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PHARMACOGNOSTICAL AND CHEMICAL COMPARISON OF THE SEED AND ARIL OF *MYRISTICA FRAGRANS* HOUTT.

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ABSTRACT

Myristica fragrans Houtt., commonly known as Jaiphal and Javitri in India, belongs to the family Myristicaceae. Objective of the present study is to generate information based on botanical, physico-chemical and HPTLC data needed for proper identification and authentication of seed and aril of *Myristica fragrans*. The botanical study comprises of macroscopic analysis and powder microscopic studies of the two plant materials. The physico-chemical parameters such as loss on drying at 105℃, total ash, acid insoluble ash, water soluble ash, water soluble extractive, pH of water extract, alcohol soluble extractive, fibre content, swelling index, foaming index and volatile oil were determined by following standard methods. HPTLC studies of the defatted chloroform extracts of the seed and aril were conducted at 254 nm, 366 nm and 575 nm after derivatisation using vanillin-sulphuric acid reagent and the results were documented. The study shows that macroscopy, powder microscopy and most of the physico-chemical parameters of both the plant materials are different. HPTLC studies reveal that there are so many compounds having the same R_f values and colour indicating the presence of similar compounds in both the plant materials. The bands which are not common indicate that the corresponding compounds are different. It is also observed that larger numbers of phytochemicals are present in seed than aril. The given results help in accurate identification of these medicinally important plant materials by pharmacognostical and chemical methods.

INTRODUCTION

Myristica fragrans Houtt. (Family: Myristicaceae) is a tropical aromatic evergreen tree that reaches about 10-20 m height. It is cultivated widely in India for its fruit. It is found mostly in Tamil Nadu and to some extent in Kerala, Andhra Pradesh and Assam. *M. fragrans* is a native of the E. Moluccas and Banda Islands. It is seldom found truly wild. It is cultivated in tropical regions such as Malay Peninsula, Malay Islands, Indonesia, Grenada in the West Indies and Sri Lanka. In India it is found only in a few localities, where the climate is sufficiently hot and moist (Purseglove, 1968; Chopra *et al*., 1999; Bown, 1995). The plants are usually dioecious. They do not give flowers until around 9 years old, but once start flowering they continue to do so for further about 75 years. The trees bear 2 to 3 crops a year. The leaves are fragrant when crushed. The seed known as Nutmeg and its fleshy aril known as Mace are used as spices and in medicines. Aril is the net like skin covering on the kernel of the seed (Purseglove, 1968).

The vernacular names of *M. fragrans* seed are Sanskrit: Jatisasya, Jatiphala; Assamese : Jaiphal, Kanivish; Bengali : Jaiphala, Jaitri; English : Nutmeg Fruit; Gujrati : Jaiphala, Jayfar; Hindi : Jaiphal; Kannada : Jadikai, Jaykai, Jaidikai; Kashmiri: Jafal; Malayalam : Jatikai; Marathi : Jaiphal; Oriya : Jaiphal; Punjabi : Jaiphal; Tamil : Sathikkai, Jathikkai, Jatikkai, Jadhikai, Jadhikkai; Telugu: Jajikaya and Urdu: Jauzbuwa, Jaiphal and that of aril are Sanskrit : Jatipatri; Bengali: Jotri; English: Mace; Gujrati: Jaepatri; Hindi: Jaepatri; Kannada: Jaypatri; Kashmiri : Jowwatri; Malayalam: Jatipathri; Marathi: Jaepatri; Oriya: Jaipatri; Punjabi: Jauntari; Tamil: Jadipathri and Telugu : Jaepatri (Nadkarni, 1988)

The leaves of *M. fragrans* are pointed dark green arranged alternately along the branches and are borne on leaf stems about 1 cm long. Upper leaf surfaces are shiny. Flowers are usually single sexed. The flowers are bell-shaped, pale yellow and somewhat waxy and fleshy. Occasionally male and female flowers are found on the

same tree. Female flowers arise in groups of 1 to 3. Male flowers occur in groups of 1 to 10. Staminate flowers are arranged in groups of one to ten each 5–7 mm long. The fruits are fleshy, drooping, yellow, and smooth 6 to 9 cm long with a longitudinal ridge. When ripe, the succulent yellow fruit coat splits into two valves along a ridge running the length of the fruit, revealing a purplish-brown shiny seed surrounded by a red aril. Seeds or nutmegs (Fig. 1) are broadly ovoid (2 to 3 cm long), firm, fleshy, whitish and transversed by red-brown veins. The fruit has a fleshy husk. ound on the same tree. Female flowers arise in groups of 1 to 3; males in groups of 1 to 10. Flowers are pale yellow, fruit and mace is the fleshy red, net like skin covering (aril) on the kernel. It is a spreading aromatic evergreen tree usually growing to 5 to 13 metres high, occasionally 20 metres. The pointed dark green leaves are arranged alternately along the branches and are borne on leaf stems about 1 cm long. Upper leaf.

