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Pharmacognostic, phytochemical, physicochemical and TLC profile study Mace (Aril) of *Myristica malabarica* Lamk. (Myristicaceae)

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ABSTRACT

The plant *Myristica malabarica* Lamk. is traditionally used as a medicine and spices in food. It is belonging to family *Myristicaceae*. The plant is native to India and endangered trees are mostly found in western ghats. Extracted with various solvents by successive soxhlet hot extraction process with increasing order of polarity on phytochemical investigation. The extract has shown alkaloids, saponin, tannin and flavones glycosides. It has important medicinal uses like Ayurvedic Medicines. It is traditionally used as anticancer, anti-Inflammatory, anti-Oxidant, Sedative hypnotics, Antimicrobial, Antifertility, Hepatoprotective and cytotoxicity. The chemical constituents such as Malabaricones, Malabaricanol, Isoflavones are isolated. *Myristica* Fragrans also known as fragrant Nutmeg or true Nutmeg. The present study i.e. Pharmacognostic, Phytochemical, Physicochemical and TLC Profile Study of Mace (Aril) Of *Myristica malabarica* Lamk. is helpful in the characterization of the crude drug. Physicochemical and phyto-chemical analysis of mace confirm the quality and purity of plant and its identification. The information collected is useful for further pharmacological and therapeutical evaluation of mace (Aril) Of *Myristica malabarica* Lamk. and anthology of quality control of crude drug.

Keywords: Myristica fragrans, Malabaricone, Malabaricanol, Phytochemical screening, Microscopical study, TLC.

INTRODUCTION

Myristica malabarica is commonly called as Malabar nutmeg or Jatiphala. *Myristica malabarica* seed and seed aril is used as spice in Indian foods. They enhance the taste and aromatic flavor of the food. Recent scientific studies proved their biological activity according to their traditional claim. They are now known to possess Gastroprotective, anticancer, antioxidant, antifungal, anthelmintic. Plant have played a significant role in maintaining human health & improving the quality of human life for thousands of years & have served humans well as valuable components of medicines. Herbal Medicine is based on the premise that plant contain natural substances that can promote health. Malabar Nutmeg is a tree about 25 m tall. Bark is greenish-black, smooth; blaze reddish. Branches are horizontal, branchlets round, hairless. Red sap oozes from cut end of bark. Alternately arranged leaves, 9.5-22 x 3.7-10 cm, are elliptic or elliptic-oblong, tip pointed, base narrow or flat, margin entire, glossy above, hairless and glaucous beneath, leathery. Leaf-stalks are 1.0-1.5 cm long. Midrib is raised above, secondary_nerves 8-14 pairs. Flowers are unisexual, urn-shaped, and white. Male flowers are numerous, smaller than female flowers, borne in cymes in leaf axils. Female flowers are borne in 5-6 flowered umbels. Capsule is 5-7.5 x 1.8-3.5, oblong, velvety, with one oblong seed. Aril covering the seed yellow and stringy. Malabar Nutmeg is endemic to the Western Ghats - South and Central Sahyadris [1-2].

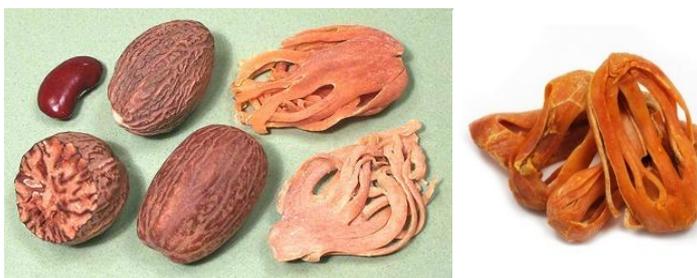


Figure 1: Mace (Aril) of *Myristica malabarica* Lamk.

Taxonomical Classification

Plant Taxonomy

Kingdom	: Plantae
Super division	: Angiosperms
Phylum	: Tracheophyta
Class	: Magnoliopsida
Order	: Mangoliales
Family	: Myristicaceae
Genus	: Myristica
Species	: <i>Myristica malabarica</i>

Synonyms

Myristica dactyloides Wall
Myristica notha Wall
Myristica tomentosa J.Grah
Palala malabarica Kuntze

Common Names

Malabar nutmeg, false nutmeg, Bombay mace.

Vernacular Names

Hindi : Ramptri, Bambah-jayphal.
Kannada : Kanage, Doddajajikai.
Malayalam : Ponnampooovu, Kottappannu, Pathiripoovu, Panampalka.
Sanskrit : Bandhukapushpa, Gostani.
Tamil : Colaivenkai, Kattujatikikai.
Telugu : Adavijaikaya, Adividzajikaya.

