ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

HUMAN

Human Journals
Research Article
August 2016 Vol.:7, Issue:1

Pharmacognostical, Phyto-Physicochemical Profile of the Leaves of *Michelia champaca* Linn

ISSN 2349-7203
IJPPR
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Submission: 5 August 2016
Accepted: 10 August 2016
Published: 25 August 2016

www.ijppr.humanjournals.com

Keywords: *Michelia champaca*, Magnoliaceae, Leaf, Microscopic characters

ABSTRACT

Michelia champaca (Hindi: Champa, Tamil: Sambagam) belongs to the family-Magnoliaceae. It consists of 12 genera and 220 species of evergreen trees and shrubs, native to tropical and subtropical South and Southeast Asia. Leaves are traditionally used as stomachic, expectorant, diuretic and anti-ulcer etc. Present study aimed to establish in detail the macro and micromorphology including phyto and physicochemical analysis of *Michelia champaca* to be performed as per WHO and pharmacopoeial guidelines. The leaves (30.5 × 10.2cm) are ovate, alternately arranged with pointed apex. Microscopic evaluation revealed the presence of unicellular unbranched covering trichome in lower epidermis and upper epidermis. The vascular system consists of adaxially flattened semicircular cylinder. The vascular bundles are wedge shaped or triangular which has compactly arranged wide, thin walled angular xylem elements and small nests of phloem. In lamina, palisade layer consists of 6 or 7 layers of small lobed cells which linked with each other forming aerenchymatous tissue. Powder microscopy showed the presence of non glandular trichome, globular oil body and fragment of adaxial epidermal cell. Vein islet number, vein termination number, stomatal number and index and other physicochemical parameters like ash values, loss on drying, extractive values were also determined. Preliminary phytochemical screening of appropriate solvent extracts showed the presence of Carbohydrates, Sterols, Tannins, Flavonoids, Volatile oil and absence of Alkaloids, Proteins, Glycosides and Fixed oil. All these findings provided referential pharmaco-botanical information for correct identification of the leaves of *Michelia champaca* Linn.

1. INTRODUCTION

Michelia champaca Linn. (Fam: Magnoliaceae) is commonly known as champaca. It consists of 12 genera and 220 species of evergreen trees and shrubs, native to tropical and subtropical South and Southeast Asia, including Southern China [1, 2]. In India, it is highly distributed in Eastern Himalayan tract, Assam, Myanmar, Western Ghats, South India, Arunachal Pradesh and Bihar [3]. *M.champaca* is cultivated in gardens and near the temples for its fragrant flowers and handsome foliage in India. Traditionally, this plant leaves used for the treatment of fever, colic, leprosy, post partum protection and in eye disorders. Juice of the leaves is given with honey in colic. Leaves are also applied in andolent swellings. Leaves of champaka were including in a vagina pessary recommended for treating vaginal infections [4, 5].

Vernacular names [6]

Tamil	:	Sambagam
Hindi	:	Champ, Champa
Telugu	:	Champakmu
Bengal	:	Champaca
Assam	:	Phulchop
Gujarati	:	Pitochampo

Taxonomy [7]

Kingdom	:	Plantae
Subkingdom	:	Tracheobionata
Division	:	Mangoliphyta
Class	:	Magnoliopsida
Subclass	:	Magnolidae
Order	:	Magnolioles
Family	:	Magnoliaceae
Genus	:	*Michelia*
Species	:	*champaca*

Leaves of this plant have been reported as anti-inflammatory [8], antifertility [9], antiulcer [10, 11], anthelmintic [12], antimicrobial [13, 14], analgesic [15], diuretic [16], cytotoxic [17] and antidiabetic [18]. Various phytoconstituents has been reported in this plant such as Sesquiterpenes - michelia- A, liriodenine, parthenolide and guaianolides[19], Volatile oil containing compounds like benzyl acetate, linalool and isoeugenol [7].