The most important part of the plant in terms of its pharmacological activity is the dried kernel (seed). Intoxication from the use of the aril of the fruit has also been reported, but only rarely. The oil of seed is generally used for medicinal purposes since it contains the pharmacologically active components. It is used as a spice in various dishes, as components of tea and soft drinks or mixed in milk and alcohol. In traditional medicine, the seed is used as a stomachic, stimulant, carminative as well as for intestinal catarrh and colic, to stimulate appetite, to control flatulence and it has a reputation as an emmenagogue and abortifacient (Nadkarni, 1988; The Wealth of India, 1995; Chopra *et al*., 1999).

Aril is widely used in a folk medicine and as a flavouring agent. It is reported to possesses antipapillomagenic, anticarcinogenic (Hussain & Rao, 1991) and anti-inflammatory activities (Ozaki et al., 1989).

Seed is found to contain about 12% essential oil and aril about 10%. Essential oils of both seed and aril are reported to contain sabinene, pinenes, camphene, p-cymene, phellandrene, terpinene, limonene, myrcene, linalool, geraniol, terpineol, myristicin(methoxy-safrole), elemicin, safrole, eugenol and eugenol derivatives (Qiu *et al*., 2004; Wang *et al*., 2004; Forrester, 2005; Yang *et al*., 2008). Both seed and aril contain about 2% of lignans called diarylpropanoids. These are non volatile dimers of phenylpropanoid constituents of the essential oil (The Wealth of India, 1995). commonly known as Jaiphal and Javitri in India, belongs to the family Myristicaceae. It produces two spices, nutmeg and mace.

Both seed and aril exhibit strong antimicrobial activity against animal and plant pathogens, food poisoning and spoilage bacteria including *Bacillus subtilis*, *Escherichia coli*, *Saccharomyces cerevisiae*, multi-drug resistant *Salmonella typhi* and *Helicobacter pylori* (Preetee Jaiswal *et al*., 2009). Studies show that the seed of *M. fragrans* has hypolipidaemic (Ram *et al*., 1996) and hypocholesterolemic (Sharma *et al*., 1995) action. Moreover, *M. fragrans* possess antidepressant activity (Dhingra and Sharma, 2006), antidiabetic activity (Han *et al*., 2008), aphrodisiac activity (Tajuddin *et al*., 2005), memory enhancing activity (Parle *et al*., 2004) and cytotoxicity (Lee *et al*., 2005).

In the present study an attempt is made to compare the seed and aril of *M. fragrans* by generating information based on macroscopic, microscopic, physico-chemical and HPTLC data which are the tools needed for proper identification of plant materials.

MATERIALS AND METHODS

Plant materials

The fresh seed and aril of *M. fragrans* were collected and authenticated by the Dept. of Pharmacognosy, Siddha Regional Research Institute, Thiruvananthapuram. The plant materials were cut, crushed, dried and kept in airtight containers and used for all experimental purposes.

Macroscopy

The different macroscopic characters of seed and aril of *M. fragrans* were studied and recorded systematically (Khandelwal and Nirali, 2008).

Powder microscopy

The powder microscopic studies of the two samples were carried out using standard procedure (Tyler *et al*., 1977) by capturing the images of different fragments of tissues and diagnostic characteristic features were recorded.

Physico-chemical study

The physico-chemical parameters such as loss on drying at 105℃, total ash, acid insoluble ash, water soluble ash, water-soluble extractive, pH of water extract, alcohol soluble extractive, fibre content, swelling index, foaming index and volatile oil were determined as per WHO guidelines (WHO, 1998).

High-performance thin layer chromatographic analysis (HPTLC)

HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials and is the simplest separation technique today available to the analyst (Camag, 2015).

a. Preparation of extract of the drug materials for HPTLC analysis

4 gm each of the dried and powdered sample was defatted and soaked in 40 ml chloroform at room temperature for overnight. The contents were filtered through separate filter papers and each filtrate was concentrated on a water bath to 4 ml. These extracts were used for chromatographic studies (Wagner and Bladt, 1996).

b. Development of HPTLC

15 µl and 20 µl of chloroform extract of each plant material was spotted in the form of bands with Camag microliter syringe on a precoated silica gel 60 F254 (Merck) plates with Automatic TLC Sampler 4 (ATS4). Mobile phase selected for the study was Toluene: Ethyl acetate: Formic acid (5: 2: 0.1). Linear ascending development was done in twin trough glass chamber saturated with the specified mobile phase. The plate was air dried and kept under UV short (254 nm) and UV long (366 nm) and white light (575 nm) after derivatization using vanillin-sulphuric acid reagent and photo documentation was done. The plates were scanned at UV 254 nm, 366 nm and 575 nm after derivatization using TLC Scanner 4 with winCATS software for interpretation of data.

RESULTS AND DISCUSSION

Macroscopy

The seed (Fig. 1) is ellipsoid, externally greenish brown in colour and sometimes marked with small irregular dark brown patches or minute dark points and lines slightly furrowed reticulately. A small light-coloured area is observed at one end of the seed indicating the position of the radical. A groove running along the line of raphe to the darker chalaza at the opposite end, surrounded by a thin layer of perisperm within foldings appearing as dark ruminations in the abundant greyish-brown endosperm. Embryo is in an irregular cavity, small with two widely spreading crumpled cotyledons and a small radical. It is strong and aromatic in odour, and pungent and aromatic in taste. Seed is oblong, obtuse and testa is shiny.