Habit and Habitat

It is a large 15-25 mts tall perennial tree found evergreen forests upto 800mts. It is a swamp and lowland forest habitat tree. Large trunks with greyish black color. Flowering and fruiting season starts at feb-aug month. It is vulnerable species listed according to ICUN list due to drainage of swampforests for agricultural purposes.

Ethano Medicinal Uses

The plant *Myristica malabarica* is traditionally used as medicine and spices in food. The aril is used as febrifuge, cooling, expectorant. In Ayurveda, aril is used for many conditions related to vata such as, fever, bronchitis, cough and burning sensation. The fat extracted from seed is used to treat indolent ulcers, analgesics and for rheumatism. In Ayurveda, for disorders in vata seed fat is used for myalgia, sprains and sores. The plant is also used for anti-inflammatory, Analgesic, anti-ulcer, sedative, hypnotic, and antimicrobial actions [1-2]. The nutmeg is bitter, aromatic, astringent. They are useful in inflammations, cephalgia, helminthiasis, halitosis, dyspepsia, flatulence, nausea, vomiting, diarrhea, dysentery, colic, asthma, catarrh, neuralgia, lumbago, stangury, amenorrhea, menorrhagia, dysmenorrhea, ulcers, liver and splenic disorders, eye diseases, impotency, skin diseases, freckles, cracks in feet, insomnia, delirium tremens, hyperdypsia, cardiac disorders, fever and general debility. Concrete oil of nutmeg is used in cases of mild ringworm, chronic rheumatism, paralysis and sprains. Nutmeg and mace spice contains many plant derived chemical compounds that are known to have been antioxidant, disease preventing, and health promoting properties. The spicy nut contains fixed oil trimyristin and many essential volatile oils such as which gives a sweet aromatic flavor to nutmeg like myristicin,

elemicin, eugenol and safrole. The other volatile oils are pinene, camphene, dipentene, cineole, linalool, sabinene, safrole, terpeniol [10-11].

Pharmacological actions

Aphrodisiac, anti-inflammatory, anodyne, antipyretic, anthelmintic, deodorant, digestive, carminative, stomachic, expectorant, diuretics, emmenagogue, antispasmodic, febrifuge, narcotic, stimulant, ophthalmic, anticonvulsant, antiseptic, constipating and tonic. Antihepatotoxic, hypolipidemic and antifungal. The active principles in nutmeg have many therapeutic applications in many traditional medicines as antifungal, anti-depressant, aphrodisiac, digestive, and carminative functions. *Myristica fragrans* is a good source of minerals like copper, potassium, calcium, manganese, iron, zinc and magnesium. Potassium is an important component of cell and body fluids that helps control heart rate and blood pressure. Manganese and copper are used by the body as co-factors for the antioxidant enzyme, superoxide dismutase. Iron is essential for red blood cell production and as a co-factor for cytochrome oxidases enzymes. It is also rich in many vital B-complex vitamins, including vitamin C, folic acid, riboflavin, niacin, vitamin A and many flavonoid anti-oxidants like beta-carotene and cryptoxanthin that are essential for optimum health [12-13].

MATERIALS AND METHODS

Collection of plant material

Dried aril part of *Myristica malabarica* were collected from local Ayurvedic shop in Alephata Junner and authenticated by Pharmacognosy department of Vishal Institute of Pharmaceutical Education And Research. The dried aril portions of Jatiphala were cleaned, coarsely powdered and used for microscopic and macroscopical characterization, preliminary phytochemical evaluation and TLC.

Extraction

Powdered plant material was extracted with 250 ml of petroleum ether then ethanol using Soxhlet apparatus for 72hr and extract was filtered through cotton wool. The filtrate was dried and concentrated under reduced pressure at 50° C.

Description

(A) Macroscopic Examination

Botanical description

Morphology were mention in Table1.

(B) Microscopic Examination

The fragments of aril with oil cells encircled by epithelial cells embedded with oil globules, endosperm cells with starch, aleurone grains and fragments of scleriform xylem tracheids and thin walled fibers are scattered as such throughout (in Table 2) [3, 4, 5].

(C) Physico-Chemical Parameter

Crude powdered drug of mace(Aril) was used for the determination of various physicochemical parameters such as

total ash value, acid insoluble ash value, water soluble ash value, loss on drying, foreign matter, pH, moisture content and extractive values. The results of the physicochemical analysis were mentioned in the Table 3 [6, 7].

