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Macro-microscopic evaluation, physicochemical analysis, standardization and HPTLC finger printing of aril part of *Myrstica fragrans Houtt.*

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Abstract:

Myrstica fragrans Houtt. Family Myristicaceae is an evergreen moderate sized aromatic tree with grayish black bark having lenticular spots on the outside and red juice on inner side. Fruits yellow, globose or pyriform, pericarp fleshy, seeds oblong, testa shiny aril yellowish red, irregular lobed. The fruit portion and aril portion have been significantly used in the treatment of various ailments including male infertility.

Key words:

Myristica fragrans, HPTLC fingerprinting, Pharmacognostic, Standardization, Quality control.

Introduction

Myristica fragrans Houtt. is a well known medicinal plant known as Jayaphala in Hindi.[1] It is a evergreen moderate sized aromatic tree with grayish black bark having lenticular spots on outer side and red juice on inner side.[2] The plant is native of Moluccas now cultivated in many tropical countries of both hemispheres. In India, it is grown in Nilgiri, Coimbatore, Salem, Ramanatha puram, Tirunelveli, Kanyakumari etc[3]. In Sharangadhara samhita Purvakhanda, Deepana pachanadi adhyaya, Jatiphala is mentioned as Shukrasthmabaka dravya[4] and also in the madhyama khanda, churna Kalpana adhyaya it is mentioned that the Jatiphaladi churna is beneficial in

kasa,shwasa, aruchi, kshaya etc.conditions.

In Atisara chikitsa of Bhavaprakasha samhita, uttarardha, Jatiphala is mentioned as one of the dravya of Vijayava leha[5].

M.fragrans has been reported with the chief chemical constituent Myristicin.[6] other than that, some of other phytochemical constituents present in different parts of Myristica fragrans are;Alpha-terpinine,Alpha-thujene,Amylodextrin,caprylic acid,Malabaricane B, Malabaricane C, Calcium, carbohydrates etc.

And also the kernels of Myristica malbarica Lam. are sometimes mixed with the material. Also the fruits of Myristica dactyloides Gaerth are mixed with the fruits of Myristica malabarica Lam for the purpose of adulteration[7],[8].With this background, detailed quality control studies were undertaken for this traditional raw drug with the aim of developing standards of authenticity.

Materials and Methods:

Collection and Identification

Dried aril part of Jatiphala were collected from local Ayurvedic shop in Udupi, Karnataka. The plant material was authenticated at Pharmacognosy department of SDM Centre for Research in Ayurveda and Allied Sciences, Udupi,Kuthpady, Karnataka. And (a specimen code 16032903) is being mentioned for further reference.The dried aril portions of Jatiphala were cleaned, coarsely powdered and used for macroscopic and microscopical characterization, phytochemical analysis and HPTLC.

Macro-microscopic analysis:

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

Microscopy

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

A pinch of powder was warmed with drops of chloral hydrate on a microscopic slide and mounted in glycerine. Slides observed under microscope and diagnostic characters were observed and 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

Physico chemical analysis: The results of the Physico chemical Analysis are mentioned in the below mentioned Table-1.

HPTLC finger printing

1gm of *Jatiphala* powder was extracted with 20 ml of alcohol, kept overnight and filtered . 4, 8 and 12µl 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 n-hexane: Chloroform (1.0: 1.0). The developed plates were visualized in UV 254, 366, and then derivatised with anisaldehyde sulphuric acid reagent and scanned under UV 254nm, 366nm and 620nm. R_f, colour of the spots and densitometric scan were recorded.