Arils (Fig. 2) are flat, smooth, irregularly slit, slightly flexible or brittle and somewhat translucent. Aril is yellowish red in colour irregularly lobed and extending to the apex of the seed. It is rich in oil and therefore exude a reddish or orange oily colour when pressed. It bears some odour and taste as that of seeds. When fresh, the aril is bright scarlet becoming more horny, brittle and in yellowish-brown colour when dried.

Fig. 1: Seed

Fig. 2: Aril

Powder Microscopic Characters

The powder of seed is brown, oily and shows fragments of endosperm cells containing prismatic crystals and starch grains. A few cells of endosperm contains brown contents. Starch grains are numerous, oval to rounded, measuring upto 20 µm in diameter having 2 to10 components. A few cells containing oil globules and a few aleurone grains are present. The powder of aril is fine, homogeneous and yellowish-brown with a strong aroma and slightly bitter in taste. The powder when seen under the microscope shows abundance of thick-walled cells. Starch and aleuronic grains are absent. The powder microscopic characters of the seed and aril of *M. fragrans* are given in Fig. 3 and Fig. 4 respectively.

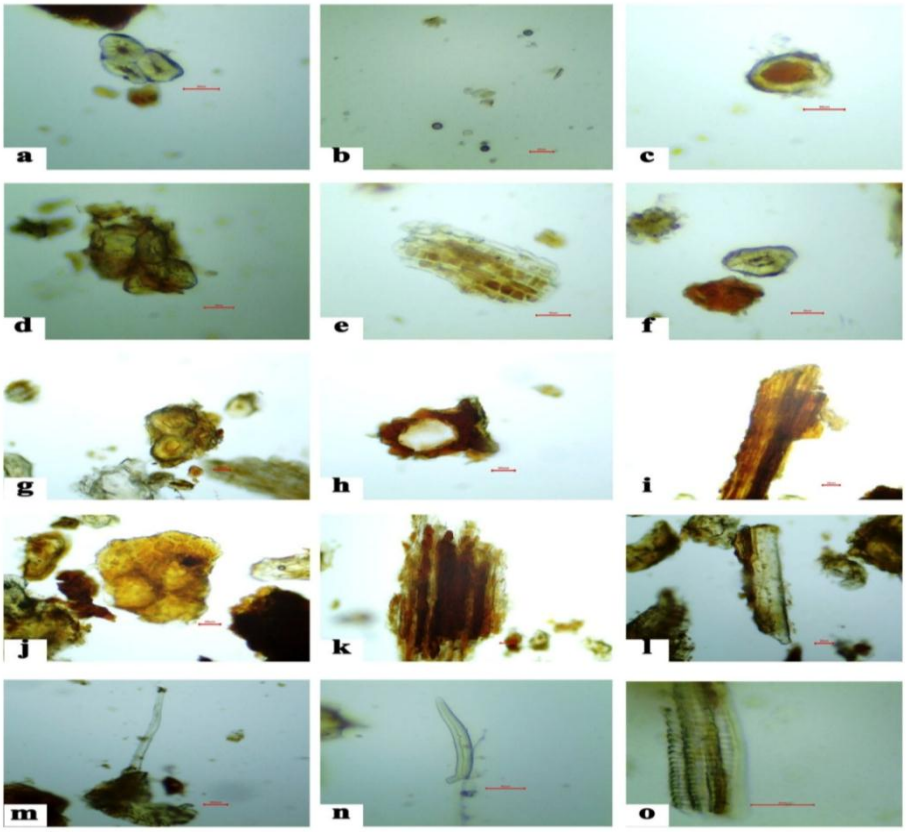


Fig. 3: Powder Microscopy of *M. fragrans* (seed): a) Stone Cells Cluster with depositions; b) Clump of starch grains; c) Oil globules; d) Stone Cells; e) Epidermal cells embedded with oil globules; f) Stone cells; g)Stone cells; h) Fragmented stone cell with lumen; i) Fibre; j) Seed coat/ Testa; k) Lignified cell; l) Fibre; m) Trichome with base; n) Trichome; o) Xylem vessels with spiral thickening.