As mentioned earlier several reports have been published regarding chemical constituents and different biological activities *in-vitro* and *in-vivo*. An investigation to explore its pharmacognostic examination is inevitable. 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 *M.chempaca* leaves to treat various diseases and disorders.

2. MATERIALS AND METHODS

2.1: 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.

2.2: Collection of Specimens and authentication: The leaves of the selected plant were collected from in and around Shevaroy hills, Salem and were identified and authenticated by Dr. P. Jayaraman, Director of Plant Anatomy Research Institute, Tambaram, Chennai, Tamil Nadu, India.

2.3: Macroscopic analysis: Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste etc was noted [20].

2.4: Microscopic analysis [21, 22]: The leaves were fixed in FAA (Formalin - 5 ml + acetic acid - 5 ml + 70% ethyl alcohol - 90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol (TBA). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C), until TBA solution attained supersaturation. The specimens were cast into paraffin blocks.

Sectioning: The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12 µm. After de-waxing, the sections were stained with toluidine blue. Since toluidine blue is a polychromatic stain, the staining results

were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc.,

Photomicrographs: Photographs of different magnifications were taken with Nikon lab-photo 2 microscopic Unit. For normal observations, bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background.

2.5: Powder microscopy: Coarse powder of the leaf was used to study the microscopical characters [23, 24].

2.6: Physicochemical analysis: Total ash, acid insoluble ash, water soluble ash, loss on drying and extractive values were determined [25, 26].

2.7: Preliminary phytochemical screening: Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure [27, 28].

3. RESULTS

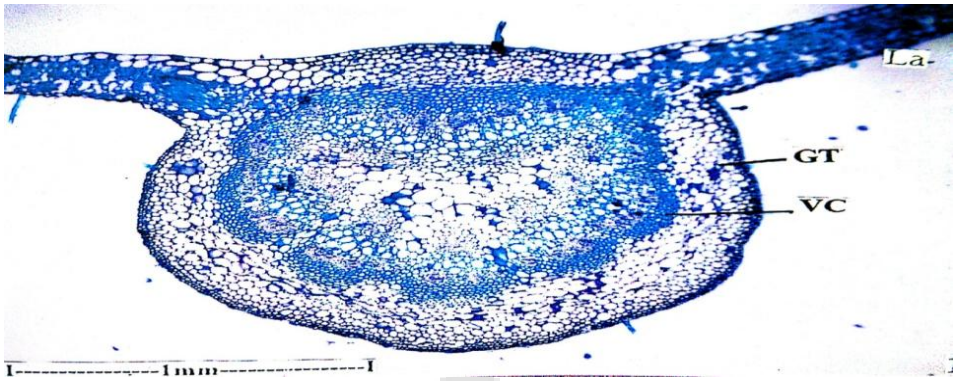
3.1. Macroscopic character

M.chempaca is a tall, handsome, evergreen tree with a straight trunk. Leaves (30.5 cm×10.2cm) are simple, alternate, 15- 25 by 5- 9 cm, lanceolate, acute, entire, glabrous; petioles are 18-25mm in long. Flowers are about 5-6 cm in diameter, very fragrant, grayish yellow pubescent. Sepals and petals are 15 or more deep yellow or orange.

