ABSTRACT

The present study is carried out with the motto to study in-vitro evaluation parameters for an Indian spice Myristica fragrans Houtt. (Myristicaceae). The study design includes Pharmacognostic, Quality Control and Analytical Evaluation of the crude drug. This information will be helpful in lying down the primary parameters for standardization of the crude drug, so we have undertaken this task of generating information in a scientific manner. The study revealed the micro finding of Transverse section, Lateral section and Powder characteristics of seed kernels of Myristica Fragrance Houtt. We have also documented the Microscopic fingerprints of the said crude drugs. The quality control parameters revealed the values for moisture content, total ash, acid insoluble ash, water soluble extractive, methanol soluble extractive, hexane soluble extractive, petroleum ether extractive. The analytical data includes details of UV, IR, TLC and HPTLC of extract.

Key words: Myristica fragrans Houtt, quality control, microscopy, HPTLC, myristicine.

INTRODUCTION

In every Indian home we find abundant herbal medicines which are still used for minor ailments or infections. The herbal remedies include natural substances such as fruits, vegetables, herbs and spices. These herbs and spices have abundant of curative properties that eliminate the disturbing elements of the body from the root. In present work we are focused on study of different in-vitro evaluation parameters for an Indian spice Myristica fragrance Houtt. (Jaiphala).

The plant Myristica Fragrance Houtt. Fig.1, its seed kernel and mace are used since ancient time in India and is known as Jaiphala (Marathi), magic, muscier, nutmeg, muskatbaum and mace in different regional languages. In traditional Indian medicine an aqueous extract of nutmeg is used for infantile diarrhea. It is used as condiment, spice, as a flavoring agent in dessert preparations like ladoo, keer, payasam. Burkill et al reported uses of Nutmeg as stimulant, carminative, astringent and aphrodisiac. It is used in tonics and electuraries and forms a constituent of preparations prescribed for dysentery, stomach ache, flatulence, nausea, vomiting, malaria, rheumatism and early stage of leprosy.

Nutmeg is the dried, ripe seed kernel of Myristica Fragrans Houtt. belonging to family- Myristicaceae. The fruit, which is called as drupe or a nutmeg apple, is similar in appearance to a peach or an apricot Fig 2. When the mature fruit splits open, the nutmeg (stony endocarp or seed surrounded by a red, slightly fleshy network or aril) is exposed. The dried aril alone is called mace.

Reported activities of Myristica fragrans includes; soothing, anti-inflammatory and so used to treat joint pain and muscle pain, anti diarrhoeal, analgesic and antithrombic activity in Rhodents. K. K. Lee et al (1999), found that methanolic extract (IC50, 284.1 µg/ml) may be potent inhibitor of the anti-ageing process in skin by utilizing human leukocyte elastase and also used for anti-inflammatory activity (chloroform extract), hyperlipidemia in Albino rats, platelet anti-aggregatory activity, antimicrobial activity and sedative property (petroleum ether) 10. The antibacterial activity of Nutmeg (water extract) against 20 serotypes of E. coli revealed that they were sensitive to the extract at 100 %, 75% concentrations and against serotypes of Salmonella, Aeromonas hydrophila, Listeria monocytogenes, Salmonella weltevreden and Salmononella Worthington 11. Essential oil showed anti-microbial activity of against Bacillus subtilis, Escherichia coli, Salmonella typhi, Klebsielle pneumoniae, as well as potent anti-oxidant activity 13.

Chung J. H. et. al. found that the mace lignan is effective antioxidant agents Streptococcus mutans at a concentration 3.9 mcg/ml which was much lower concentration than those of other natural anticariogenic agents viz.; Sanguinarine (15.6 mcg/ml), Eucalyptol (250 mcg/ml), Menthol and thymol (500 mcg/ml) 14. The anticariogenic compound was isolated from methanol extract of Myristica fragrance Houtt. by repeated silica gel chromatography. The literature data suggests that the drug possess potent antimicrobial activity and this is the reason why various sweet preparations in India like Ladoo, Payasam, Shrikhans, Basundi etc. contains small amount of nutmeg power as a flavoring and stabilizing agent.

