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Research Article

SCIENTIFIC VALIDATION OF *JATIPHALA* AND *JAVITRI* IN HYPER PIGMENTATION

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ABSTRACT

Traditionally used medicinal plants have recently received the attention of the pharmaceutical and scientific communities. There is a need to develop new effective traditional drugs with scientific validation. The seed and aril of *Jatiphala* (*Myristica fragrans* Houtt.) of family Myristicaceae have the utilized therapeutically as medicine as well as a spice. Research into the scientific validation of nutmeg used in the treatment of Antidiarrheal, Hypnotic, Analgesic, Antimicrobial, Antidepressant and Sexual dysfunction has been done. In the present study Pharmacognostic study, Phytochemical study, Physico-chemical study and High performance thin layer chromatography are taken for the scientific validation of nutmeg.

The Pharmacognostic study of macroscopic and microscopic identity of nutmeg was taken in order to set the quality standards of the market sample. The Phytochemical screening and its nutrients was done using Standarad methods. Qualitative analysis of Aqueous and Milk extracts of Seed and Aril of the nutmeg was studied for the presence of the Alkaloids, Carbohydrates, Proteins and Amino acids, Tannins, Saponins, Flavonoids and Fixed oils. Physico-chemical study involves the determination of identity, purity, and quality of nutmeg by the evaluation of Loss on Drying, Total Ash, Acid Insoluble Ash, Water Soluble Matter and Alcohol Soluble Matter parameters. High performance thin layer chromatography is one of the Sophisticated instrumental techniques for Identification and Quantitative determination of Phytochemical constituents of Seed and Aril part of the Nutmeg (*Myristica fragrans* Houtt).

KEYWORDS: Seed and aril of nutmeg, Scientific validation, Pharmacognosy, Phytochemical study, Physico- chemical study, HPTLC.

INTRODUCTION

Medicinal plants have been a major source of treatment for Human disease since time immemorial. One fourth of the world population people are dependent on traditional medicines, particularly plant drugs for curing ailments. Medicinal plants are promising choice over modern synthetic drugs. They show no side effects and are considered to be safe. Generally Medicinal plant formulations involve the use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. The process of standardization can be achieved by Pharmacognostic studies, Phytochemical, Physico-chemical and High performance thin layer Chromatography (HPTLC) Standardization is a system to ensure that every packet of medicine that is sold and will induce its therapeutic effect. The Seed and aril of *Jatiphala* is traditionally used to treat in Hyperpigmentation and possesses valuable medicinal properties.

The Pharmocognosy is "an applied science which deals with the biological and biochemical of natural drugs and their constituents." In a restricted sense, it implies particular knowledge of methods of identification and evaluation of drugs.

In most of the books dealing with the Materia Medica of Ayurveda the correct identity of the botanical source has become very difficult on account of the synonyms and the use of vernacular names. For this a scientific investigation of the medicinal plants embodying proper identification of all source plants and correlating them properly to the drugs described in Ayurvedic literature is absolutely necessary.

For this, proper identification of plants and raw materials at the basic level with the help of microscopic and morphological characteristics is essential. The identification of adulterants from crude plant drugs and powdered drugs is also essential. Hence, various Pharmacognostic standards may be applied to standardize and maintain the 'quality control' of the single plant drugs. Though Pharmacognostic standards alone may not always be adequate to ensure their quality but can play a major role to standardize a plant drug.

Phytochemicals are chemical compounds that occur naturally in plants (phyto means “plant” in Greek). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. More than thousand known phytochemicals. Plants produce these chemicals to protect itself but recent research demonstrate that they can protect humans against diseases. They are not essential nutrients and are not required by the human body for sustaining life. Phytochemicals are the biologically active substances in plants that are responsible for giving them color, flavor and natural disease resistance. There are many phytochemicals and each works differently. These are some possible actions: Antioxidant, Hormonal action, Stimulation of

enzymes, Interference with DNA replication, Anti-bacterial effect and Physical action.

Medicinal plants play a vital role in preventing various diseases. Many activities of the medicinal plants are due to the presence of the secondary metabolites. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs and are responsible for the desired therapeutic properties.

The preliminary phytochemical studies were performed for testing the different chemical groups present the drug. 10% (w/v) solution of extract was taken unless otherwise mentioned in the respective individual test.

General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them. (Trease and Evan, 1983)

Determination of extractive values, Loss on Drying, ash residues and active components plays a significant role for standardization of the indigenous crude drugs. For the useful application of the plant parts in modern medicine, physico - chemical standardization is very important, so that the medicinal benefits of the plant may be used properly and scientifically and reach to the larger populations of the world. Therefore, in the present research work was to evaluate the physicochemical parameters of the seed and aril of *Myristica fragrans* Houtt.

High performance thin layer chromatography (HPTLC) is an enhanced form of thin layer chromatography (TLC). A number of enhancements can be made to the basic method of thin layer chromatography to automate the different steps, to increase the resolution achieved and to allow more accurate quantitative measurements.

PHARMOCOGNOSTIC STUDY

AIMS AND OBJECTIVES

- To study the Macroscopic study of the seed and aril of the nutmeg
- To study the Microscopic study of the seed and aril of the nutmeg
- To study the Powder study of the seed and aril

MATERIAL AND METHODS

Trail drugs

The drugs *Jatiphala* and *Javitri* for the study were acquired from Kerala drug market. For this study, the dried drugs were taken and were pounded to powder form.

OBSERVATION AND RESULTS

Pharmacognosy of seed of *Jatiphala* (*Myristica fragrans* Houtt.)

Name of the Sample : *Jatiphala*

Scientific Name : *Myristica fragrans* Houtt.

Family : Myristicaceae

Plant part : Seed

Drug description: The entire dried pieces of seed shows brownish, ellipsoidal patch and which are marked with irregular patches.

Powder -Light brown in colour, fine and aromatic.

Macroscopic Properties

Size: Average length of seeds - 20.4 mm (Ref. 20-30mm) Average breadth of seeds - 18mm (Ref. about 20mm)

Shape : Ellipsoidal

Colour: Light Brown

Odour : Strongly aromatic

Taste: Pungent and aromatic

Texture of the Powder : Fine

Seed externally marked with small irregular dark brown patches or minute dark spots and slightly furrowed reticulate lines. One of the end is light in colour indicating the position of radicle and the other darker end indicates the position of chalaza. A groove is running along the line of raphae from the point of radicle to chalaza of the opposite end. After breaking the seed, inner side shows an abundant greyish-brown endosperm surrounded by a thin layer of perisperm with infoldings appearing as dark colour ruminations. An embryo shows in an irregular small cavity with two widely spreading crumpled cotyledons and a radicle inside.

Microscopic Properties

A. T.S. of Seed: Transverse Section of Seed is done by Free hand Section cutting and Simple staining procedure and findings are as mentioned below.

- Seed externally covered with a seed coat consisting of epicarp
- Epicarp is composed of small square shaped cells covered with a thin cuticle
- Epicarp is followed by radially elongated palisade like compact thin walled cells.
- Tannin presents in some of the parenchyma cells that are in continuous sheath.
- Vascular bundles with scalariform vessels and phloem elements are seen distributed diffusely in parenchymatous tissue of the seed coat.
- Below the seed coat heterogenous peripheral perisperm composed of few layers of small flattened polyhedral parenchyma cells is present. It is followed by layers of squarish cells containing brown content and prismatic crystals of calcium oxalate.
- Inner part of perisperm is composed of cells of variable shapes and sizes, thin walled and consists of dark amorpous content.
- Inner part of perisperm is showing infoldings in to the tissue of endosperm to form ruminations which are lobed or vermiform in appearance. These ruminations are containing numerous very large oil cells with cell walls.
- These ingrowths are having collateral vascular strands with xylem and phloem elements
- Inner to the perisperm, abundant mass of Endosperm is present, as it becomes lobed due to infoldings of perisperm it is called as “Ruminate endosperm”.
- Endosperm is composed of thin walled parenchyma cells. Occasionally tannin containing thin & brown walled idioblasts are present in between the endosperm cells.