(D) Phytochemical Screening of Mace (Aril) extract

The phytoconstituents present in the alcoholic extract of were expressed in the Table:4 Phytochemical screening procedure

1) Test for alkaloids

To the extract dilute hydrochloric acid will be added and filtered. The filtrate will be treated with various alkaloid reagents

- Mayer's test:** The filtrate will be treated with Mayer's reagent; appearance of cream colour indicates the presence of alkaloids.
- Dragendroff's test:** The filtrate will be treated with Dragendroff's reagent; appearance of reddish brown precipitate indicates the presence of alkaloids.
- Hager's test:** The filtrate when treated with Hager's reagent, appearance of yellow colour precipitate indicates the presence of alkaloids.

2) Test for carbohydrates and reducing sugar

The small quantities of the filtrate will be dissolved in 4ml of distilled water and filtered. The filtrate will be subjected to

- Molisch's test:** A small portion of the filtrate will be treated with Molisch's reagent and sulphuric acid. Formation of a violet ring indicates the presence of carbohydrates.
- Fehling's test:** The extract will be treated with Fehling's reagent A and B. The appearance of reddish brown colour precipitate indicates the presence of reducing sugar.

3) Test for steroids

Liebermann bur chard's test: The extract will be treated with 3ml of acetic anhydride, few drops of glacial acetic acid followed by a drop of concentrated sulphuric acid. Appearance of bluish green colour indicates the presence of steroids.

4) Test for proteins

- Biuret test:** The extract will be treated with copper sulphate solution, followed by addition of sodium hydroxide solution; appearance of violet colour indicates the presence of proteins.
- Millon's test:** The extract will be treated with Millon's reagent; appearance of pink colour indicates the presence of proteins.

5) Test for tannins

The extract will be treated with 10% lead acetate solution; appearance of white precipitate indicates the presence of tannins.

6) Test for phenolic compounds

- The extract will be treated with neutral ferric chloride solution; appearance of violet colour indicates the presence of phenolic compounds.
- The extract will be treated with 10% sodium chloride solution; appearance of cream colour indicates the presence of phenolic compounds.

7) Test for flavonoids

- 5ml of extract will be hydrolyzed with 10% sulphuric acid and cooled. Then, it will be extracting with diethyl ether and divided in to three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution will be added to the first, second and third test tubes respectively. In each test tube. Development of yellow colour demonstrated the presence of flavonoids.
- Shinoda's test:** The extract will be dissolved in alcohol, to which few magnesium turnings will be added followed by concentrated HCL drop wise and heated, and appearance of magenta colour shows the presence of flavonoids.

8) Test for gums and mucilage

The extract was treated with 25 ml of absolute alcohol, and filtered. The filtrate will examine for its swelling properties.

9) Test for glycosides

When a pinch the extract was treated with glacial acetic acid and few drops of ferric chloride solution, followed by the addition of conc. Sulphuric acid, formation of ring at the junction of two liquids indicates the presence of glycosides.

10) Test for saponins

Foam test: About 1 ml of the extract was diluted to 20 ml of distilled water and shaken well in a test tube. The formation of foam in the upper part of test tube indicates the presence of saponins.

11) Test for Triterpenoids

The substance was warmed with tin and thionyl chloride. Pink colour indicates the presence of triterpenoids [4, 6, 7, 8].

(E) Thin layer chromatography

TLC was performed according to the method described in WHO guidelines with slight modifications 5µl extract was spotted in the TLC plate. Extract were developed in Chloroform: Methanol: Formic acid (2.89: 2.5: 1) mobile phase and observed under U.V. 366nm and after spraying with vanillin sulfuric acid. The Rf values and colour of the spots were recorded with Rf value 0.612 (Fig.4) [7, 8, 9, 10].

RESULTS

Morphological description:

The Mace (Aril) Of *Myristica malabarica* Lamk. were Dark orange in colour. The mace was Aromatic, Pungent, Irregular in shape and rough in surface (Fig:1).

Microscopic examination:

The fragments of aril with oil cells encircled by epithelial cells embedded with oil globules, endosperm cells with starch, aleurone grains and fragments of scleriform xylem tracheids and thin walled fibers are scattered as such throughout (Fig. 2).

Physico-Chemical Parameter:

The results of physicochemical analysis were mentioned in the Table: 3 which signify that the quality and purity of the raw material was good.