Fig.1. Plant of Nutmeg
Ehlers D. et al studied HPLC analysis of nutmeg and mace oils produced by supercritical \( \text{CO}_2 \) extraction and compared with steam distilled oils, comparison of typical fingerprints of East Indian West Indian and Papuan Oils found to contain; myristicin, safrole, elemicin, eugenol, methyl eugenol, isoeugenol, methylisoeugenol, methoxyeugenol and isoellemicin in different amounts and ratios which was found by using HPLC. Other constituents found are terpenes, terpene alcohols and oleoresins. The narcotic and poisonous effect of numeg and mace at high dosage is particularly attributed to their content of myristicin, safrole and elemicin. Essential oil, fixed oil and aroma glycosides of nutmeg were separated and studied by Anathakumar et al.

The present study is carried out to get a complete pharmacognostic picture of drug and its identification and confirmation by various means; morphology, microscopy, microchemical tests, powder characteristics, phytochemical analysis, quality control parameters, analytical parameters like UV, IR, TLC and HPTLC. The important phytochemical have been isolated from this drug and some of them are characterized.

This paper reports the pharmacognostic investigation and various quality control parameters of Indian \textit{Myristica Fragrance} Houtt.

**MATERIALS AND METHODS**

**Plant Material:**
The dried seed kernels of \textit{Myristica Fragrance} were purchased from local market of Santacruz (E.) Mumbai -400 098, and was authenticated at Agharkar research institute, Pune and the allotted Anth. No. is 08-125. The voucher specimen (P-18) was retained in the museum at Dept. of Pharmacognosy, Bombay College of Pharmacy, Mumbai-400 098. The chemicals used for the study were of AR and LR grade.

**In-vitro Morphological and Microscopical Evaluation:**
Initial weight variation test was performed for seed kernel of nutmeg by using Single pan electronic balance. The Morphological Evaluation includes observation of colour, odor, taste, shape and size and extra features by using simple lens. In microscopy, the histological sections were taken manually and also by using Microtome apparatus Courtesy Marico Pvt. Ltd. BCP center. Photomicrographs were documented for stained sections and powder by using Compound binocular inverted microscope attached to Computer and Camera. The sections taken manually were thinner than taken by means of Microtome apparatus. The Phytochemical evaluation was carried out as per tests in official books.

**Quality Control and Analytical Parameters:**
The quality control parameters were carried out as per procedures cited in Indian Pharmacopoeia, FT/IR analysis, UV analysis, TLC and HPTLC analysis by using instruments viz., Soxhlet apparatus, Muffle furnace, Hot air oven, Jasco FTIR 5300, UV spectrophotometer Jasco-530, CAMAG Linomat 5, Hamilton syringe and WinCATS Software respectively.

**TLC**
Methanolic extract, few \( \mu l \) (microlitre) of stock solution (1 mg/ml) was manually spotted on precoated aluminum (10/5cm) TLC plates (Silica gel G.E. Merck). The chromatogram was developed by ascending technique until the solvent front moved through a distance of 8.5 cm on the plate of 10.0 cm in a chamber saturated with vapors of mobile phase (toluene: ethyl acetate of ratio 9.3:0.7). The plate were observed in UV chamber.

**HPTLC**
Initially different concentrations of methanolic extract were prepared as 30, 60, 90, 120, 150, 1000 microgram/ml in 10 ml volumetric flask. Few microlitre of these dilutions were applied as band on HPTLC plate with the help of CAMAG Linomat 5 applicator Hamilton syringe (Fig.6). Further the chromatogram was developed by using the same solvent system as that of TLC, then the plate was dried and scanned by using Camag TLC scanner and WINCATS 4 software for interpretation of data.

The reported \( R_f \) values for myristicin, eugenol was compared with literature data.

**RESULTS AND DISCUSSIONS**
The seed kernel was broadly oval brown to grayish brown externally, marked with numerous minute dark reddish brown points and lines; strong and aromatic odor; pungent, aromatic and slightly bitter in taste. About 2-3 cm long, 2 cm wide. External surface is reticulately furrowed. A small circular depression at one end indicates the position of the ‘radicle’ of the embryo. A groove marking the line of raphe extends from the opposite end of the kernel where the ‘Chalaza’ is situated Fig.2.

![Figure:2 Seed kernel of Nutmeg](image)

Average weight of seed kernel was recorded as 3.00 gm, minimum weight was 2.5gm and maximum weight was 3.2 gm calculated with the 50 such seeds.