- Cells of endosperm consists of numerous simple rounded and compound starch grains with 2-10 components. Some of the cells are also containing aleuron grains
- Embryo is shrivelled with collapsed parenchyma. Fig.4 and 5

B. Powder Analysis: Powder Analysis is carried out in Glycerin mount, Iodine solution mount and Saffranin solution mount and the following characters are identified.

- Numerous simple rounded and compound starch grains with 2-10 components, fragments of parenchymatous cells filled with starch grains, fragments of vessels with scalariform thickenings, fibers isolated and also in bundles, prismatic crystals of calcium oxalate and oil globules. Fragments of endosperm having cells filled with starch grains. Fig. 1,2and 3

Pharmacognosy of *Javitri* (Aril of *Myristica fragrans* Houtt.)

Name of the Sample : *Javitri*
Scientific Name : ***Myristica fragrans* Houtt.**
Family : Myristicaceae
Plant part : Aril

Drug description: The drug received is dried, entire and fragmented, flat ribbon like, branched and reddish colour pieces of aril.

Powder drug is fine, yellowish brown and strongly aromatic.

Macroscopic Properties:

Size : 2 to 4 cm
Shape : Flat, ribbon like and branched
Colour : Reddish to dark Red in colour
Odour : Strongly aromatic
Taste : Pungent & aromatic
Texture of the Powder : Fine

The Aril is fused in to wide bowl-shaped at the base where it attaches to seed and becomes free above; generally it loosely adheres on the seed and can easily be separable.

Microscopic Properties

C. T.S. of Aril: Transverse Section of Aril is done by Free hand Section cutting and Simple staining procedure and findings are as mentioned below.

- Transverse section of aril is more or less iso-bilateral and flat like a T.S. of a Leaf.
- Externally both the surfaces are covered by a single layered epidermis composed of Small Square shaped, thin walled cells having shallow foldings.
- Epidermal layers are externally covered with a prominent cuticle
- In between both the epidermal layers parenchymatous ground tissue is present.
- Ground tissue consists of cells of unequal sizes i.e. some are small and some are larger.
- The larger cells are oil bearing idioblasts, they are angular in outline and thick walled.
- In between the idioblasts small angular or spindle shaped parenchymatous cells are present.
- In the middle part of aril prominent discrete strands of vascular bundles are present.
- In the vascular bundles small strands of xylem and Phloem elements are present
- Circular secretory oil cavities surrounded by epithelial cells are present in ground tissue.
- Some of the ground parenchymatous cells containing aromatic compounds are stained darker in colour. Fig.8 and 9.

C. Powder Analysis: Powder Analysis is carried out in Glycerin mount, Iodine solution mount and Saffranin solution mount and the following characters are identified.

- Numerous fragments of tissue composed of thick walled cells filled with reddish content, fragments of vessels with scalariform thickenings, fibers isolated and also in bundles, prismatic crystals of calcium oxalate and oil globules. Starch grains and aleuron grains are absent. Fig.6 and 7.

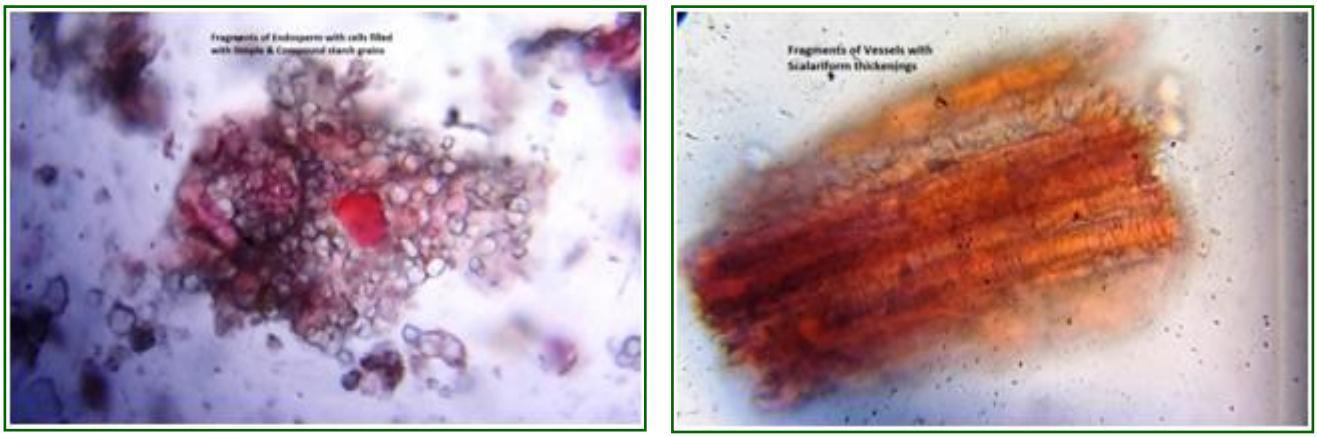
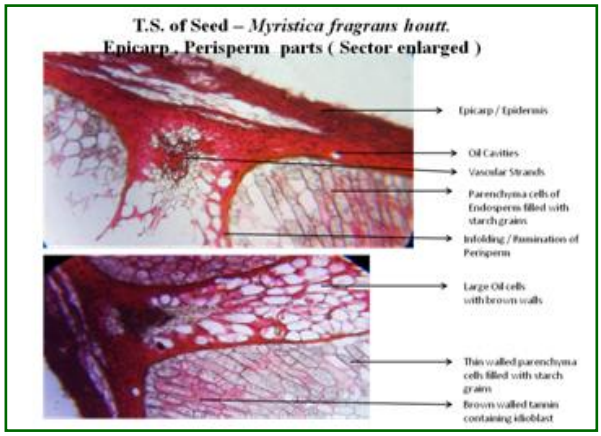


Fig 1, 2: *Jatiphala* seed Powder analysis



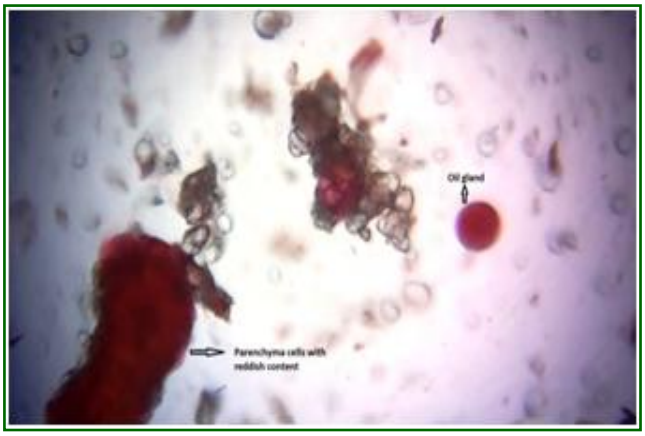
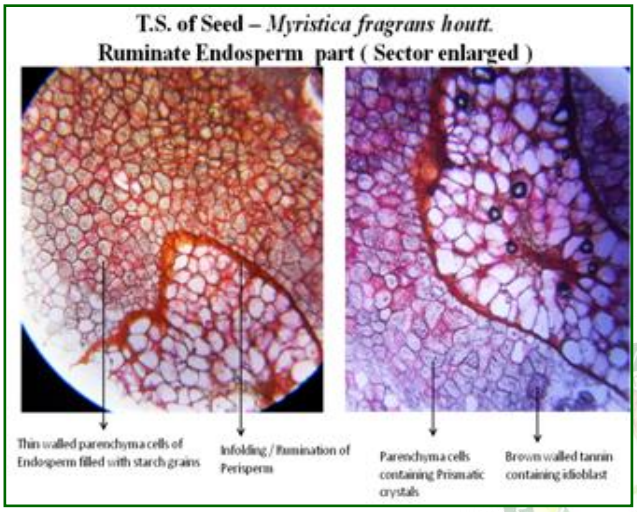


Fig 3, 4: T. S. of Seed analysis – *Myristica fragrans* Houtt.