Part C: Results

Figure 1: Macroscopy of *Myristica fragrans*

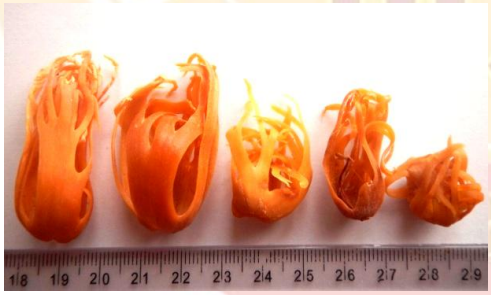

Figure 2: Microscopy of Aril of *Myristica fragrance*

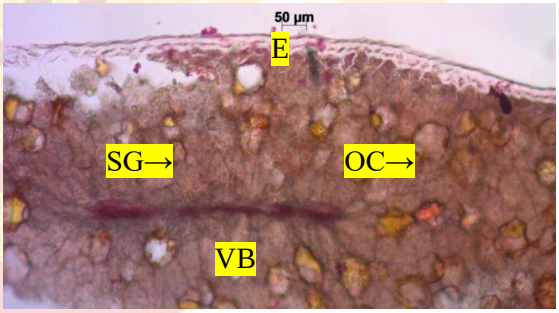

Fig 2a: T.S of aril

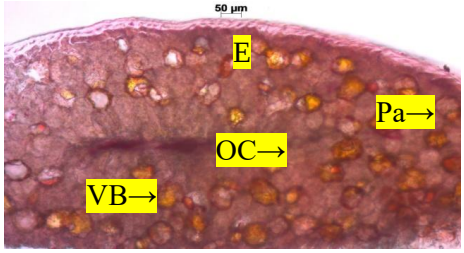

Fig 2b: A portion enlarged

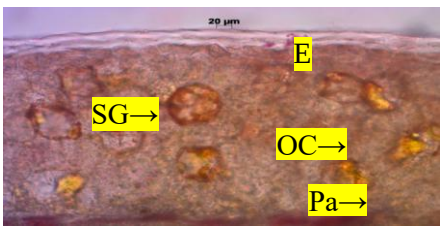

Fig 2c: Outer portion enlarged

E – epidermis; OC – oil cell; Pa – parenchyma; SG – starch grains; VB – vascular bundle

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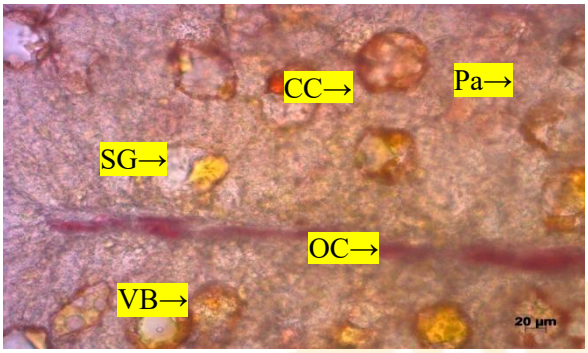
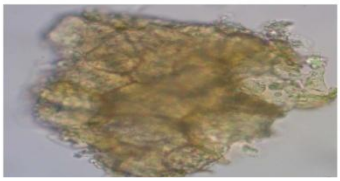


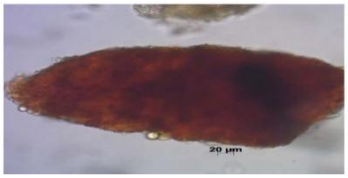
CC – content cell; Ep – epidermis;
OC – oil cell; Pa – parenchyma;
SG – starch grains; VB – vascular bundle

Fig 2d: Inner portion enlarged

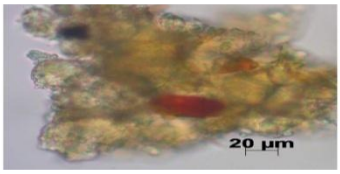
Figure 3: Powder microscopy of Aril of *Myristica fragrance*



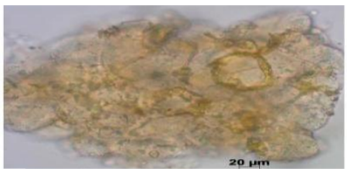
Parenchyma cells with content



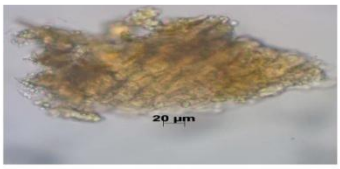
Parenchyma cells with starch



Parenchyma cells with oil



Polygonal parenchyma cells



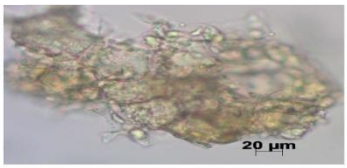


Table 1. Results of standardization parameters of Aril part of *Jatipatra*

Parameter	Results n = 3 %w/w
Loss on drying	11.04
Total Ash	2.04
Acid Insoluble Ash	0.30
Water soluble Ash	1.00
Alcohol soluble extractive value	12.60
Water soluble extractive value	9.56
Volatile oil percentage	4 %v/w