Phytochemical Analysis - Qualitative

The results of qualitative analysis of extracts of Mace (Aril) Of *Myristica malabarica* Lamk. are illustrated in Tables 3 and 4 respectively. On basis of the intensity of the reaction product of qualitative tests, the data were graded as present (+) and absent (-). The phytochemical tests employed indicated that ethanolic extract contained most of the secondary metabolites. However the extractions carried out with petroleum ether indicated little amount of phytoconstituents. The major phytoconstituents detected were alkaloids, glycoside, tannin and phenolic compound, flavonoids, protein and amino acid, phytosterols, terpenoids and carbohydrates. Gum & mucilage were absent in all solvent extractions (Fig.3). The observations from quantitative analysis suggested that ethanol could effectively extract flavonoids and phenolics. The results also indicated that the Mace (Aril) Of *Myristica malabarica* Lamk. is a comparatively richer source for flavonoids and phenolic. The relatively higher efficiency of ethanol in extraction of flavonoids and phenolics could be attributed to the better solubility level of these constituents in ethanol than in aqueous medium. Ethanol may have more penetration capacity than water to release the phytochemicals from intracellular regions where they are stored. All the secondary metabolites detected in the present study are reported to have many bioactive and therapeutic properties. The medicinal property of the Mace (Aril) Of *Myristica malabarica* Lamk. as evident from ethno botanical and folkloric uses, could be attributed to the presence of these secondary metabolites [10-13]. The plant used in the present study is widely used to cure many diseases in folkloric medicine. Mace (Aril) Of *Myristica malabarica* Lamk. are antihelminthic as well as antiseptic and this might be due to the antimicrobial properties owing to its high alkaloid content [10-13].

Thin layer chromatography

The Rf values and colour of the spots were recorded with Rf value 0.612 (Fig.4).

Table 1: Morphology of Mace (Aril) Of *Myristica malabarica* Lamk.

Sr. No.	Characters	Apperance
1.	Colour	Dark orange
2.	Odour	Aromatic
3.	Taste	Pungent
4.	Shape	Irregular
5.	surface	Rough

Table 2: Staining / Diagnosis/ Microchemical Test

Sr. No.	Reagents	Observations	Characteristics
1	Phloroglucinol+Hcl (1:1)	Pink	Lignified tissues:xylem (vascular bundle)
2	Sudan Red III	Pink	Cutin/cuticle
3	Ruthenium red	pink	Mucilaginous cells of epidermis

4	Sulphuric acid	Needle shape crystals from calcium sulphate are formed.	Calcium oxalate crystals
5	Alcoholic	Picric acid Yellow	Aleurone Grains
6	Iodine	Blue	Starch
7.	Acetic Acid	Insoluble	Calcium oxalate crystals

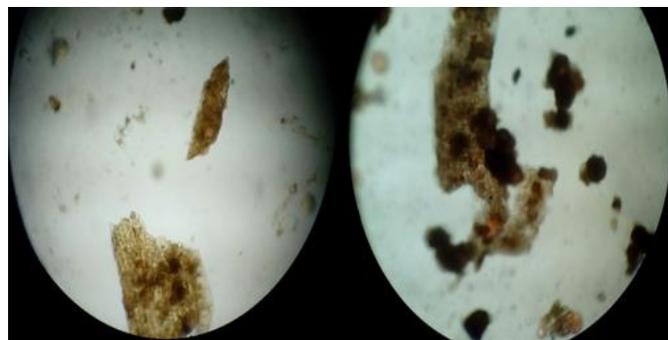


Figure 2: Microscopic Examination of Mace (Aril) of *Myristica malabarica* Lamk.

Table 3: Physico-Chemical constant Mace (Aril) Of *Myristica malabarica* Lamk.

Sr. No.	Parameters	Results
1	Loss on drying	11.04
2	Total ash	2.04
3	Acid insoluble ash	0.30
4	Water soluble ash	1.00
5	Alcohol soluble extractive value	12.60
6	Water soluble extractive value	9.56

Table 4: Phytochemicals present in the successive extracts of Mace (Aril) of *Myristica malabarica* Lamk.