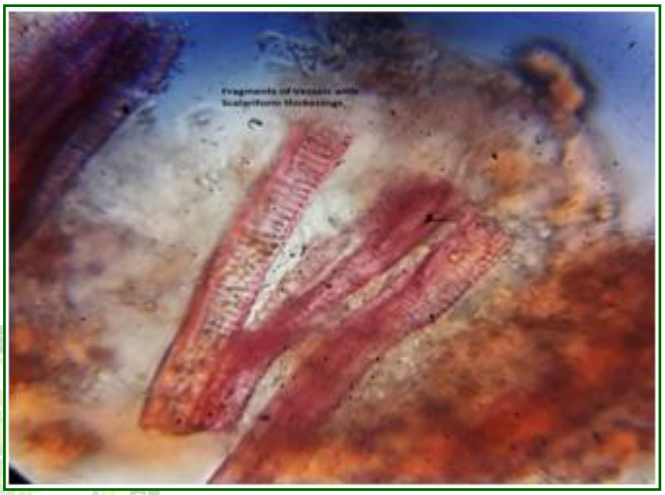
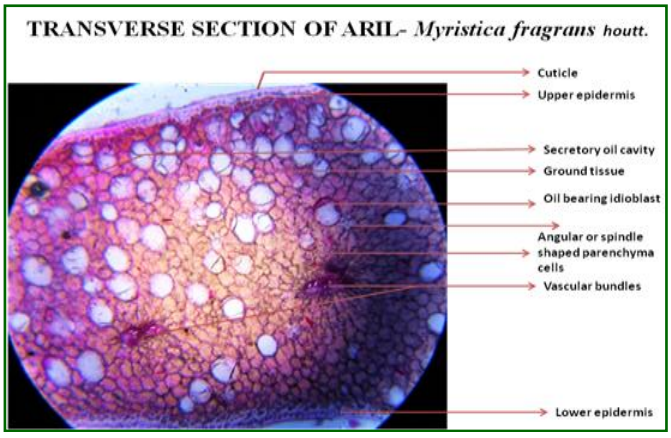


Fig 5, 6: T. S. of Seed analysis – *Myristica fragrans* Houtt.

Fig 7: *Javitri* Powder analysis



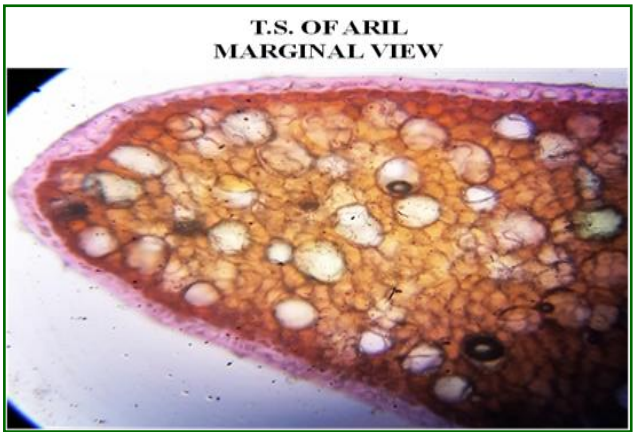


Fig 8, 9: Transverse Section of Aril *Myristica fragrans* Houtt.

PHYTOCHEMICAL STUDY

AIMS AND OBJECTIVES

The research drug *Jatiphala* seed and *Javitri* (aril) are subjected to preliminary Phytochemical screening for the detection of various chemical constituents present.

MATERIALS AND METHODS

I. Tests for Alkaloids

Mayer's Test: To1 ml of the extract, 3 ml of Mayer's reagent was added, the formation of full white precipitate confirmed the presence of alkaloids. (Result in Table No.1 and 2)

II. Test for Carbohydrates

Molisch Test: To 2 ml of the extract, 1 ml of α-naphthol solution was added, and concentrated sulphuric acid through the sides of test tube. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates. (Result in Tab. No.1 and 2).

III. Tests for Proteins and Amino Acids

1.Biuret Test: To 1 ml of the extract add 1 ml of 40% sodium hydroxide solution was added followed by 2 drops of 1% copper sulphate solution. Formation of a violet colour showed the presence of proteins. (Result in Tab. No.1 and 2).

2.Ninhydrin Test: Two drops of freshly prepared 0.2% Ninhydrin reagent was added to the extract solution and it was then heated. Development of blue colour revealed the presence of proteins, peptides or amino acids. (Result in Tab. No.1 and 2).

1V. Test for Saponins

About 1 ml of methanol extract was diluted separately with distilled water to 20 ml, and shaken in a graduated cylinder for 15 minutes. A1% 1 cm layer of foam indicated the presence of Saponins. (Result in Tab. No.1 and 2).

V. Test for Flavonoids

Shinoda Test: To 1 ml of the extract, magnesium turnings were added followed by 1-2 drops of concentrated hydrochloric acid. Formation of red colour showed the presence of flavanoids. (Result in Tab. No.1 and 2).

VI. Test for Tannins and Phenolic compounds

To 1 ml of the extract, ferric chloride was added, formation of a dark blue or greenish black colour product showed the presence of tannins. (Result in Tab. No.1 and 2).

V11.Test for Fixed Oils

Spot Test: A small quantity of extract was pressed between two filter papers. Oil stains on paper indicated the presence of fixed oils. (Result in Tab. No.1 and 2).

RESULTS AND OBSERVATIONS: Table No : 1

Table 1: Showing the Phytochemical analysis results in Water extracts

S.No.	Test	*Jatiphal*	*Javitri*
1	Alkaloids	+	_
2	Carbohydrates	_	+
3	Proteins and Amino acids	_	_
4	Tannins	_	+
5	Saponins	_	_
6	Flavonoids	+	+
7	Fixed oils	+	_
8	Acid test (pH)	6	6

+Positive ; -Negative

Results and observations

1. *Jatiphal* - It showed the presence of Alkaloids, Flavonoids, and Fixed oils, with pH value as 6.
2. *Javitri* - It showed the presence of Carbohydrates, Tannins, Flavonoids and pH value as 6.

Table 2: Showing the Phytochemical analysis results in Milk extracts

S.No.	Test	*Jatiphal*	*Javitri*
1	Alkaloids	_	_
2	Carbohydrates	Green colour	Orange
3	Proteins and Amino acids	_	_
4	Tannins	+	+
5	Saponins	_	_
6	Flavonoids	_	_
7	Fixed oils	_	_
8	Acid test (pH)	7	7

+Positive; -Negative

Results and observations

1. *Jatiphal* - It showed the presence of Tannins and Carbohydrates (Green colour). with pH value as 7.
2. *Javitri* - It showed the presence of Carbohydrates (Orange colour) and Tannins. with pH value as 7.

PHYSICOCHEMICAL STUDY

AIMS AND OBJECTIVES

To study the Identity, Purity and strength of seed and aril of *Jatiphala* (*Myristica fragrans* Houtt.)

METERIALS AND METHODS

1. Loss on drying at 105 C/ Moisture content

10 gm of trail drug samples are placed after accurately weighing it in a tarred evaporating dish. After placing the above said amount of sample in a tarred evaporating dish is dried at 105 C for 5 hours and it is weighed. After drying tarred evaporating dish was allowed to cool in desiccators for 30 minutes and then weighed the remnant material. (Result in Table No.3).

$$\text{The \% of Loss on drying} = \frac{\text{Difference in weight after heating}}{\text{Weight of sample taken}} \times 100$$

2. Determination of Ash

1. Determination of Total Ash

About 2.0g of powdered drugs was weighed and placed in three separate previously ignited and tarred silica crucibles. The samples were spread evenly and then ignite or incinerate it to a constant temperature not exceeding 450C until it is white indicating the absence of carbon. The crucible then cooled in desiccators and final weighed. The results were then calculated the content of total ash in terms of percentage w/w of the air-dried drug.

