxial epidermal layer and slightly thin abaxial epidermis (Fig 5.)

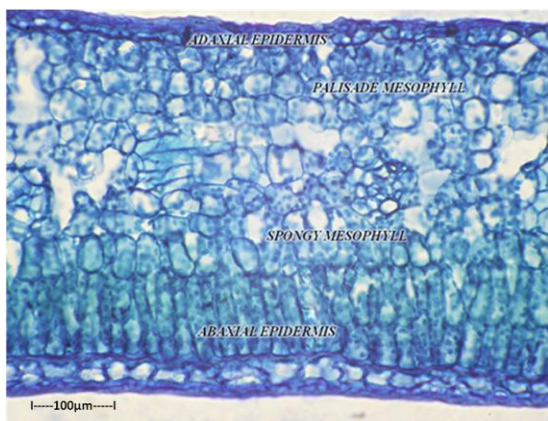


Figure 5: t.s of lamina

Mesophyll : Differentiated in to adaxial narrow single vertical row of compact columnar cells of palisade zone and abaxial spongy 8 to 10 rows of spherical, lobed, less compact mesophyll zone.

Calcium oxalate prismatic crystals: They are sparsely distributed in mesophyll tissue and around the bundle sheath fibers (Fig 6).

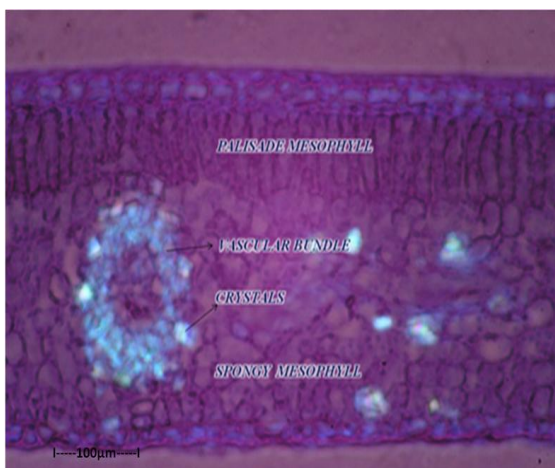


Figure 6: t.s of lamina under the polarized light to show crystals

Venation pattern: The lamina consist of thick straight veins forming wide polygonal vein-islet having prominent straight vein boundaries. The vein terminations are well developed repeatedly forked forming dendroid outline of termination.

T.S of petiole (Fig 7, 8)

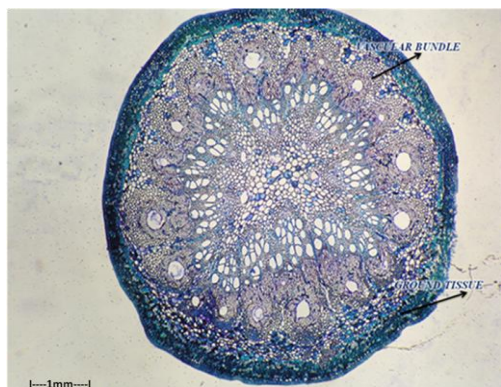


Figure 7: t.s of petiole-entire view

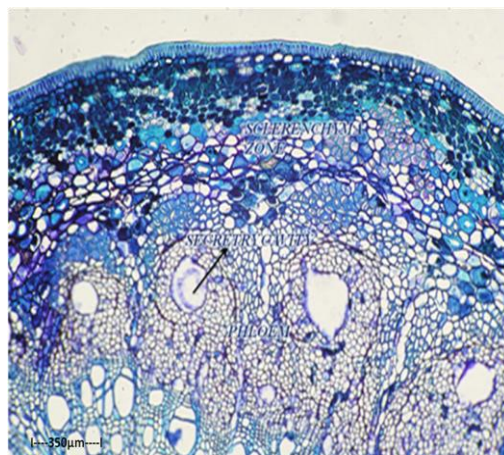


Figure 8: t.s of petiole-outer portion

Shape: Circular, smooth and even 1.8 mm thick.

Epidermis: Small thick walled lignified cells with prominent cuticle.

Ground tissue: Wide, complex in tissue organization with dense tannin containing outer zone cells parenchymatous inner zone with brachysclereids and gelatinous fibers.

Vascular bundle

Xylem: Several thick radial segments with inner xylem with wide thin wall radially elongated vessel enter in sclerenchyma tissue.

Phloem: Outer wide mass of phloem, wide circular lysigenous secretory canals are located within phloem arc of sclerenchyma cap is present on each bundle.

Pith: The central core consist of radiating mass of parenchyma cells.

Crystals: Diffused in distribution, calcium oxalate prism as well as druses are present in sclerenchyma zone.