Phytochemicals	Test	Petroleum Ether	Ethanol
Alkaloids	Dragendorff's test	+	+
	Hager's test	+	+
	Mayer's test	+	+
Glycoside	General test	+	+
	Ferric chloride test	-	+
Saponins	Foam Test	-	+
	Lead Acetate Test	-	+
Flavonoids	Shinoda Test	+	+
	Lead Acetate Test	+	+
Carbohydrates	Fehling's Test	-	+
	Molisch Test	-	+
	Million's Test	-	+
Proteins & Amino Acid	Biuret Test	-	+
	Salkowaski test	-	+
Gum & mucilage	Ruthenium red test	-	-
Terpenoids	Liebermann-Buchardt Test	+	+

(Duration of extraction = 72 h; Treatment temperature = 50°C)
(-)=Absent (+) =Present

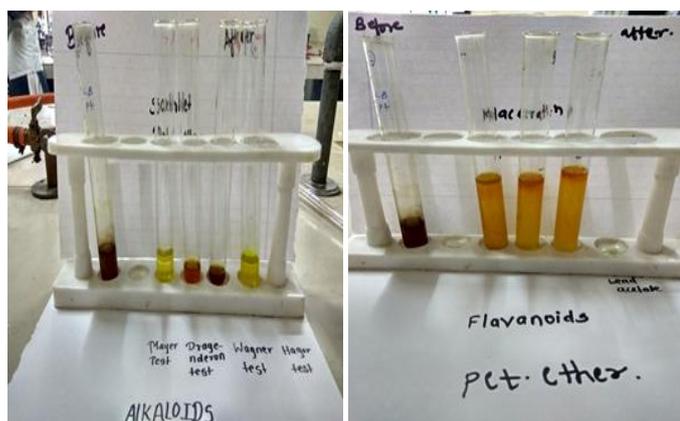


Figure 3: Phytochemical screening of alcoholic extract of Mace (Aril) of *Myristica malabarica* Lamk.



Figure 4: Thin layer chromatography of Mace (Aril) of *Myristica malabarica* Lamk.

DISCUSSION

Now-a-days there has been drastic increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the availability of modern techniques, it is more reliable to identify a plant drug by pharmacognostic evaluation. A complete and systematic study of a crude drug which comprises of collection, preservation, storage, macroscopical, microscopical, organoleptic characters, etc. is claimed to be the scientific or pharmacognostic evaluation. Standardization is an essential measure for quality, purity and sample identification [14]. Standardization of herbal drugs is a very challenging task for herbal drug industry because of complex nature and variation of chemical constituents. Microscopical evaluation is one of the simplest methods for identification of drugs. According to WHO, the macroscopic and microscopic evaluation is the first step to be carried out to establish its identity and purity. The evaluation of physico-chemical constants is an important parameter in detecting adulteration or improper handling of drugs. The extractive values are immensely useful to evaluate the chemical constituents that are present in the crude drug. These extractive values are also helpful in the estimation of specific constituents soluble in particular solvent [15]. The total ash value obtained from the study can be used to detect foreign organic matter and adulteration with sand and earth, therefore, reflecting the kind of care that must be taken in preparing the plant for drug. Ash value varies within equitably wide limits and is therefore an important parameter for the evaluation of crude drug [16]. All crude drugs were standardized for the active phytoconstituents. Here preliminary phytochemical studies confirmed the presence of alkaloids, glycoside, tannin and phenolic compound, flavonoids,

protein and amino acid, phytosterols, terpenoids and carbohydrates. These findings are not only helpful in the pharmacological and therapeutic evaluation of the mace but also assist in standardization for quality, purity and sample identification. In the present investigation various standardization parameters such as Pharmacognostic, Phytochemical, Physicochemical and TLC Profile of Mace (Aril) Of *Myristica Malabarica* Lamk. could be help in authentication of Mace (Aril) of *Myristica malabarica* Lamk. The result of present study will also serve as reference material in preparation of monograph.

CONCLUSION

The study concluded that of Mace (Aril) of *Myristica malabarica* Lamk. is an interesting source of secondary metabolites with potential for use as a medicinal plant. Pharmacognostical studies can serve as a basis for proper identification, collection and investigation of the plant. The present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. In other words, the pharmacognostic features examined in the present study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations. These parameters, which are being reported, could be useful in the preparation of the herbal monograph for its proper identification. The present study i.e., Pharmacognostic, Phytochemical, Physicochemical and TLC Profile of Mace (Aril) Of *Myristica Malabarica* Lamk. is helpful in the characterization of the crude drug. Physicochemical and phyto-chemical analysis of mace confirm the quality and purity of plant and its identification. The information collected is useful for further pharmacological and therapeutical evaluation of mace (Aril) of *Myristica Malabarica* Lamk. and anthology of quality control of crude drug. The Mace (Aril) was screened for phytochemical constituents and found to be good source of medicinally active elements which can be further exploit to isolate and synthesize modern medicines. This work justifies the need to isolate and characterize the medicinally active compounds and TLC Profile also confirm medicinal bioactive content.

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