Figure 4. HPTLC photo documentation of ethanol extract of *Jatipatra*

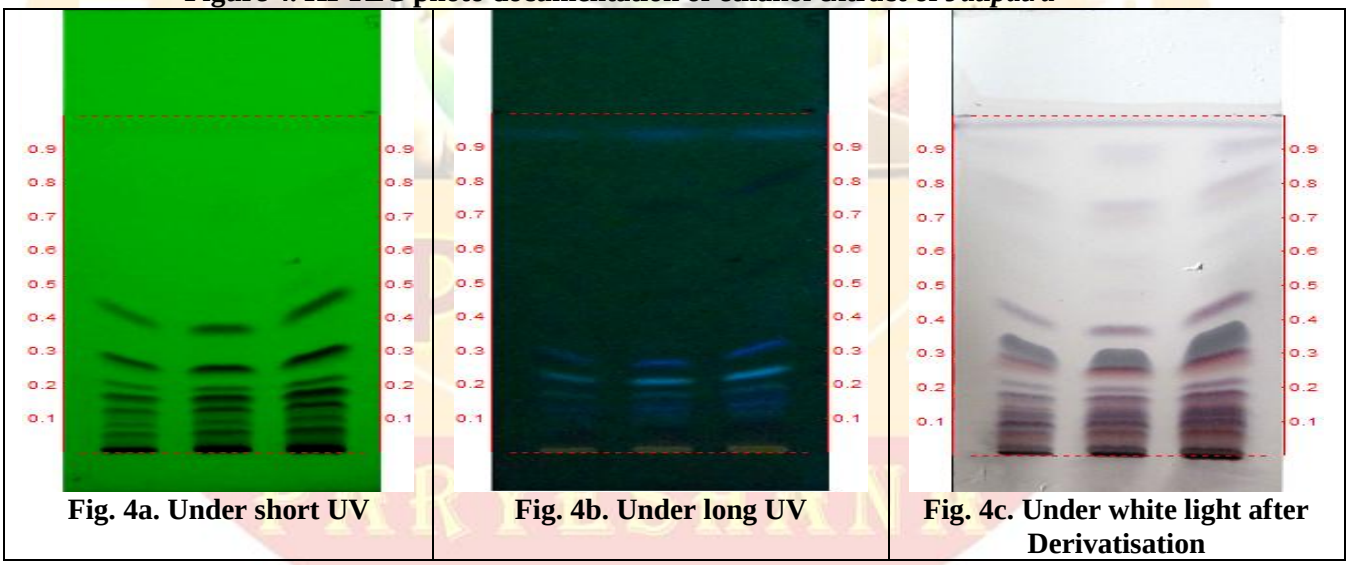


Fig. 4a. Under short UV | **Fig. 4b. Under long UV** | **Fig. 4c. Under white light after Derivatisation**

Track 1- Jatipatra– 4µl; Track 2– Jatipatra – 8µl; Track 3- Jatipatra – 12µl

Solvent system: n-hexane: Chloroform (1.0: 1.0)

Epidermal and hypodermis cells

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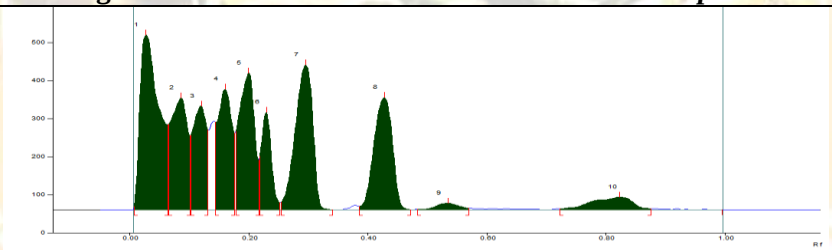
Table 2: R_f values of ethanol extract of *Jatipatra*

Under short UV	Under long UV	Under white light after Derivatisation
0.08 (D. green)	0.08 (FD. purple)	0.08 (D. pink)
0.10 (D. green)	-	0.10 (D. purple)
-	-	0.12 (D. purple)
0.13 (D. green)	0.13 (FD. blue)	0.13 (D. purple)
0.17 (D. green)	0.17 (FD. green)	0.17 (D. purple)
-	0.21 (F Aq. blue)	0.21 (D. purple)
0.26 (D. green)	0.26 (F. blue)	0.26 (D. pink)
-	-	0.30 (D. grey)
0.37 (D. green)	-	0.37 (D. purple)
-	-	-
-	0.47 (FD. blue)	-
-	-	0.57 (L. purple)
-	-	0.70 (L. purple)
-	0.73 (FD. blue)	0.73 (L. purple)
-	-	0.91 (L. purple)
-	0.95 (F. blue)	-