2. Determination of Extractable Matter in water and alcohol

About 4.0g of coarsely powdered air dried samples, was accurately weighed in three glass Stoppard conical flask and macerated with 100ml of the solvent (Water, Methanol, Ethanol, Hydro alcoholic, Ethyl acetate, Chloroform, Benzene and Hexane) specified for the plant material concerned for 6 hours, shaking frequently and then allowed to stand for 18 hours. Filtering was done by what man paper, taking care not to lose any solvent, and then transfer 25 ml of filtrate to tarred flat bottomed shallow dish. The extracted matter was dried at 105C for 6 hours, cooled in desiccators for 30 minutes and then weighed. The percentage extractable matter was calculated. (Result in Table No.3)

RESULTS AND DISCUSSIONS:

Table 3: Results of Identity, Purity and Strength

Parameter	**Results**	
	***Jatiphala* (seed)**	***Javitri* (Aril)**
Loss on Drying	1.9 %	17.9 %
Total Ash	2.75 %	4 %
Acid Insoluble Ash	1.25 %	2 %
Water Soluble Matter	3 %	11 %
Alcohol Soluble Matter	54 %	43 %

HPTLC (High performance thin layer chromatography)

High performance thin layer chromatography (HPTLC) is an enhanced form of thin layer chromatography (TLC). A number of enhancements can be made to the basic method of thin layer chromatography to automate the different steps, to increase the resolution achieved and to allow more accurate quantitative measurements.

Automation is useful to overcome the uncertainty in droplet size and position when the sample is applied to the TLC plate by hand. One recent approach to automation has been the use of piezoelectric devices and inkjet printers for applying the sample.

The spot capacity (analogous to peak capacity in HPLC) can be increased by developing the plate with two different solvents, using two-dimensional chromatography. The procedure begins with development of sample loaded plate with first solvent. After removing it, the plate is rotated 90° and developed with a second solvent.

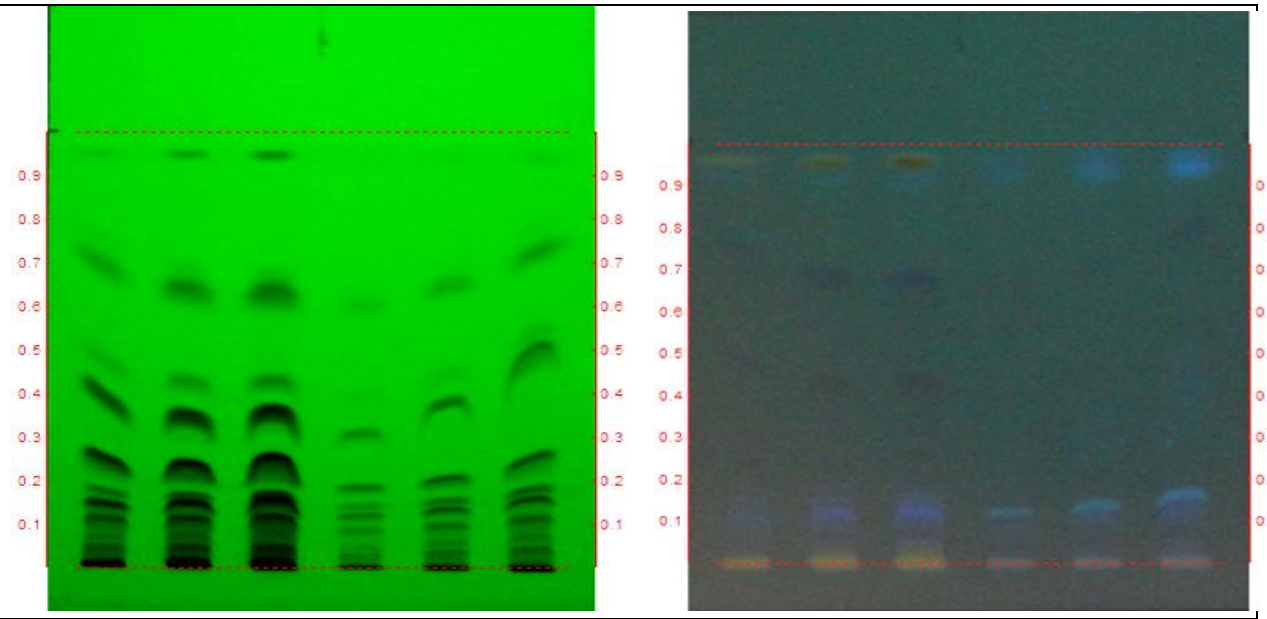
AIMS AND OBJECTIVES

- To study the Identification and Quantitative determination of phytochemical constituents of Seed and Aril part of the Nutmeg (*Myristica fragrans* Houtt.)
- **HPTLC**

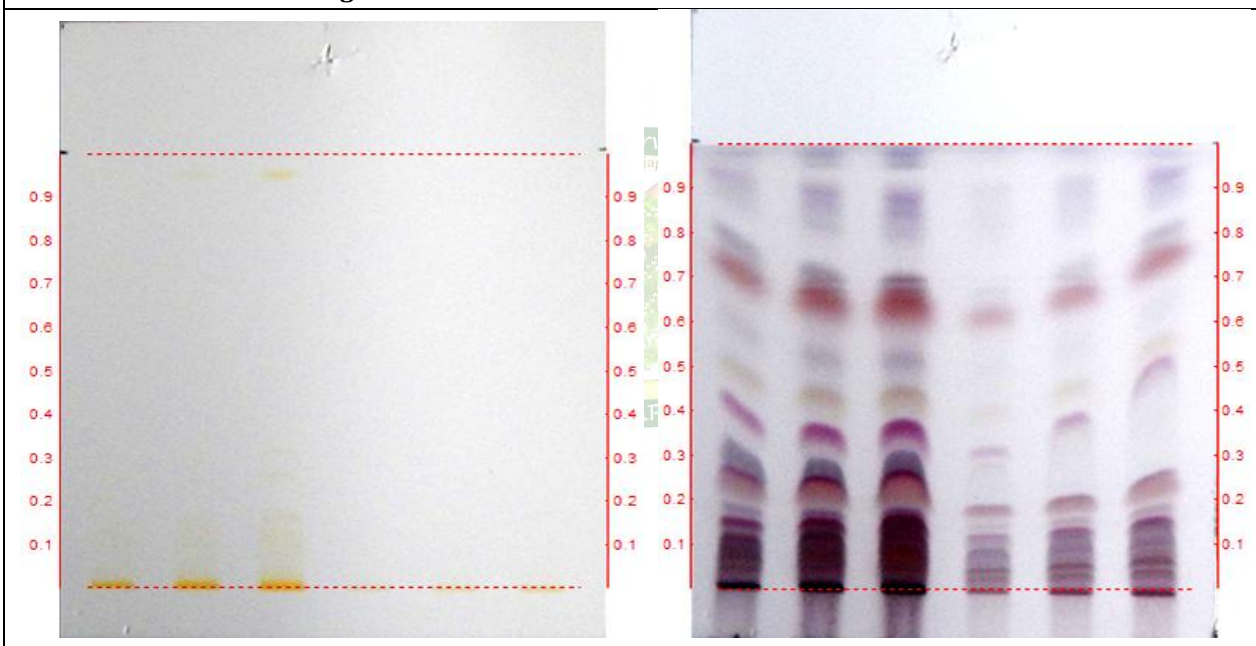
  1g of sample was extracted with 10 ml of chloroform. 3, 6 and 9 µl of the above extract was applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in *n*-hexane: chloroform (1:1) as per the methods for identification of Myristicin standard*. The developed plates were visualized in UV 254, 366, and then derivative with vanillin sulphuric acid and scanned under UV 254 and 366 nm. R_f, colour of the spots and densitometric scan were recorded.

  *Quality Standards of Indian Medicinal Plants, 2003, Vol 1, New Delhi: Indian Council of Medical Research, p. 156-7.