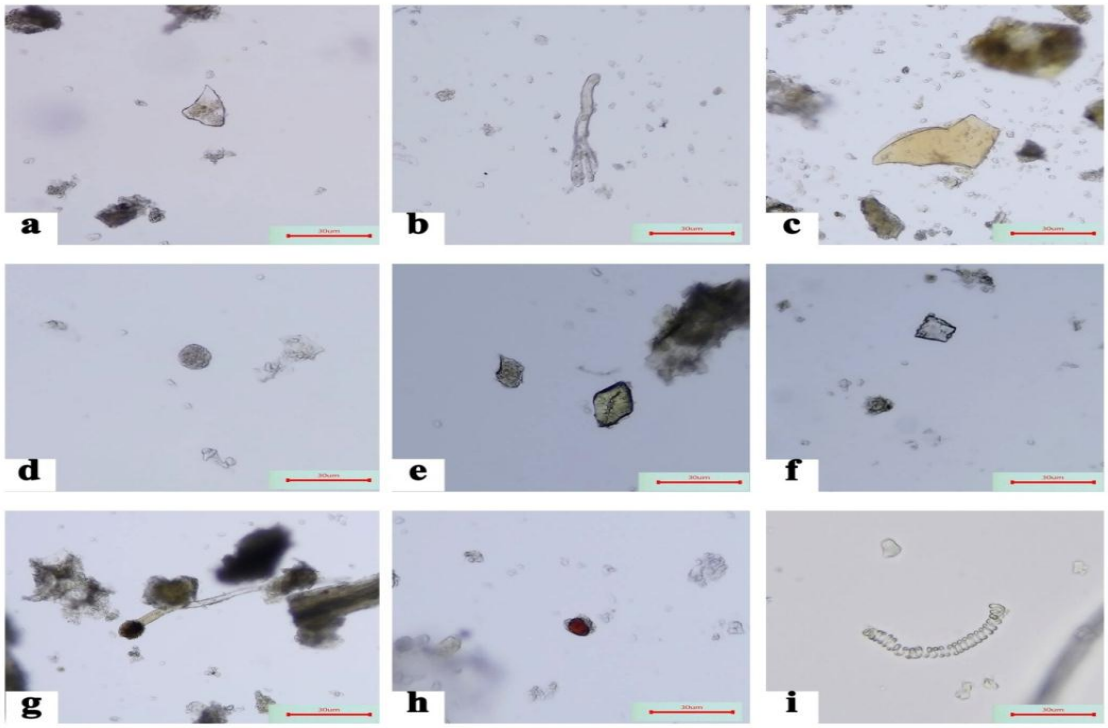


Fig. 4: Powder Microscopy of *M.fragrans* (aril): a) Crystal; b) Glandular trichome; c) Fragment of an epithelial cell; d) Aleurone grain; e) Sclerids; f) Stone cells; g) Glandular trichome; h) Oil globule; i) Xylem vessels with spiral thickening.

The fine powder of both seed and aril had a characterstic aromatic odour and slightly bitter taste. The seed powder is brown in colour and aril powder is yellowish-brown. Brachysclereids or clustered stone cells are scattered in the seed powder. But the aril powder had irregular sclereids. Glandular trichomes were scattered in the aril powder and trichome with only base in the seed powder. The epidermal cells of the seed are embedded with oil globules. Numerous large brown oil globules and occasional crystal occur in the aril powder. Abundant starch granules - some simple spherical but mostly compound - are present in the seed powder. In the aril powder, small aleurone grains were found scattered. No starch granules were found. Fragment of epithelial cells were observed in the aril powder and were not found in the seed powder. Xylem vessels with spiral thickening were noted in both.

Table 1: Physico-chemical parameters.

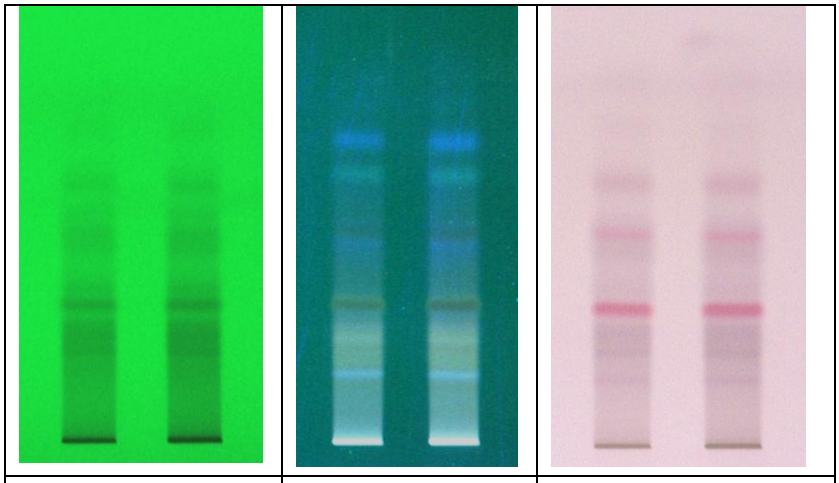
Sl. No.	Parameter	Aril	Seed
1	Loss on drying at 105˚C (%)	10.0	8.90
2	Total ash (%)	2.65	1.91
3	Acid insoluble ash (%)	Nil	0.31
4	Water soluble ash (%)	0.80	1.49
5	Water soluble extractives (%)	11.00	20.16
6	Alcohol soluble extractives (%)	22.90	28.42
7	pH of water extract	5.38	6.16
8	Volatile oil (%)	1.00	4.00
9	Fibre content (%)	5.30	10.09
10	Swelling index (ml/gm)	4.00	5.80
11	Foaming index	<100	<100

The results of the physico-chemical analysis of the seed and aril of *M. fragrans* are represented in Table 1. The low value of total ash indicates less amount of minerals and earthy materials attached to it. Acid-insoluble ash usually represents the amount of silica present as sand and dust contaminated (Rizvi *et al*., 2015) to the sample which are absent for aril and only 0.31 obtained for seed. Loss on drying at 105°C shows the presence of moisture content and volatile oil in the drug. The water-soluble extractive value indicates the presence of more polar constituents such as tannin, sugar, plant acid, mucilage and glycosides. The alcohol-soluble extractive values indicated the presence of phenols, alkaloids, steroids, glycosides, flavonoids etc. The water-soluble extractive value of the seed was 20.16 % and that of the aril was 11.00 % indicating the presence of more polar phytochemicals in the seed. The alcohol-soluble extractive values of the seed and aril are 28.42 % and 22.90 % respectively showing that the alcohol-soluble components are also higher in seed than in aril. These values are a measure of the quantity of the chemical constituents soluble in alcohol. Presence of volatile oil was detected in both the plant materials and it was found that the volatile oil content is also higher in seed compared to aril. Ash values are also higher for seed indicating the presence of more inorganics in seed than in aril. The study reveals that the physico-chemical parameters of both plant materials are different.