F- fluorescent; D – dark; L – light

Fig 5. Densitometric scan of ethanol extract of *Jatipatra*



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	9.2 AU	0.03 Rf	458.0 AU	17.08 %	0.06 Rf	23.3 AU	10592.5 AU	20.04 %
2	0.07 Rf	224.8 AU	0.09 Rf	294.0 AU	10.97 %	0.10 Rf	94.0 AU	5805.8 AU	10.98 %
3	0.10 Rf	194.9 AU	0.12 Rf	271.7 AU	10.14 %	0.13 Rf	09.9 AU	4531.5 AU	8.57 %
4	0.15 Rf	229.6 AU	0.16 Rf	317.3 AU	11.83 %	0.18 Rf	01.4 AU	5524.9 AU	10.45 %
5	0.18 Rf	205.2 AU	0.20 Rf	358.8 AU	13.38 %	0.22 Rf	29.5 AU	6673.4 AU	12.62 %
6	0.22 Rf	139.9 AU	0.23 Rf	256.2 AU	9.56 %	0.25 Rf	19.4 AU	3085.0 AU	5.84 %
7	0.25 Rf	20.0 AU	0.30 Rf	379.9 AU	14.17 %	0.34 Rf	0.3 AU	7990.9 AU	15.12 %
8	0.39 Rf	9.4 AU	0.43 Rf	294.6 AU	10.99 %	0.47 Rf	0.1 AU	6521.0 AU	12.33 %
9	0.48 Rf	0.8 AU	0.54 Rf	17.4 AU	0.65 %	0.57 Rf	3.9 AU	479.2 AU	0.91 %
10	0.72 Rf	2.7 AU	0.82 Rf	33.2 AU	1.24 %	0.88 Rf	2.6 AU	1662.1 AU	3.14 %

Fig 5a. At 254nm

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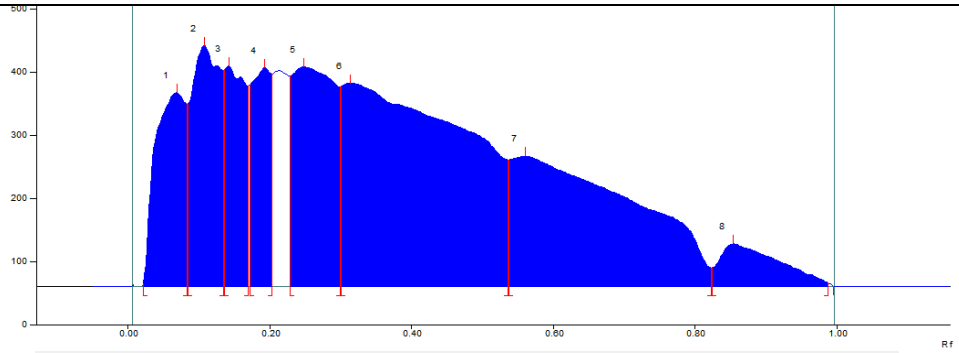
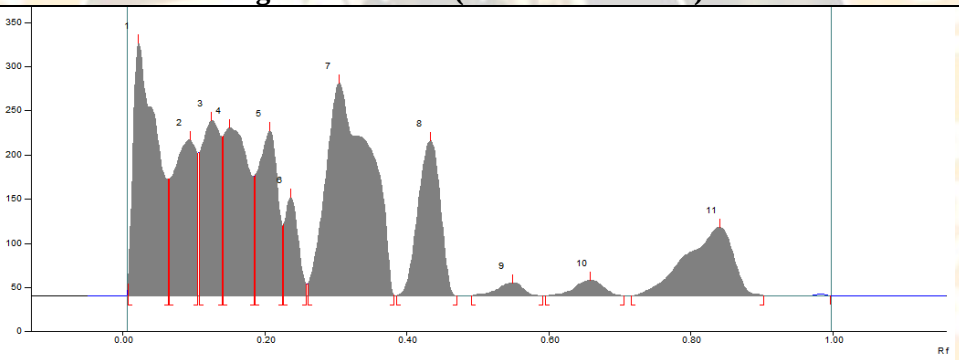