T.S. of whole seed shows marble like appearance Fig. 3a. and constitutes perisperm, endosperm and veins Fig. 3b. Perisperm is a thin layer about 0.12 mm thick is divided into 2 parts outer perisperm and inner perisperm. The outer perisperm constitutes cells which are polyhedral and much flattened dark brown colored, and some of them contain a prismatic / disc shaped crystal thought of Potassium acid tartrate. Inner perisperm shows numerous extensive lamellae, corresponding to the furrows on the surface, grows into endosperm to form the blunt ended ruminations/veins). The lamellae composed of parenchymatous cells within thin brown walls and numerous large oval oil-cells filled with the volatile oil and in their outer part vascular strands composed of lignified spiral vessels.

![Figure:3a T.S. of Whole Seed Kernel](image)

![Figure:3b T.S. showing Perisperm and endosperm](image)

![Figure: 4 L.S. showing lamellae containing oval oil cells](image)
Endosperm-It contains polyhedral parenchyma, the cell of which contains fat, aleurone and starch grains. The surface of LS appeared as lustrous and like a marble Fig. 4. It shows the embryo which lies in a small cavity in the endosperm and its cotyledons gradually grow up into the endosperm, as they absorb the food it contains. Rest all other characters were same as that of T.S. The sections were subjected to different micro-chemical tests by using various staining reagents and observed for different stains under microscope (Table 1).

<table>
<thead>
<tr>
<th>Part</th>
<th>Reagent</th>
<th>Test for</th>
<th>Colour</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.S.</td>
<td>Iodine solution</td>
<td>Starch</td>
<td>Blue</td>
<td>+</td>
</tr>
<tr>
<td>Phloroglucinol+ conc. HCL (1:1)</td>
<td>Lignin</td>
<td>Pink</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sudan red-III</td>
<td>Oil glands</td>
<td>Pink</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Alkana extract</td>
<td>Oil glands</td>
<td>Red</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Dil. Acetic acid</td>
<td>Calcium oxalate</td>
<td>Insoluble</td>
<td>+</td>
<td></td>
</tr>
</tbody>
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Table: 1 Micro-chemical tests of Section of seed kernel of M. fragrance

The Powdered Drug Fig. 5 has shown presence of a) Fibers-few, long and coiled and Starch grains-simple and compound type (cluster particles), b) Parenchymatous cells- abundant, brown colored, surrounded by circular small cells, c) Crystals- few calcium oxalate crystals with clear and lustrous surface and special Crystals (may be of Potassium acid tartrate).

Nutmeg extract was found to contain various phytochemical; Sugars (reducing sugars, hexose, pentose), phenols, lipids (fixed oil), drying oil, volatile oil (terpenoids), carbohydrates (starch, mucilage), glycosides, tannins, flavonoids (phenolics) and Proteins (amino acid).

Figure: 5 Powder features a)Fibre and starch grains b) Parenchymatous cells c)Calcium oxalate Crystal d) Crystal

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Fragmentation m/z</th>
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Table: 2 Mass Fragmentation Pattern

Figure: 6 Chromatogram of Myristicine

The UV spectroscopic analysis of methanolic extract exhibited the λ max 274 nm, 271 nm and reported values are 211 nm, 274 nm and 271 nm by using UV-Visible spectrophotometer, Jasco V-530. The data obtained matches with the literature data hence it is extract of Myristica fragrance Houtt.

Figure: 7 HPTLC Pattern of Nutmeg extract at 254 nm

The HPTLC studies revealed the presence of Myristicine, allyl tetramethyl benzene, elemicin, apiole and eugenol in the methanolic extract of Myristica fragrance based on Rf values as; 0.80, 0.45, 0.40, 0.75, 0.5 respectively.
CONCLUSION:
This paper reports the complete Pharmacognostic and Analytical picture of Myristica fragrans Houtt. and its identification and confirmation also. The diagrammatic representation and photographs is one of the Microscopic fingerprints and is invaluable information. This data can be interpreted as Myristica fragrans Houtt. is a tough seed kernel and contains less moisture so it will not deteriorate easily and can be stored for years together in quarantine area / laboratory storage area as well as in kitchen. The ash value indicates presence of fewer amounts of silica and other inorganic particles. Comparing the extractive values we can comment that fat content is very high and Volatile components becomes less in stored seeds, these may be responsible for very less smell of seed if stored for long time but intense smell can be felt in the room if broken in mortar with pestle. The Analytical data obtained by UV, IR, HPTLC may help in identification of chemical constituents as Myristicine, eugenol, elemicin, apiol in the extract.

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