Figure 1. HPTLC photo documentation of Chloroform extract of *Jatipatra* and *Jatiphala*



Under short UV Under long UV



Under white light After derivatisation with vanillin sulphuric acid

Track 1- *Javitri* – 3 µl; Track 2– *Javitri* – 6 µl; Track 3- *Javitri*– 9 µl

Track 4- *Jatiphala*– 3 µl; Track 5– *Jatiphala* – 6 µl; Track 6- *Jatiphala* – 9 µl

Solvent system: *n*-Hexane: Chloroform (1:1)

Table 1: R_f values of sample At 254 nm

Javitri	*Jatiphala*
0.04(D Green)	0.04(L Green)
-	0.08(L Green)
0.09(L Green)	0.09(L Green)
0.12(D Green)	0.12(L Green)
0.16(D Green)	0.16(L Green)
0.18(L Green)	0.18(L Green)
-	0.24(L Green)
0.26(D Green)	-
0.37(D Green)	-
0.43(D Green)	-

-	0.51(L Green)
-	0.55(L Green)
0.65(Green)	-
0.68(D Green)	-
-	0.74(L Green)
0.94(D Green)	0.94(L Green)

***L-Light, D-Dark**

Table 2: R_f values of sample At 366 nm

Javitri	*Jatiphala*
0.05(F L Red)	0.05(F L Violet)
0.08(F L Red)	-
0.13(F L Violet)	0.13(F L Violet)
0.18(F L Violet)	0.18(F Blue)
0.24(F L Red)	0.24(F L Violet)
0.29(F L Red)	-
-	0.34(F L Violet)
-	0.38(F L Violet)
0.44(F L Violet)	0.44(F L Violet)
-	0.53(F L Violet)
0.69(F L Violet)	-
-	0.71(F L Violet)
0.78(F L Brown)	-
-	0.83(F L Red)
0.92(F L Violet)	-
0.96(F Brown)	0.96(F Violet)

***L-Light, F-Fluorescence**

Table 3: R_f values of sample At 540 nm

Javitri	*Jatiphala*
0.08(L Brown)	-
0.16(L Brown)	-
0.25(L Brown)	-
0.31(L Brown)	-
0.95(L Brown)	-

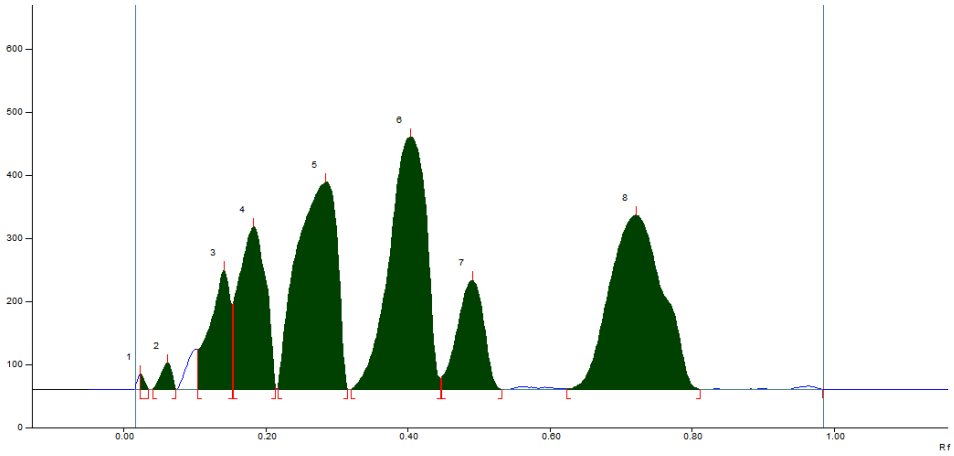
***L-Light**

Table 4: R_f values of sample After Derivatisation

Javitri	*Jatiphala*
0.04(Red)	0.04(L Red)
0.07(Red)	0.07(L Red)
0.12(Red)	0.12(L Violet)
0.17(Red)	0.17(L Red)
-	0.19(L Violet)
0.22(Brown)	-
0.25(Red)	0.25(L Red)
0.31(L Violet)	-
0.38(Pink)	-
0.45(Brown)	-
0.52(L Violet)	0.52(L Red)
-	0.56(L Yellow)
-	0.65(L Violet)
0.67(Red)	-
0.70(L Violet)	-
0.75(Pinkish Red)*	0.75(Pinkish Red)*
0.79(L Violet)	-
-	0.81(L Violet)
0.85(L Violet)	-
0.89(L Violet)	0.89(L Violet)
0.94(L Violet)	0.94(L Violet)

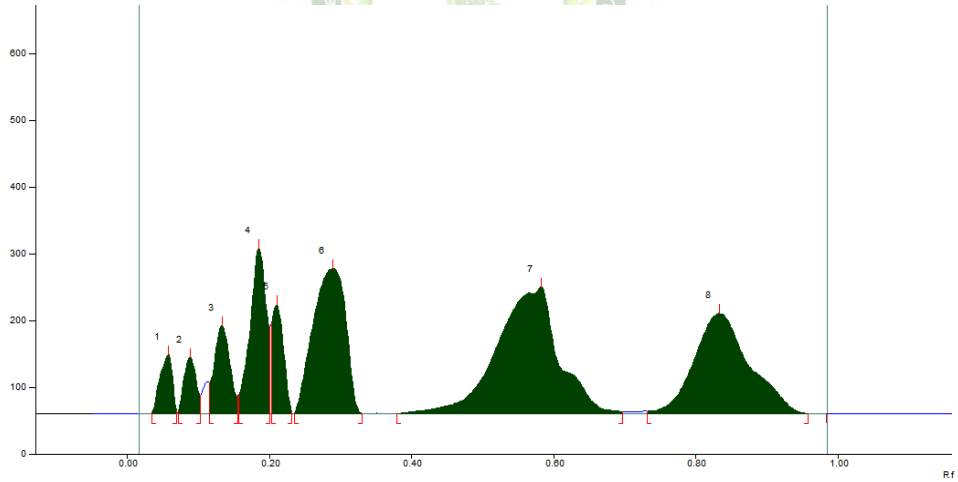
***Myristicin; L-Light**

Figure 2. Densitometric scan of the samples at 254 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	24.5 AU	0.02 Rf	24.5 AU	1.45 %	0.04 Rf	1.1 AU	110.1 AU	0.19 %
2	0.04 Rf	0.5 AU	0.06 Rf	42.5 AU	2.51 %	0.07 Rf	0.6 AU	453.5 AU	0.77 %
3	0.10 Rf	63.6 AU	0.14 Rf	189.5 AU	11.21 %	0.15 Rf	34.5 AU	3859.2 AU	6.59 %
4	0.15 Rf	136.6 AU	0.18 Rf	257.4 AU	15.23 %	0.21 Rf	3.3 AU	6836.2 AU	11.67 %
5	0.22 Rf	4.8 AU	0.29 Rf	328.2 AU	19.41 %	0.32 Rf	0.7 AU	13184.1 AU	22.51 %
6	0.32 Rf	0.4 AU	0.40 Rf	399.9 AU	23.65 %	0.45 Rf	18.0 AU	14722.6 AU	25.14 %
7	0.45 Rf	19.3 AU	0.49 Rf	172.9 AU	10.23 %	0.53 Rf	0.1 AU	4587.6 AU	7.83 %
8	0.62 Rf	0.9 AU	0.72 Rf	275.8 AU	16.32 %	0.81 Rf	0.2 AU	14804.2 AU	25.28 %