Fig 1: Leaves and flowers of *M.chempaca*

3.2: Microscopy of the leaf of *M. champaca*

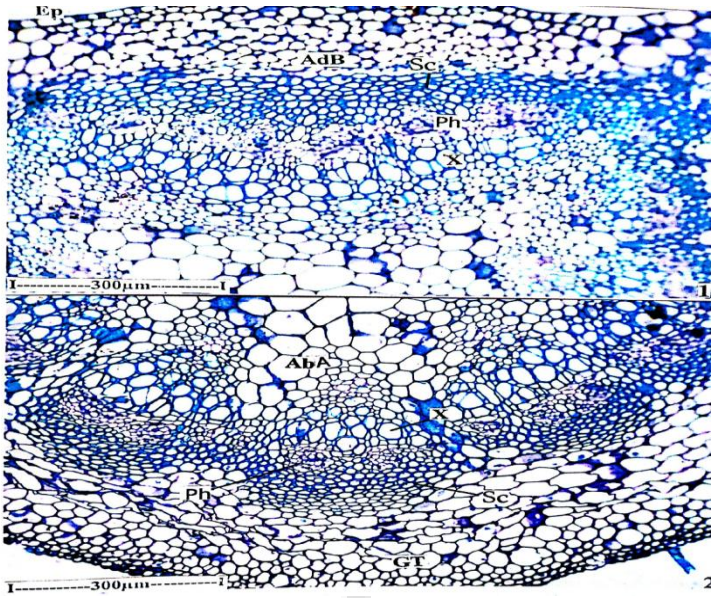
T.S of Midrib with lamina: The midrib is 1.5mm in vertical plane and 1.75 mm in horizontal plane. It is flat on the adaxial side and bulged into semicircular body on the abaxial side.



(GT- Ground tissue, VC- Vascular cylinder, La- Lamina)

Figure 2: T.S of leaf through midrib with lamina

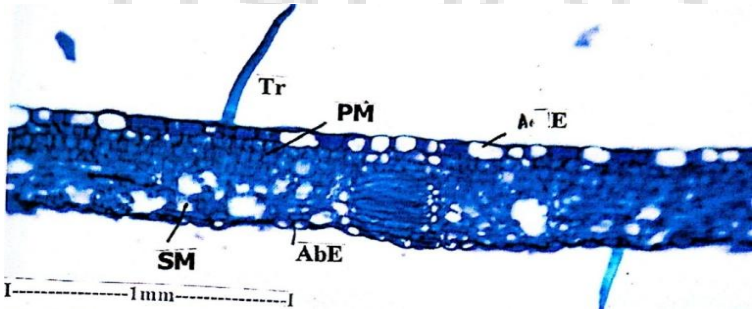
The epidermal layer of the midrib is thin and the cells are small and thick walled (Figure 2). Two or three layers of cells inner of the epidermis are smaller thick walled and collenchymatous. Remaining ground tissue is parenchymatous. The cells are variable in shape and size and compact. The vascular system consists of adaxially flattened semicircular cylinder. The lower semicircular cylinder has about light discrete vascular bundles placed very close each other. The adaxial flat part has a continuous plate of vascular tissues. The vascular bundles are wedge shaped or triangular; they have compactly arranged wide, thin walled angular xylem elements and small nests of phloem (Figure 3). A thick sclerenchyma cylinder is ensheathed the vascular system forming a continuous cylinder. The abaxial bundles are 250µm in radial plane and 150µm in tangential plane. The adaxial plate is 300µm thick. The metaxylem elements are 40µm wide.



(Aba- Abaxial arc of vascular tissue, AdB-Adaxial vascular bundle, GT-Ground tissue, Ep-Epidermis, Ph-Phloem, Sc-Sclerenchyma, X-Xylem)