Physicochemical analysis:

Physicochemical parameters were found as follows: total ash 8.17%w/w, acid insoluble ash 6.46% w/w, water soluble ash 6.34%w/w, ethanol soluble extractive value 14.62%w/w, water soluble extractive value 19.14%w/w, loss on drying 7.68%w/w and foreign organic matter was nil. Leaf constants were as follows vein islet number 3, vein termination number 14, stomatal number (lower epidermis) 43 and stomatal index (lower epidermis) 23.9.

Preliminary phytochemical screening:

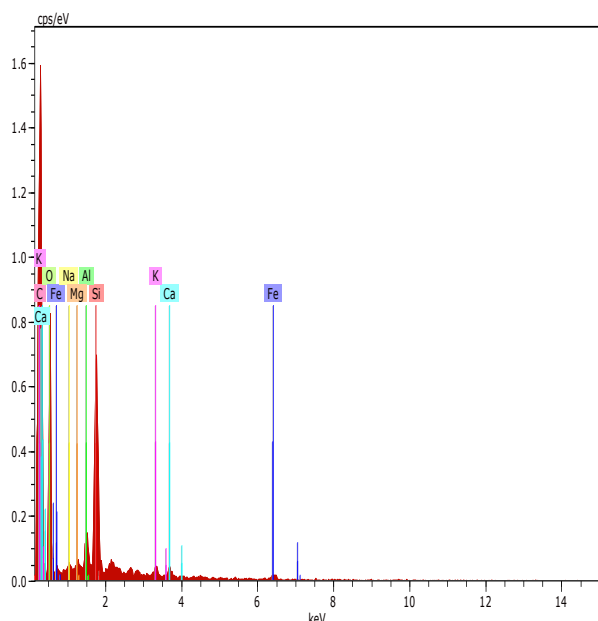
Preliminary phytochemical screening showed the presence of flavonoids, terpenoids, steroids, volatile oil, mucilage, tannin, saponins, carbohydrates, reducing sugars, proteins and amino acids and absence of alkaloids, cyanogenetic glycosides, anthroquinone glycosides, cardiac glycosides and fixed oils.

Determination of trace elements by Energy Dispersive Spectrometer

Estimation of the elements like Ca, Fe, Na, Mg, Al, Si, K, showed the following weight percentage : 1.22, 2.36, 0.86, 0.67, 2.17, 12.85, 1.12 (Plate-9).

FIGURE-9: ENERGY DISPERSIVE SPECTRUM (EDS) ANALYSIS OF *M. indica* LEAVES

ELEMENT	WEIGHT%	ATOMIC%
Ca K	1.22	3.06
Fe K	2.36	5.89
Na K	0.86	2.15
Mg K	0.67	1.68
Al K	2.17	5.43
Si K	12.85	32.17
K K	1.12	2.79



Elemental spectrum

DISCUSSION

Adulteration and misidentification of medicinal plants can cause serious health problems to consumers and legal problems for the pharmaceutical industries. The past decade has witnessed the introduction and implementation of new Good Manufacturing Practices (GMP) in quality control of raw materials, intermediates and finished products of botanical origin. The initial step in quality control of medicinal plants is ensuring the authenticity of the desired species for the intended use. It can be conducted via a variety of techniques, namely macro and microscopic identification and chemical analysis especially description of microscopic botanical aspects to determine definitively the proper species of plant material while it is still in its non extracted form. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs. These characters are especially important for identification of powdered drugs, because in these cases most of the morphological diagnostic features are lost [22].

Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials. In our present work we selected the plant *M.indica* L. var *Alphonso*(Anacardiaceae). The macroscopic and organoleptic characters of the leaf can serve as diagnostic parameters. Presence of cyclocytic stomata in the lower epidermis, numerous more or less uniform secretory cell, thick lateral vein terminals mostly forked once or twice and some of them form dendroid endings, thick walled fibres are diagnostic value. The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. The ash values is particularly important to find out the presence or absence of foreign inorganic matter such as metallic salts and or silica (earthy matter). The extractive values are primarily useful for the determination of exhausted or adulterated drug. Preliminary phytochemical screening will reveal the useful information about the chemical nature of the drug. Preliminary phytochemical screening showed the presence of volatile oil, steroids, flavonoids, terpenoids, saponins, mucilage, reducing sugars, carbohydrates, protein and aminoacids and absence of alkaloids and fixed oils.

In conclusion, the present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. Microscopical evaluation and physicochemical standards and preliminary phytochemical reports can be useful to substantiate and authenticate drug [23].

Conflict of interest statement:

We declare that we have no conflict of interest.

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