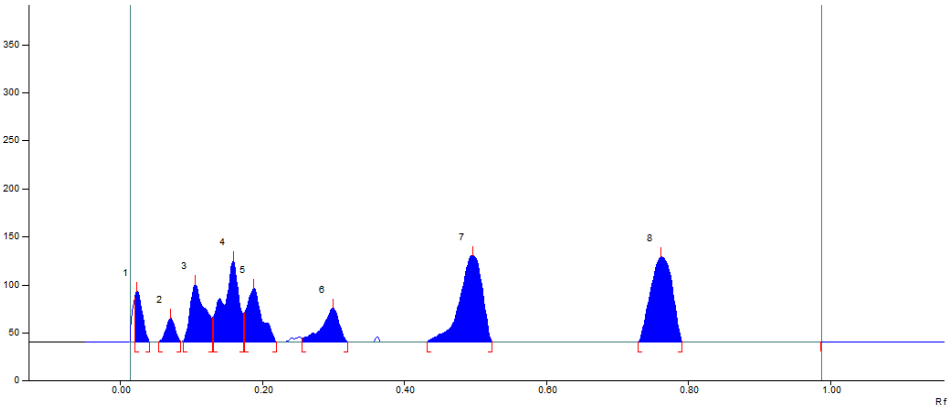
Fig 2.a *Javitri*



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.8 AU	0.06 Rf	88.5 AU	6.97 %	0.07 Rf	5.0 AU	1096.0 AU	2.92 %
2	0.07 Rf	1.8 AU	0.09 Rf	84.2 AU	6.63 %	0.10 Rf	27.7 AU	1045.3 AU	2.78 %
3	0.12 Rf	46.5 AU	0.13 Rf	131.1 AU	10.33 %	0.16 Rf	25.6 AU	2099.8 AU	5.59 %
4	0.16 Rf	28.2 AU	0.19 Rf	247.3 AU	19.47 %	0.20 Rf	29.6 AU	4065.5 AU	10.82 %
5	0.20 Rf	133.9 AU	0.21 Rf	162.7 AU	12.82 %	0.23 Rf	2.7 AU	1926.7 AU	5.13 %
6	0.24 Rf	0.1 AU	0.29 Rf	216.6 AU	17.06 %	0.33 Rf	0.5 AU	7143.7 AU	19.02 %
7	0.38 Rf	0.0 AU	0.58 Rf	189.7 AU	14.94 %	0.70 Rf	3.1 AU	11820.9 AU	31.47 %
8	0.73 Rf	3.5 AU	0.83 Rf	149.8 AU	11.79 %	0.96 Rf	0.3 AU	8361.5 AU	22.26 %

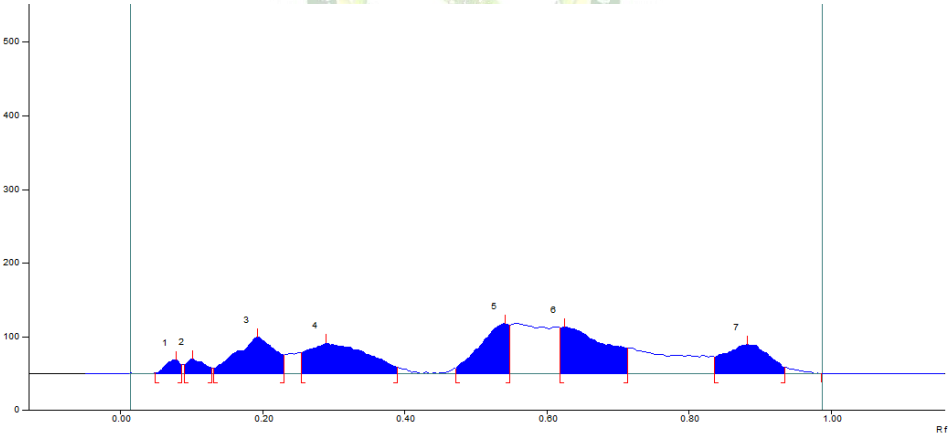
Fig 2.b *Jatiphala*

Figure 3. Densitometric scan of the samples at 366 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	46.6 AU	0.02 Rf	53.1 AU	10.81 %	0.04 Rf	0.1 AU	405.1 AU	4.91 %
2	0.05 Rf	0.4 AU	0.07 Rf	24.8 AU	5.04 %	0.09 Rf	0.5 AU	221.7 AU	2.69 %
3	0.09 Rf	0.7 AU	0.11 Rf	59.9 AU	12.20 %	0.13 Rf	24.8 AU	904.4 AU	10.96 %
4	0.13 Rf	26.7 AU	0.16 Rf	84.3 AU	17.15 %	0.17 Rf	29.6 AU	1327.5 AU	16.08 %
5	0.18 Rf	30.4 AU	0.19 Rf	55.5 AU	11.30 %	0.22 Rf	0.3 AU	830.4 AU	10.06 %
6	0.26 Rf	3.9 AU	0.30 Rf	35.1 AU	7.14 %	0.32 Rf	0.3 AU	601.2 AU	7.28 %
7	0.43 Rf	0.2 AU	0.50 Rf	90.1 AU	18.35 %	0.52 Rf	0.9 AU	2046.4 AU	24.79 %
8	0.73 Rf	0.1 AU	0.76 Rf	88.5 AU	18.01 %	0.79 Rf	0.5 AU	1917.8 AU	23.23 %

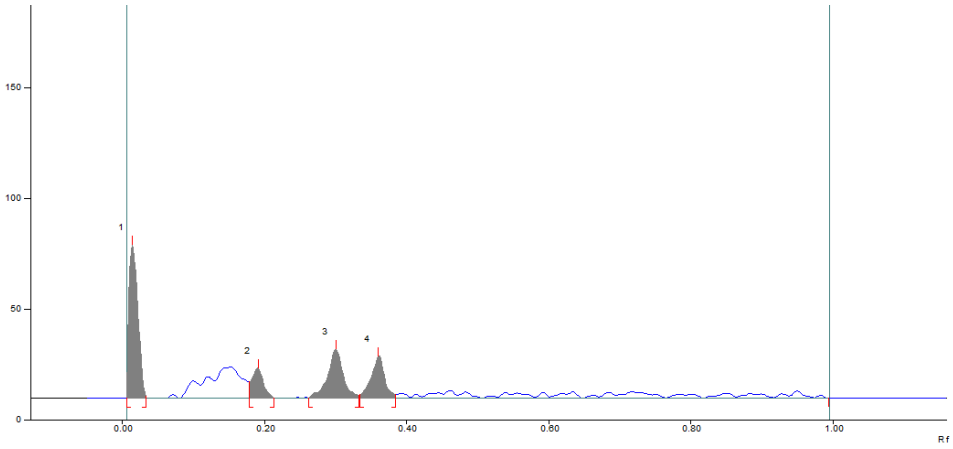
Fig 3.a *Javitri*



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	1.0 AU	0.08 Rf	18.9 AU	6.27 %	0.09 Rf	12.0 AU	276.9 AU	2.37 %
2	0.09 Rf	12.8 AU	0.10 Rf	19.8 AU	6.58 %	0.13 Rf	7.9 AU	362.8 AU	3.11 %
3	0.13 Rf	7.3 AU	0.19 Rf	49.8 AU	16.53 %	0.23 Rf	25.1 AU	1908.4 AU	16.36 %
4	0.25 Rf	28.4 AU	0.29 Rf	41.6 AU	13.79 %	0.39 Rf	8.7 AU	2500.3 AU	21.43 %
5	0.47 Rf	6.8 AU	0.54 Rf	67.9 AU	22.52 %	0.55 Rf	65.7 AU	1976.6 AU	16.94 %
6	0.62 Rf	61.8 AU	0.63 Rf	63.8 AU	21.18 %	0.71 Rf	34.1 AU	2879.1 AU	24.68 %
7	0.84 Rf	22.4 AU	0.88 Rf	39.5 AU	13.12 %	0.94 Rf	8.3 AU	1762.0 AU	15.10 %