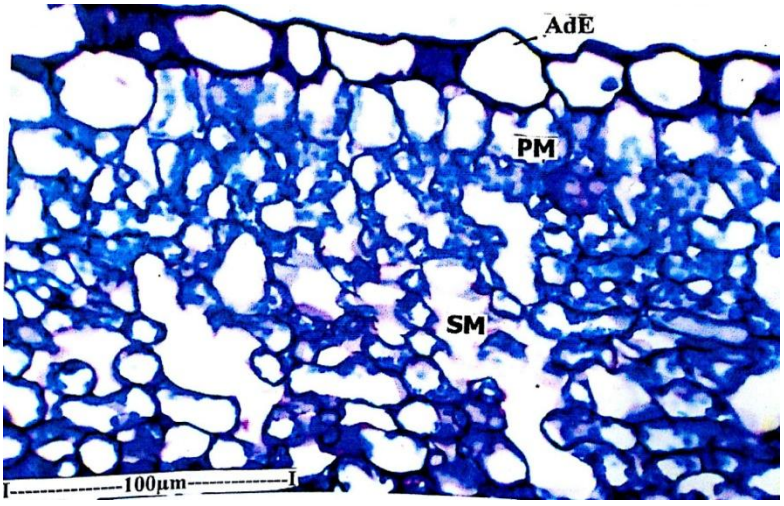
Figure 3: T.S OF MIDRIB

LAMINA: The lamina is 150µm thick. It consists of thick adaxial epidermal layer of wide, circular to squarish cells with prominent cuticle. The cells are 20µm thick. The abaxial epidermis is thin with spindle shaped cells (Figure 4, 5). The mesophyll is differentiated into narrow palisade zone and wide spongy parenchyma. The palisade layers consist of 6 or 7 layers of small lobed cells which linked with each other forming aerenchymatous tissue (Figure 5).



(Tr-Trichome, PM- Palisade mesophyll, AdE- Adaxial epidermis, SM- Spongy mesophyll, AbE-Abaxial epidermis)

Figure 4: T.S of lamina



(AdE- Adaxial epidermis, PM- Palisade Mesophyll, SM- Spongy Mesophyll)

Figure 5: T.S OF LAMINA- MAGNIFIED

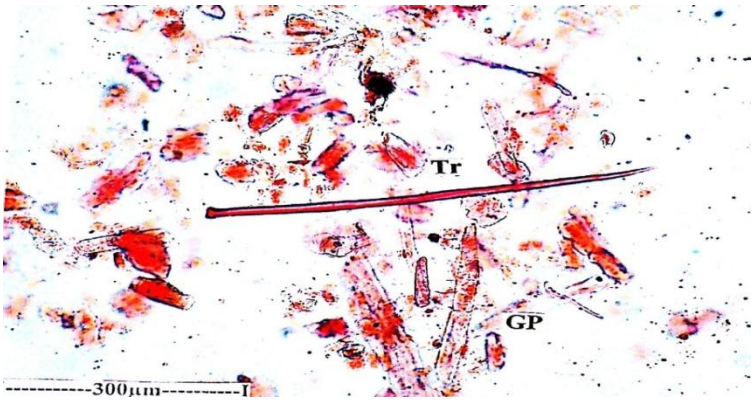
3.3: Powder microscopy:

The powder of the leaf exhibits the following characteristics

Epidermal trichomes: non glandular trichomes are sparingly seen in the powder. They are unicellular unbranched, narrow and pointed their walls fairly thick and lignified. They are 500-600µm long and thick (Fig 6).

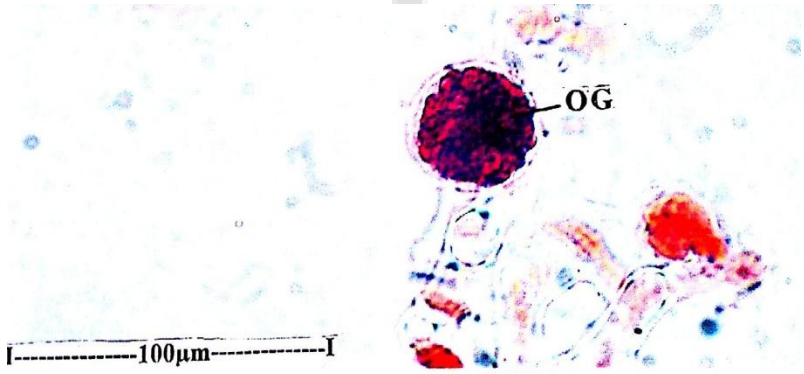
Oil granules: These circular thin walled cells which are filled with dense globular bodies, these bodies seem to contain oil substance. The cells with such oil bodies belong to the mesophyll tissue of the leaf (Fig 7).

Epidermal fragment: small fragments of epidermal peeling are frequently seen in the powder. The peeling has large epidermal cells with sinuous walls so that the cells appear amoeboid in outline (Fig 8).