Swelling index is a measure of the swelling properties of the plant material due to the presence of gums, mucilage, pectin or hemicellulose. The low value of swelling index for seed and aril indicates the less amount these materials. Foaming index measures the foaming ability of an aqueous decoction of the plant material. Foaming ability is due to the presence of saponins that can cause persistent foam when an aqueous decoction is shaken. The value of foaming index is <100 indicating the absence of saponins in both the plant materials.

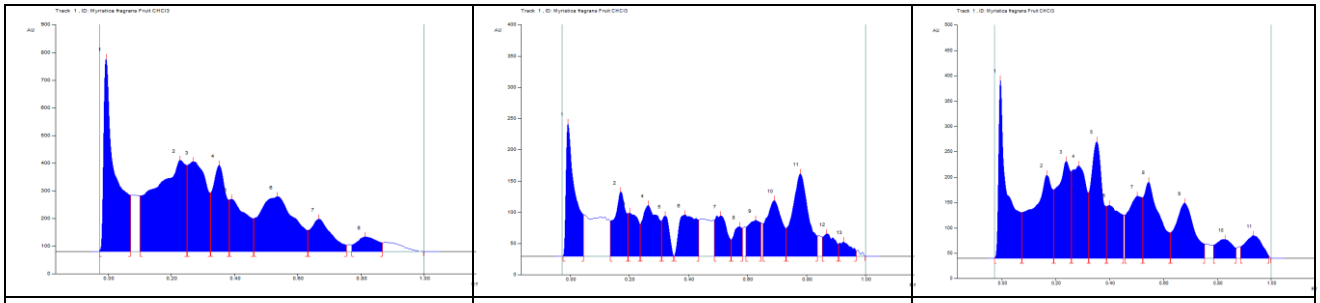
High-Performance thin layer chromatographic analysis (HPTLC)

Firstly the HPTLC was performed using the chloroform extract. But the resolution was very poor due to the presence of oil content. Hence the chloroform extracts were defatted using petroleum ether and the remaining fractions were used for HPTLC studies. 15 µl and 20 µl of chloroform extract of each plant material was spotted in the form of bands on precoated silica gel plates in track 1 and 2 respectively. The results indicated that both seed and aril contain an appreciable amount of phytochemicals. The developed HPTLC plates of seed and aril at 254 nm, 366 nm and after derivatization using vanillin-sulphuric acid viewed in visible light at 575 nm are represented in Fig. 5 and 6 respectively. The R_f values and peak area percentages obtained at different wavelengths for seed and aril are given in Table 2 and 3 respectively.



Under UV short (254 nm)	Under UV long (366 nm)	Under white light after derivatisation (575 nm)

Fig. 5: HPTLC photodocumentation of defatted chloroform extract of *M. fragrans* (seed); Viewed in UV short; Viewed in UV long; After derivatisation using vanillin-sulphuric acid viewed in visible light;Solvent system: Toluene: Ethyl acetate: Formic acid (5: 2: 0.1)



Under UV short	Under UV long	Under white light after derivatisation

Fig. 5a: Chromatogram of defatted chloroform extract of *M. fragrans* (seed); Viewed in UV short; Viewed in UV long; After derivatisation using vanillin-sulphuric acid viewed in visible light;Solvent system: Toluene: Ethyl acetate: Formic acid (5: 2: 0.1).

Table 2: R_f values and peak area percentages of defatted chloroform extract of *M. fragrans* (seed).

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	2.0 AU	-0.00 Rf	697.6 AU	31.41 %	0.07 Rf	03.5 AU	17707.0 AU	19.09 %
2	0.10 Rf	200.8 AU	0.23 Rf	329.8 AU	14.85 %	0.25 Rf	11.6 AU	23166.4 AU	24.97 %
3	0.25 Rf	311.8 AU	0.27 Rf	324.4 AU	14.61 %	0.32 Rf	11.1 AU	12846.8 AU	13.85 %
4	0.32 Rf	211.4 AU	0.35 Rf	311.9 AU	14.04 %	0.38 Rf	86.4 AU	9034.0 AU	9.74 %
5	0.38 Rf	186.7 AU	0.39 Rf	188.6 AU	8.49 %	0.46 Rf	18.8 AU	7138.5 AU	7.69 %
6	0.46 Rf	119.3 AU	0.54 Rf	198.6 AU	8.94 %	0.63 Rf	76.2 AU	15025.6 AU	16.20 %
7	0.63 Rf	76.3 AU	0.67 Rf	117.3 AU	5.28 %	0.76 Rf	22.7 AU	5383.4 AU	5.80 %
8	0.77 Rf	23.9 AU	0.81 Rf	52.7 AU	2.37 %	0.87 Rf	31.5 AU	2470.7 AU	2.66 %