Fig 3.b *Jatiphala*

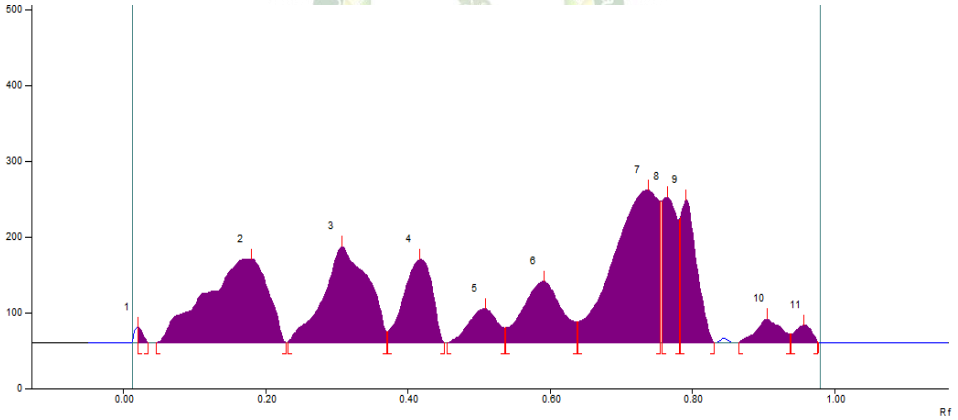
Figure 4. Densitometric scan of the sample at 540 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.01 Rf	69.5 AU	56.02 %	0.03 Rf	0.7 AU	628.2 AU	45.31 %
2	0.18 Rf	7.4 AU	0.19 Rf	13.5 AU	10.85 %	0.21 Rf	0.1 AU	159.6 AU	11.51 %
3	0.26 Rf	0.1 AU	0.30 Rf	22.1 AU	17.86 %	0.33 Rf	1.3 AU	341.5 AU	24.63 %
4	0.34 Rf	1.5 AU	0.36 Rf	18.9 AU	15.27 %	0.39 Rf	1.6 AU	257.3 AU	18.56 %

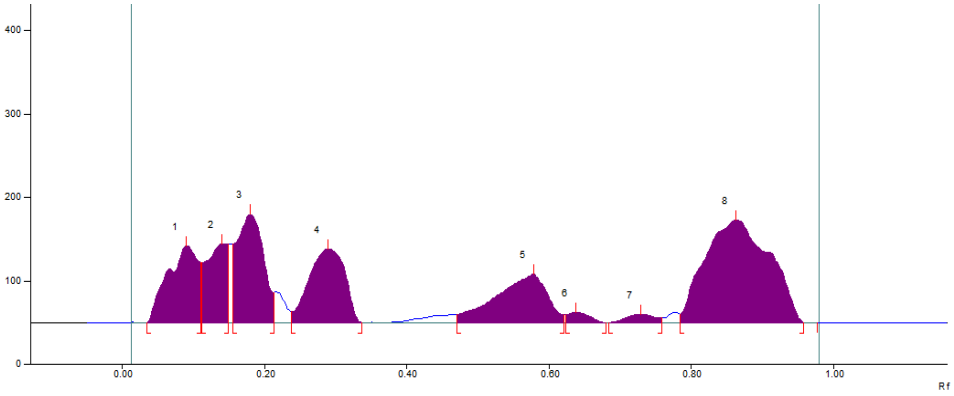
Fig 4.a *Javitri*

Figure 5. Densitometric scan of the sample at 620 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	20.2 AU	0.02 Rf	20.2 AU	1.79 %	0.04 Rf	0.6 AU	112.7 AU	0.31 %
2	0.05 Rf	0.0 AU	0.18 Rf	110.5 AU	9.80 %	0.23 Rf	0.0 AU	6952.4 AU	19.09 %
3	0.23 Rf	0.7 AU	0.31 Rf	127.1 AU	11.27 %	0.37 Rf	14.8 AU	5644.2 AU	15.50 %
4	0.37 Rf	14.9 AU	0.42 Rf	109.7 AU	9.72 %	0.45 Rf	0.6 AU	3113.9 AU	8.55 %
5	0.46 Rf	0.1 AU	0.51 Rf	44.7 AU	3.97 %	0.54 Rf	19.5 AU	1289.9 AU	3.54 %
6	0.54 Rf	19.6 AU	0.59 Rf	80.9 AU	7.17 %	0.64 Rf	27.7 AU	3149.8 AU	8.65 %
7	0.64 Rf	27.7 AU	0.74 Rf	200.7 AU	17.80 %	0.76 Rf	86.2 AU	9067.0 AU	24.89 %
8	0.76 Rf	186.5 AU	0.77 Rf	191.9 AU	17.02 %	0.78 Rf	62.1 AU	2895.4 AU	7.95 %
9	0.78 Rf	164.3 AU	0.79 Rf	187.9 AU	16.66 %	0.83 Rf	0.3 AU	3033.0 AU	8.33 %
10	0.87 Rf	0.5 AU	0.91 Rf	31.1 AU	2.76 %	0.94 Rf	11.3 AU	778.2 AU	2.14 %
11	0.94 Rf	11.5 AU	0.96 Rf	23.0 AU	2.04 %	0.98 Rf	1.8 AU	388.0 AU	1.07 %

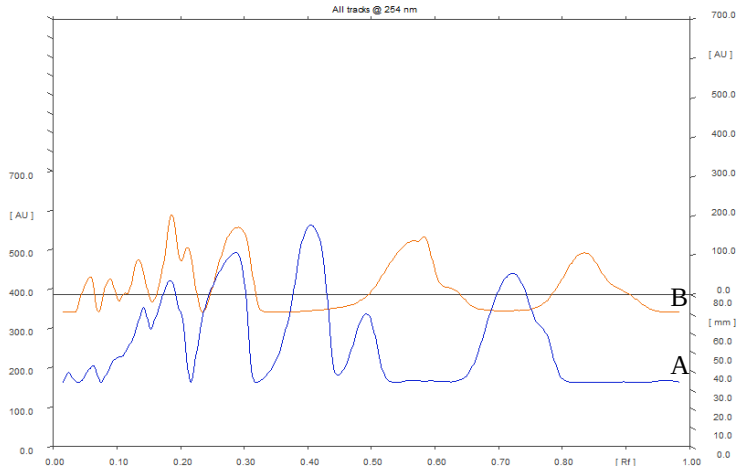
Fig 5.a *Javitri*



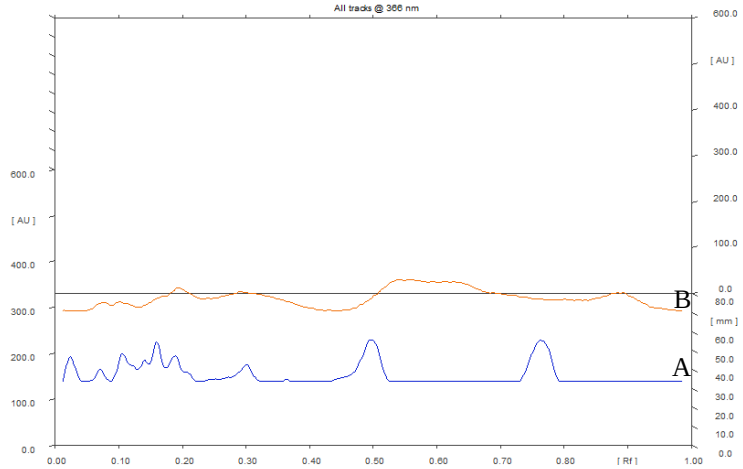
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.3 AU	0.09 Rf	92.1 AU	15.13 %	0.11 Rf	72.1 AU	2831.8 AU	12.15 %
2	0.11 Rf	72.1 AU	0.14 Rf	94.6 AU	15.53 %	0.15 Rf	94.2 AU	2034.5 AU	8.73 %
3	0.16 Rf	94.3 AU	0.18 Rf	129.7 AU	21.29 %	0.21 Rf	36.4 AU	3517.2 AU	15.09 %
4	0.24 Rf	13.2 AU	0.29 Rf	88.3 AU	14.50 %	0.34 Rf	0.2 AU	3217.4 AU	13.81 %
5	0.47 Rf	10.0 AU	0.58 Rf	58.2 AU	9.55 %	0.62 Rf	9.5 AU	3002.8 AU	12.88 %
6	0.62 Rf	9.5 AU	0.64 Rf	12.6 AU	2.07 %	0.68 Rf	0.1 AU	278.9 AU	1.20 %
7	0.69 Rf	0.2 AU	0.73 Rf	10.4 AU	1.71 %	0.76 Rf	5.7 AU	303.4 AU	1.30 %
8	0.79 Rf	10.5 AU	0.86 Rf	123.2 AU	20.23 %	0.96 Rf	0.2 AU	8119.4 AU	34.84 %