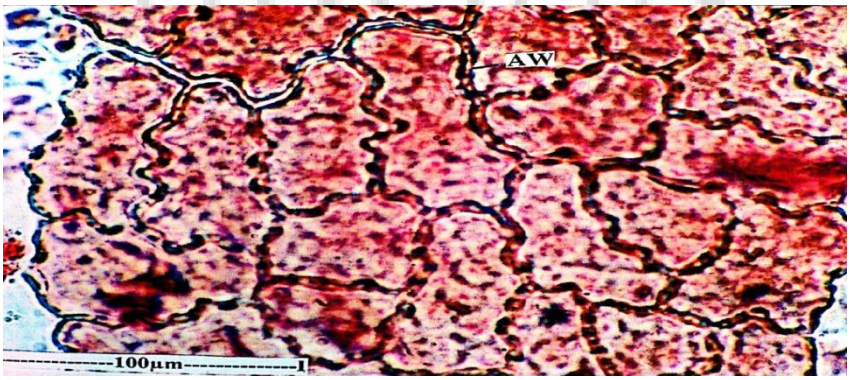
(Tr-Trichome, GP- Ground Parenchyma)

Fig 6: Non glandular epidermal trichome and ground parenchyma cell



OG – Oil Granules

Fig 7: Globular oil body

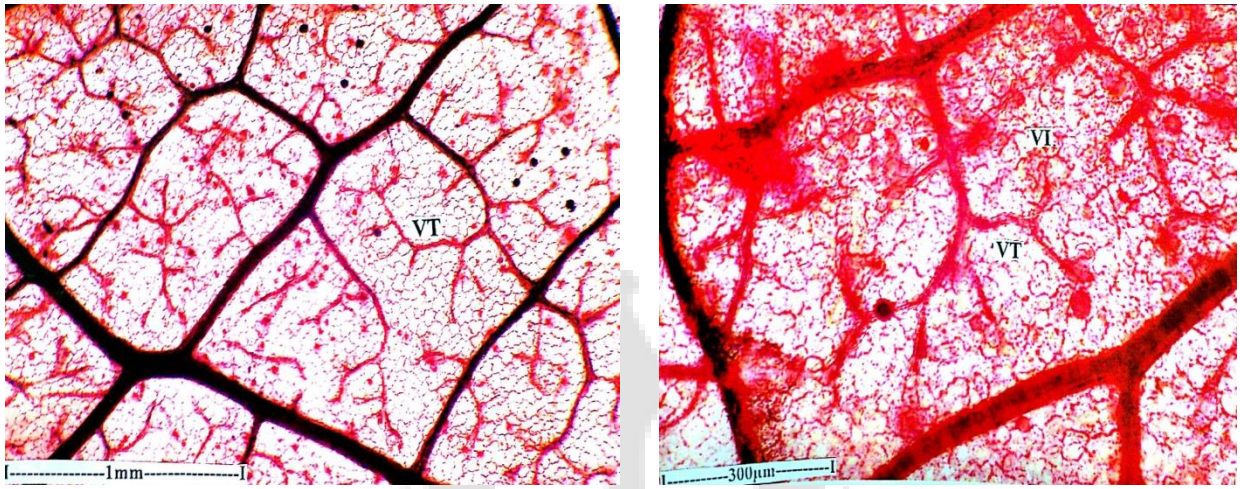


(AW- Anticlinal Wall)

Fig 8: Fragment of adaxial epidermal cell

Venation pattern

The primary lateral veins are thick and straight. The secondary and tertiary veins are thin and branch profusely into smaller units. These branched veins constitute the veins terminations (Fig 9)



(VI- Vein –islet, VT- Vein termination)

Fig 9: Vein-islet and Vein termination number

3.4: Physicochemical parameter

3.4.1: Ash Value and LOD of Leaves of *M.champaca* L.