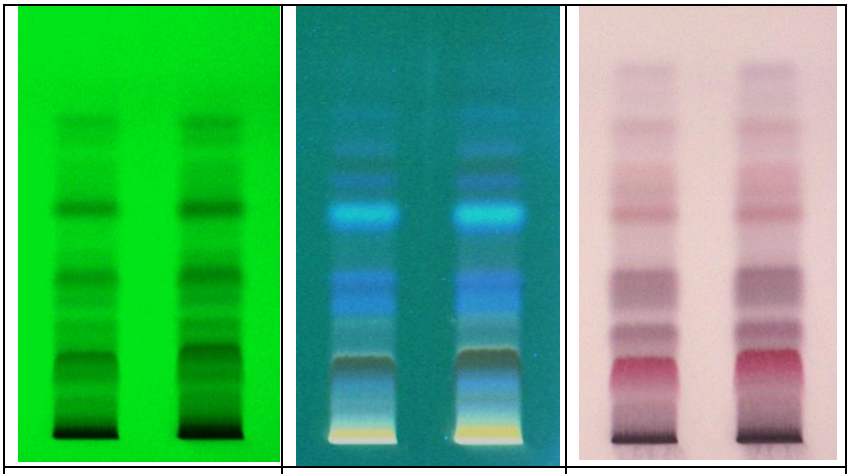
Under UV short

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	0.0 AU	-0.01 Rf	212.4 AU	20.28 %	0.05 Rf	66.6 AU	4452.0 AU	14.97 %
2	0.14 Rf	56.4 AU	0.17 Rf	102.5 AU	9.79 %	0.19 Rf	68.1 AU	2698.1 AU	9.07 %
3	0.20 Rf	68.2 AU	0.20 Rf	69.8 AU	6.67 %	0.24 Rf	51.2 AU	1599.2 AU	5.38 %
4	0.24 Rf	51.4 AU	0.26 Rf	81.6 AU	7.79 %	0.31 Rf	56.2 AU	2955.6 AU	9.94 %
5	0.31 Rf	56.5 AU	0.32 Rf	64.4 AU	6.15 %	0.35 Rf	0.8 AU	1021.7 AU	3.44 %
6	0.35 Rf	0.6 AU	0.39 Rf	66.5 AU	6.35 %	0.44 Rf	58.0 AU	2730.9 AU	9.19 %
7	0.49 Rf	57.4 AU	0.51 Rf	64.7 AU	6.18 %	0.55 Rf	26.7 AU	1851.8 AU	6.23 %
8	0.55 Rf	26.7 AU	0.57 Rf	47.5 AU	4.54 %	0.58 Rf	45.1 AU	982.1 AU	3.30 %
9	0.60 Rf	46.2 AU	0.63 Rf	57.9 AU	5.53 %	0.65 Rf	53.4 AU	1661.3 AU	5.59 %
10	0.65 Rf	52.0 AU	0.69 Rf	89.3 AU	8.53 %	0.73 Rf	44.6 AU	3255.4 AU	10.95 %
11	0.73 Rf	44.9 AU	0.78 Rf	131.9 AU	12.60 %	0.84 Rf	32.5 AU	4896.6 AU	16.48 %
12	0.86 Rf	30.7 AU	0.87 Rf	35.7 AU	3.41 %	0.91 Rf	19.0 AU	1003.8 AU	3.38 %
13	0.91 Rf	19.9 AU	0.93 Rf	23.0 AU	2.20 %	0.97 Rf	8.9 AU	621.7 AU	2.09 %