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	4.9 AU	0.07 Rf	306.1 AU	13.18 %	0.08 Rf	87.3 AU	9287.8 AU	7.78 %
2	0.09 Rf	287.5 AU	0.11 Rf	380.0 AU	16.36 %	0.14 Rf	40.8 AU	11112.7 AU	9.31 %
3	0.14 Rf	342.1 AU	0.14 Rf	348.8 AU	15.02 %	0.17 Rf	16.7 AU	7322.9 AU	6.13 %
4	0.17 Rf	318.0 AU	0.19 Rf	345.8 AU	14.89 %	0.20 Rf	36.2 AU	6688.4 AU	5.60 %
5	0.23 Rf	332.8 AU	0.25 Rf	347.0 AU	14.94 %	0.30 Rf	15.7 AU	15109.2 AU	12.66 %
6	0.30 Rf	316.1 AU	0.31 Rf	321.0 AU	13.82 %	0.54 Rf	00.1 AU	39843.3 AU	33.38 %
7	0.54 Rf	200.1 AU	0.56 Rf	206.1 AU	8.88 %	0.82 Rf	29.5 AU	25882.0 AU	21.68 %
8	0.83 Rf	29.7 AU	0.85 Rf	67.4 AU	2.90 %	0.99 Rf	6.0 AU	4118.8 AU	3.45 %

Fig 5b. At 366nm (fluorescence mode)



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	13.9 AU	0.02 Rf	286.2 AU	17.05 %	0.06 Rf	32.1 AU	6733.1 AU	15.36 %
2	0.07 Rf	132.4 AU	0.10 Rf	177.0 AU	10.55 %	0.11 Rf	61.5 AU	4155.9 AU	9.48 %
3	0.11 Rf	161.7 AU	0.13 Rf	198.6 AU	11.83 %	0.14 Rf	80.1 AU	3876.0 AU	8.84 %
4	0.14 Rf	180.6 AU	0.15 Rf	190.2 AU	11.33 %	0.19 Rf	34.8 AU	4800.4 AU	10.95 %
5	0.19 Rf	135.9 AU	0.21 Rf	186.8 AU	11.13 %	0.23 Rf	78.7 AU	3699.5 AU	8.44 %
6	0.23 Rf	80.1 AU	0.24 Rf	111.9 AU	6.67 %	0.26 Rf	13.3 AU	1492.0 AU	3.40 %
7	0.26 Rf	13.5 AU	0.30 Rf	241.1 AU	14.37 %	0.38 Rf	1.0 AU	10571.7 AU	24.12 %
8	0.39 Rf	0.4 AU	0.43 Rf	176.0 AU	10.49 %	0.47 Rf	0.0 AU	3897.8 AU	8.89 %
9	0.49 Rf	0.1 AU	0.55 Rf	14.9 AU	0.89 %	0.59 Rf	0.1 AU	383.2 AU	0.87 %
10	0.60 Rf	0.2 AU	0.66 Rf	17.8 AU	1.06 %	0.71 Rf	0.0 AU	503.3 AU	1.15 %
11	0.72 Rf	0.2 AU	0.84 Rf	77.9 AU	4.64 %	0.90 Rf	0.4 AU	3717.3 AU	8.48 %

Fig 5c. At 620nm after derivatisation

Results:

The results of macro and microscopy Standardization parameters and HPTLC (Photo documentation densitometric scan of Rf values) are presented in above mentioned respective tables and figures.)

Discussion:

The seed portion and aril portion of Myristica fragrans are well known Shukrasthambaka and Atisaraghna dravya in Ayurveda System of medicine. Morphological and anatomical standardization of herbal drug needs the information from basic disciplines of plant sciences for identification of plant drug.

Simultaneously for identification of chemical nature of plant in term of physico-chemical analysis, qualitative and quantitative analysis for the detection of active constituents, expertise are required. According to Kunle et.al, Standardization of herbal drug is a series of Protocol which assure the quality, efficiency and safety of plant drug.[9]Macro-microscopic analysis helps in the identification of plant characters anatomically and helps in identification of botanical background. Standardization and authentication of plants was done by evaluating physico chemical testing.[10] The values obtained in the study will serve as constants for quality standard measures for standardization of drugs in the dried form.

High performance thin layer chromatography (HPTLC) serves as the quality assessment tool which helps in identification of variation in chemical composition of plants. TLC identity is a part of every herbal monograph of international standards.[11] HPTLC fingerprinting shows different Rf values at different wavelengths and reported values can be used as a quality indicating fingerprint for Myristica fragrans in the dried form .Findings reported in the present investigations are in support of API and QSIMP2012.[12]

Conclusion:

Pharmocognostical characterization of the Myristica fragrans has been done as per pharmacopeial methododology. Present study explores the botanical (in terms of macro microscopic observations) physico-chemical observations(in terms of total ash, AIA, ASA,ASE,USE and loss on drying) and HPTLC fingerprint profile can serve as excellent standard for the identification and authentication of drug in dried form.

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