Range	Total Ash (%)w/w	Acid Insoluble ash (%)w/w	Water soluble Ash (%)w/w	LOD
Minimum	5.2	1.7	0.9	19.5
Average	6.4	2.3	1.2	22.7
Maximum	7.2	2.8	1.9	24.9

3.4.2: Extractive Values of Leaves of *M.champaca* L.

Solvent	Method of Extraction	Extractive value (% w/w)
Petroleum ether	Continues hot percolations (**Soxhlet** apparatus)	7.5
Chloroform		8.3
Ethyl acetate		9.5
Ethanol		12.2
Water		16.3

3.5: Preliminary Phytochemical Screening of Leaves of *M.champaca* L.

Preliminary Phytochemical Screening of Different Solvent Extracts

Tests	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanolic extract	Aqueous extract
Alkaloids					
Mayers Reagent	-	-	-	-	-
Dragendorffs reagent	-	-	-	-	-
Hagers reagent	-	-	-	-	-
Wagners reagent	-	-	-	-	-
Carbohydrates					
Molishch's Test	+	+	+	+	+
Fehlings Test	+	+	+	+	+
Benedicts Test	+	+	+	+	+
Glycosides					
General Test	-	-	-	-	-
Anthraquinone	-	-	-	-	-
Cardiac	-	-	-	-	-
Cyanogenetic	-	-	-	-	-
Coumarin	-	-	-	-	-

Phytosterols					
Salkowski Test	-	-	-	-	-
Libermann Burchard Test	-	-	-	-	-
Saponins	-	-	+	+	+
Tannins	-	-	-	+	+
Proteins & Free Amino Acid					
Millons test	-	-	+	+	+
Biuret test	-	-	+	+	+
Gums & Mucilage	-	-	-	-	-
Flavonoids					
Shinoda test	-	-	+	+	+
Alkaline Reagent test	-	-	+	+	+
Terpenoids	+	+	+	+	-
Fixed Oil	-	-	-	-	-

4. DISCUSSION

Organoleptic evaluation of a crude drug is mainly for qualitative evaluation based on the observation of morphological and sensory profile [29]. Hence we have undertaken this study to serve as a tool for developing standards for identification, quality and purity of leaves of *Michelia champaca.*

Adulteration and misidentification of crude drugs can cause serious health problems to consumers and legal problems for the pharmaceutical industries. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs [30]. Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials [31].

Microscopic evaluation revealed the presence of unicellular unbranched covering trichome in lower epidermis and upper epidermis. The vascular system consists of adaxially flattened semicircular cylinder. The vascular bundles are wedge shaped or triangular which has compactly

arranged wide, thin walled angular xylem elements and small nests of phloem. In lamina, palisade layer consists of 6 or 7 layers of small lobed cells which linked with each other forming aerenchymatous tissue. Powder microscopy showed the presence of non glandular trichome, globular oil body and fragment of adaxial epidermal cell. The ash values are particularly important to find out the presence or absence of foreign inorganic matter such as metallic salts and or silica (earthy matter) [32]. Acid insoluble ash provides information about non-physiological ash produced due do adherence of inorganic dirt, dust to the crude drug [33]. Phytochemical evaluation and molecular characterization of plants is an important task in medicinal botany and drug discovery [34]. Preliminary phytochemical screening of appropriate solvent extracts showed the presence of Carbohydrates, Sterols, Tannins, Flavonoids, Volatile oil and absence of Alkaloids, Proteins, Glycosides and Fixed oil.

5. CONCLUSION

M.champaca has a wide range of phytochemicals which could be useful for treatment of various diseases. Many reports were done on screening of leaves of *Michelia champaca* both *in-vivo* and *in-vitro* exhibited its potency to cure diseases. This present study provided a platform for proper selection and authentication and to assure the quality of *M.champaca* leaves.

Conflict of interest statement: We declare that we have no conflict of interest.

Acknowledgement: The author's extent their heartful thanks for all helping hands **Dr.P.Jayaraman**, Director of Plant Anatomy Research Institute, Tambaram, Chennai for microscopical studies.

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