Under UV long

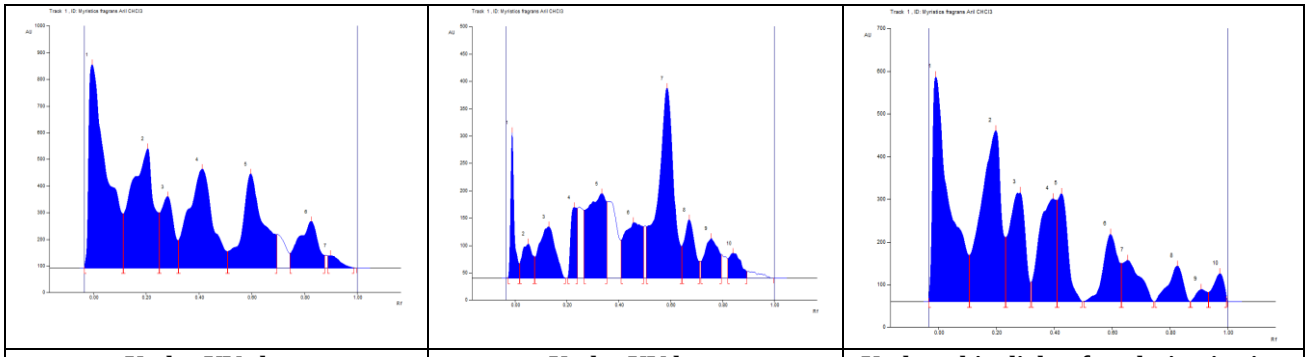
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	0.5 AU	-0.00 Rf	351.6 AU	20.82 %	0.07 Rf	90.2 AU	7997.4 AU	13.56 %
2	0.08 Rf	90.3 AU	0.17 Rf	164.2 AU	9.73 %	0.19 Rf	35.4 AU	8316.9 AU	14.10 %
3	0.19 Rf	135.5 AU	0.24 Rf	191.4 AU	11.34 %	0.26 Rf	70.7 AU	6483.8 AU	10.99 %
4	0.26 Rf	171.4 AU	0.29 Rf	182.4 AU	10.81 %	0.32 Rf	28.9 AU	6370.8 AU	10.80 %
5	0.32 Rf	129.6 AU	0.35 Rf	230.2 AU	13.64 %	0.39 Rf	02.6 AU	6736.5 AU	11.42 %
6	0.39 Rf	102.6 AU	0.40 Rf	104.7 AU	6.20 %	0.45 Rf	85.5 AU	3727.1 AU	6.32 %
7	0.46 Rf	85.4 AU	0.50 Rf	122.5 AU	7.25 %	0.52 Rf	19.3 AU	4359.3 AU	7.39 %
8	0.52 Rf	119.7 AU	0.55 Rf	150.4 AU	8.91 %	0.63 Rf	50.2 AU	6072.5 AU	10.30 %
9	0.63 Rf	50.5 AU	0.68 Rf	109.1 AU	6.46 %	0.75 Rf	28.4 AU	5317.3 AU	9.02 %
10	0.79 Rf	26.3 AU	0.83 Rf	36.9 AU	2.19 %	0.87 Rf	20.0 AU	1616.7 AU	2.74 %
11	0.89 Rf	22.7 AU	0.94 Rf	44.8 AU	2.65 %	0.99 Rf	5.0 AU	1975.4 AU	3.35 %

Under white light after derivatisation



Under UV short	Under UV long	Under white light after derivatisation

Fig. 6: HPTLC chromatogram of defatted chloroform extract of *M. fragrans* (aril); Viewed in UV short; Viewed in UV long; After derivatisation using vanillin-sulphuric acid viewed in visible light;Solvent system: Toluene: Ethyl acetate: Formic acid (5: 2: 0.1).



Under UV short	Under UV long	Under white light after derivatisation

Fig 6a: HPTLC chromatogram of defatted chloroform extract of *M. fragrans* (aril); Viewed in UV short; Viewed in UV long; After derivatisation using vanillin-sulphuric acid viewed in visible light;Solvent system: Toluene: Ethyl acetate: Formic acid (5: 2: 0.1)

Table 3: R_f values and peak area percentages of defatted chloroform extract of *M. fragrans* (aril).

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	6.7 AU	-0.00 Rf	764.3 AU	31.42 %	0.11 Rf	94.7 AU	35458.5 AU	28.64 %
2	0.12 Rf	295.0 AU	0.21 Rf	448.6 AU	18.44 %	0.25 Rf	97.6 AU	25821.7 AU	20.86 %
3	0.25 Rf	208.0 AU	0.28 Rf	269.0 AU	11.06 %	0.32 Rf	94.5 AU	8983.8 AU	7.26 %
4	0.32 Rf	195.2 AU	0.41 Rf	372.2 AU	15.30 %	0.51 Rf	62.8 AU	23567.0 AU	19.03 %
5	0.51 Rf	63.1 AU	0.60 Rf	354.7 AU	14.58 %	0.70 Rf	25.8 AU	19643.2 AU	15.87 %
6	0.75 Rf	55.9 AU	0.83 Rf	175.9 AU	7.23 %	0.88 Rf	47.9 AU	8873.1 AU	7.17 %
7	0.89 Rf	46.5 AU	0.90 Rf	47.8 AU	1.96 %	0.99 Rf	1.1 AU	1466.5 AU	1.18 %

Under UV short

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	0.7 AU	-0.01 Rf	266.5 AU	19.27 %	0.02 Rf	26.7 AU	2512.1 AU	5.64 %
2	0.02 Rf	27.0 AU	0.05 Rf	62.8 AU	4.54 %	0.08 Rf	39.4 AU	1711.5 AU	3.85 %
3	0.08 Rf	39.0 AU	0.13 Rf	94.0 AU	6.80 %	0.19 Rf	0.4 AU	4048.8 AU	9.10 %
4	0.20 Rf	0.6 AU	0.23 Rf	129.6 AU	9.37 %	0.24 Rf	27.8 AU	2250.8 AU	5.06 %
5	0.27 Rf	124.1 AU	0.34 Rf	155.2 AU	11.22 %	0.35 Rf	39.8 AU	7540.7 AU	16.94 %
6	0.41 Rf	70.4 AU	0.46 Rf	101.7 AU	7.35 %	0.50 Rf	94.1 AU	4842.7 AU	10.88 %
7	0.51 Rf	94.8 AU	0.59 Rf	347.6 AU	25.14 %	0.64 Rf	58.2 AU	14374.6 AU	32.30 %
8	0.64 Rf	58.8 AU	0.67 Rf	107.3 AU	7.76 %	0.71 Rf	30.9 AU	3028.6 AU	6.81 %
9	0.72 Rf	31.2 AU	0.76 Rf	72.5 AU	5.25 %	0.80 Rf	43.1 AU	2662.4 AU	5.98 %
10	0.82 Rf	35.6 AU	0.84 Rf	45.8 AU	3.31 %	0.90 Rf	12.5 AU	1528.9 AU	3.44 %