Fig 5.b *Jatiphala*

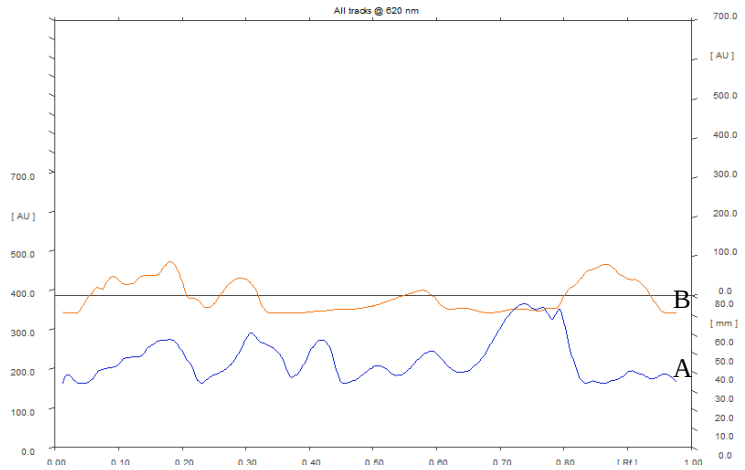
Table 5. 3-d Display of densitograms of *Javitri* and *Jatiphala*



At 254 nm



At 366 nm



At 620 nm

A- *Javitri*, B- *Jatiphala*

HPTLC comparison

		Javitri	*Jatiphala*
Photo-documentation	Under short UV	11	11
	Under long UV	11	11
	Under white light Pre-D	5	0
	Under white light Post-D	17	13
Densitometry	At 254 nm	8	8
	At 366 nm	8	7
	At 540 nm Pre-D	4	0
	At 620 nm Post-D	11	8
Total		**75**	**58**

DISCUSSION

The *Jatiphala* showed the presence of calcium oxalate crystals, large oil cells with cell wall. The endosperm contained Tannins and starch grains and aleuron grains where as the *Javitri* showed larger cells of oil bearing idioblasts, in between the idioblasts small angular or spindle shaped parenchymatous cells are present. Circular secretory oil cavities surrounded by epithelial cells and some of the ground parenchymatous cells containing aromatic compound. The powder analysis of the *Javitri* showed absence of Starch grains and aleuron grains where as *Jatiphala* endosperm showed presence of starch grains.

The presence of secretory oil cavities and oil cells in *Javitri* and *Jatiphala* was seen as it was fresh drug and slides where prepared immediately.

As seen in table no.1 and 2, the preliminary Phytochemicals was done in the department and following results obtained. It was noticed that *Jatiphala* powder with aqueous mixture had the presence of Alkaloids, Flavonoids, Fixed oils where as *Javitri* showed the presence of Carbohydrates, Tannins, Flavonoids. The absence of fixed oils in *Javitri* may be due to evaporation of volatile oils during process of pounding it into powder, therefore P^H of *Jatiphala* and *Javitri* were found to be P^H 6. The Phytochemical analysis of *Jatiphala* powder and *Javitri* powder in milk showed the presence of Carbohydrates (green colour), Tannins and Carbohydrates (Orange colour) and Tannins respectively. The P^H was found to be 7 in the both drugs. Milk extracts of the both drugs showed the absence of Flavonoids in both drugs and fixed oils. This consolidates our opinion that after adding milk the acidic nature of both drugs turned to neutral. (P^H 6 to P^H 7).

Plants are important source of potentiality bioactive constituents for the development of new chemotherapeutic agents. The first step towards this goal, Seed and Aril of *Jatiphala* was subjected to systemic physicochemical screening by aqueous and ethanolic extracts to determine the amount of soluble constituents in a given amount of medicinal seed and aril and are helpful in determining the quality and purity of a crude drug, especially in the powder form.

As seen in table no.3, Physicochemical parameters of seed and aril of *Myristica fragrans* Houtt. were estimated based on the methods. Percent weight loss on drying or moisture content value was found to be in *Jatiphala* (1.9%), *Javitri* (17.9%). This may be due to the process of making the powder of *Jatiphala* and *Javitri* where the *Jatiphala* was taken out by pesita procedure and made into pellets after drying so the moisture content was found less where compared to *Javitri* which was directly pounded to powder. Total ash of *Javitri* was found 4% this may be due to the presence of calcium oxalate crystals as seen in the pharmacognosy studies. The less value of moisture content of drugs could prevent content bacterial, fungal or yeast

growth through storage. The ash value total ash; acid insoluble ash values in *Jatiphala* and *Javitri* were found to be 2.75%, 4% ; 1.25%,2% respectively. Ash values used to find out quality, authenticity and purity of unsophisticated drug and also these values are important quantitative standards. The solubility percentage of *Myristica fragrans* Houtt. in aqueous extraction is lower (*Jatiphala*-3%, *Javitri*- 11%), when compared with ethanolic extraction. (*Jatiphala*-54%, *Javitri*-43%) The extractive values are suggest that the chemical comparison of both drug are more alcohol soluble than water soluble.

CONCLUSION

To conclude the present work it may be noticed that the purity, Quality standard of the crude drug and the powder of *Jatiphala* is in par with the API standard along with HPTLC mentioned that showed that our drug is there is no mention of *Jatiphala* and *Javitri* as such in API. This may be due to *Javitri* is a different part of same plant *Jatiphala*. The results of *Jatiphala* and *Javitri* have been mentioned above. It was noticed HPTLC study that chemical compound Myristicin is more in *Javitri* than *Jatiphala* (Number of R_f par in *Jatiphala* and *Javitri*). The extractive values of water extracts of both *Jatiphala* and *Javitri* well less compared to the alcoholic extractive values. The presence of calcium oxalate crystals showed more ash value in *Javitri* where compared to *Jatiphal*. The P^H of both *Jatiphal* and *Javitri* pure powder P^H 6 became neutral (P^H 7) when they were mixed with milk. The powder of both drugs mixed with milk showed the absence of phytochemical of milk which was seen in the powder form. Thus, the pharmocognosy, phytochemical, physico-chemical study provided the data helpful for correct identification and authentication of raw drugs of *Jatiphala* and *Javitri* as per API the HPTLC showed the presence of myristicin the important chemical composition in *Javitri* and *Jatiphala*.

REFERENCES

1. Chase, C.R. and Pratt, F.J. Fluorescence of powered vegetable drugs with particular reference to development of a system of identification J. Am. Pharm. Assoc. (38) 324-331(1949).
2. Lohar D.R. and Singh Ravindra,: Quality Control Manual For Ayurvedic, Siddha & Unani Medicine, Deptt. Of AYUSH, Ministry of Health & Family Welfare, Government of India (2008).
3. Kokaski, J, Kokoski, R and Sima, F.J. Fluorescence of powered vegetable drugs under ultra violet radiation. J. Am. Pharm. Assoc. 47(10) 715-717 (1958).
4. PSAF. Pharmacopoeial Standards for Ayurvedic Formulations. Central Council for Research in Ayurveda and Siddhha. Ministry of Health and Family Welfare, Govt of India, New Delhi. (1987).
5. Trease, G. E. and Evan, W. C. Pharmacognosy. 12th edition, English Language Book Society, Balliere, Tindall. (1983).
6. WHO. Quality Control Methods for Medicinal Plant Materials. World Health Organisation, Geneva. (1998).
7. Quality Standards of Indian Medicinal Plants, 2003, Vol 1, New Delhi: Indian Council of Medical Research, p. 156-7.

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