Under UV long

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	8.6 AU	-0.01 Rf	526.7 AU	24.97 %	0.11 Rf	88.6 AU	21136.1 AU	26.20 %
2	0.11 Rf	108.7 AU	0.20 Rf	400.6 AU	18.99 %	0.23 Rf	51.1 AU	18959.1 AU	23.50 %
3	0.23 Rf	151.1 AU	0.28 Rf	255.4 AU	12.11 %	0.32 Rf	45.0 AU	9733.5 AU	12.07 %
4	0.32 Rf	45.8 AU	0.40 Rf	240.8 AU	11.42 %	0.41 Rf	37.6 AU	9768.1 AU	12.11 %
5	0.41 Rf	237.8 AU	0.43 Rf	253.1 AU	12.00 %	0.50 Rf	0.3 AU	6207.6 AU	7.70 %
6	0.50 Rf	0.7 AU	0.60 Rf	157.8 AU	7.48 %	0.63 Rf	89.4 AU	5450.4 AU	6.76 %
7	0.63 Rf	89.4 AU	0.65 Rf	97.4 AU	4.61 %	0.75 Rf	0.4 AU	4125.7 AU	5.11 %
8	0.75 Rf	0.3 AU	0.83 Rf	83.1 AU	3.94 %	0.87 Rf	0.1 AU	2900.3 AU	3.60 %
9	0.87 Rf	0.3 AU	0.91 Rf	28.6 AU	1.35 %	0.93 Rf	21.8 AU	712.5 AU	0.88 %
10	0.94 Rf	22.3 AU	0.97 Rf	66.0 AU	3.13 %	1.00 Rf	12.9 AU	1670.5 AU	2.07 %

Under white light after derivatisation

The R_f values and colour obtained for the bands at different wavelengths for seed and aril are given in Table 4.

Table 4: R_f values and colour of bands obtained at different wavelengths.

	R_f values and colour	
Light source	***Myristica fragrans* (seed)**	***Myristica fragrans* (Aril)**
Wavelength, 254 nm	0.07 (green) **0.25** (dark green) **0.32** (light green) 0.38 (light green) 0.46 (dark green) 0.63 (light green)	0.11 (green) **0.25** (dark green) **0.32** (green) 0.51 (dark green) 0.70 (light green) **0.88** (dark green)

	0.76 (green) **0.88** (dark green)	0.98 (light green)
Wavelength, 366 nm	0.05 (orange) **0.19** (pale blue) **0.24** (pale brown) 0.31 (pale blue) **0.35** (pale brown) 0.44 (pale blue) 0.55 (pale brown) 0.58 (brown) **0.64** (blue) **0.71** (blue) 0.84 (pale blue) **0.90** (dark blue)	0.02 (purple) 0.08 (pale blue) **0.19** (pale blue) **0.24** (pale brown) **0.35** (pale brown) 0.50 (blue) **0.64** (blue) **0.71** (blue) 0.80 (pale blue) **0.90** (dark blue)
Wavelength, 575 nm	0.07 (light purple) 0.19 (light purple) 0.28 (light purple) **0.32** (pink) **0.39** (pinkish purple) 0.45 (purple) **0.50** (pink) **0.63** (purple) **0.75** (pink) **0.87** (purple)	0.11(light purple) 0.23 (light purple) **0.32** (pink) **0.39** (pinkish purple) **0.50** (pink) **0.63** (purple) **0.75** (pink) **0.87** (purple) 0.93 (purple)

HPTLC for identification of chemical constituents in the plant extracts produces fingerprints which consists of sequence of zones that have specific R_f values, colours and intensity. The fingerprints obtained for the defatted chloroform extract of the seed and aril of *M. fragrans* were compared. At 254 nm, 8 bands were obtained for seed whereas 7 bands were obtained for aril, out of which 3 bands are having the same R_f values (0.25, 0.32, 0.88) and colour. At 366 nm, 12 bands are observed in seeds and 10 bands in aril; 6 bands with R_f values 0.19 (pale blue), 0.24 (pale brown), 0.35 (pale brown), 0.64 (blue), 0.71 (blue) and 0.90 (dark blue) are similar. After derivatization at 575 nm, 10 bands were observed in seeds and 10 bands in aril. 6 bands with R_f values 0.32, 0.39, 0.50, 0.63, 0.75 and 0.87 are observed in both the plant materials with the same colour. It is found that so many compounds are having the same R_f values and colour indicating the presence of similar compounds in both the plant materials. The bands which are not common indicates that the corresponding compounds are different. It is also observed that larger number of phytochemicals are present in seed than aril.

CONCLUSION

The macro and microscopical characters and HPTLC fingerprinting profile developed along with the physico-chemical parameters can be used as a diagnostic tool to identify and to determine the quality and purity of the samples. HPTLC fingerprinting profile is a very important parameter of standardization for the proper identification of medicinal plant materials. The given results help in accurate identification of these medicinally important plant materials by pharmacognostical and chemical methods.

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