IJPSR (2015), Vol. 6, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 October, 2014; received in revised form, 17 March, 2015; accepted, 27 June, 2015; published 01 July, 2015

PHARMACOGNOSTICAL STANDARDIZATION OF NUTMEG SEEDS (*MYRISTICA FRAGRANS* HOUTT.) - A TRADITIONAL MEDICINE

Indra P Tripathi [1] and Neelesh Dwivedi [*2]

Department of Physical Science, M.G.C.G.V.[1], Chitrakoot, Satna, (M.P.), India
JRD Tata Foundation of Research in Ayurveda and Yoga Sciences [2],Chitrakoot, Satna, (M.P.), India

Keywords:

Traditional Medicine, *Myristica fragrans*, Standardization, Physicochemical, HPTLC, Fingerprints.

Correspondence to Author:
Neelesh Dwivedi

(Scientist-1), Research Lab, JRD Tata Foundation of Research in Ayurveda and Yoga Sciences, Chitrakoot, Satna (M.P.) , India

E-mail: dwivedineelesh2013@gmail.com

ABSTRACT: Resurgence of public interest in traditional medicine is increasing in both the developing and developed countries. *Myristica fragrans* belonging to the family Myristicaceae is commonly known as *jaifal* and even used as home-remedies in the rural parts of the India. The fruit is used as traditional medicine more frequently for the treatment of common ailments of children in rural folklore practices at the place. Work on standardization assumes vital significance. However, no supportive data available on microscopic characteristics and standardization of the same. In this article a study has been made to fix the parameters includes botanical identification, physicochemical analysis, preliminary phytochemical analysis, HPTLC fingerprints profile, microbial screening, aflatoxin and heavy metals in order to ensure the use of only genuine and uniform material of such herbal remedies. The obtained values/ranges can be used as standards for quality control of the drug.

INTRODUCTION: *Myristica fragrans* Houtt. (Myristicaceae) is a moderate-sized, aromatic, evergreen tree with slender branches and with beautiful appearance. Seeds are oblong, with hard shell is called a nutmeg (Jaiphala). It is cultivated in the hotter parts of India (Konkan, Madras and Karnataka) up to 750 m with a rainfall of 150-300 cm per annum. It is very much found in the forest of Malaya, Penang, Sumatra, Singapore and China[1]. The ethno botany of nutmeg was studied in the provinces of Maluka and of central and east Java[2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(7).3096-02

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(7).3096-02

The utility of nutmeg as a spice has been known since ancient times in Indonesia, and nutmeg was probably introduced into Europe during the twelfth century. Fruits are used frequently as a powder (3-6gms) in Ayurveda for Atisara, Grahini, Chardi, Mukhroga, Pinasa, Rasa, Svasa and Sukrameha. The fruit passes several pharmacological properties viz. stimulant, narcotic, carminative, astringent, aphrodisiac, acrid, astringent, sweet, bitter, thermogenic, diuretic, hypolipidaemic, antithrombotic, anti-platelet aggregation, antifungal, anti-inflammatory, anodyne, vulnerary, alternate, stomachic, laxative, carminative, digestive, anthelmintic, cordiotonic, aphrodisiac, antiseptic, febrifuge, depurative and tonic [3-5].

Earlier studies reported the presence of major active principle mainly volatile oil (5-10%) contain 4-8% myristicin, elemicine, terpene, alcohols like bereneol, geraniol and hydrocarbons pinene,

camphene and dipentene, Further volatile oil contains safrol, *α-β-* pinene, linalool, eugenol and isoeugenol [6]. The presence of two compounds, myristicin and elemicin, is related to the hallucinogenic action of nutmeg while safrole has been suspected to be carcinogenic. Essential oil from nutmeg shows hepatotoxic activity[7].

Survey of literature showed that no systematic approaches have been made to study the pharmacognostical parameters of this medicinal plant. The present investigation deals with the studies on some important pharmacognostical characteristics viz. macroscopic, microscopic characters including powder characteristics together with physicochemical parameters, preliminary phytochemical test, HPTLC finger printing, microbial screening, aflatoxin and heavy metals of the seed of *Myristica fragrans* as powdered form.

MATERIALS AND METHODS:

Drug samples were purchased from local market of Chitrakoot (Sample JF1) and Chitrakoot Pharmacy, Arogyadham Parisar, Chitrakoot (Sample JF2). The plant materials were authenticated with the help of Herbarium of Deendayal Research Institute, Chitrakoot, Satna, (M.P.). The plant materials were ground into powder, filtered by sieve 80# and the fine powder so obtained was used for further analysis. The standardization parameters were determined according to the methods detailed in the Ayurvedic Pharmacopoeia of India [8-9]. Organoleptic characters and particle size of the both samples were recorded. Quantitative analysis for loss on drying at 105^{o}C, ethanol soluble extractive value, water soluble extractive value, total ash value and acid insoluble ash values were carried out in triplicate in the studied samples. For microscopic analysis preparing slides in water, stained with iodine and mounted with glycerin. Preliminary phytochemical analysis of different extracts was performed by using specific reagents by employing standard procedures[10].

HPTLC finger prints profile was determined to assure identity of the drug. For HPTLC Powdered drug (2g) is extracted by heating under reflux for 15 min with 20 ml methanol and boiled for 5 minutes on a water bath at 105^{0}C temperature, filtered the solution and concentrated the filtrate to 5ml. 7µl of this solution were applied to the chromatogram [11]. Chromatography was performed on (5x10) aluminum packed silica gel 60F_{254} HPTLC plate (Merck, Darmstadt, Germany). Before use, the plates were dried in an oven at 105^{o}c for 5 min. and samples were applied as 9 mm band by means of sample applicator (Camag linomat- 5, Switzerland) equipped with a 100 µl micro Syringe. The developing solvent was allowed to ascend to 90 mm with Tolune: ethyl acetate (9:1) (V/V) as a mobile phase in a Twin Trough Chamber, previously saturated for 20 min by lining with thick Whatman filter paper.

The room temperature was 27^{o} C and relative humidity was 37%. After development of chromatogram, the plates were removed and completely dried in air at room temperature. Observed the spots produced before and after Derivetization with Vanillin-sulphuric acid reagent at day light and ultraviolet light at 254nm and 366nm[12]. Documented the images by means of photo documentation system (Camag Reprostar 3). Measured and recorded the distance of each spot from the point of its application and calculated the R_f value by dividing the distance travelled by the spots by the distance travelled by the front of the solvent system.

Limit tests for heavy metals, aflatoxins and microbial load were determined according to the methods given in the protocol of CCRAS and WHO guideline [13, 14].

RESULTS AND DISCUSSION:

Organoleptic and Macroscopic Characters: Fruit yellow, globose, pericarp fleshy splitting into two halves at maturity, seed oblong, obtuse, testa shiny, arial yellowish red, irregularly lobed, extending to the apex of the seed **Fig.1**. Botanical parameters revealed that the powder is fine (IS sieve no.80#) and homogeneous and yellowish brown in color with strong aroma and slightly bitter taste **Table 1**.

Powder Microscopy: The powder when seen under the microscope shows various diagnostic characters viz. Prismatic crystals of calcium oxalate, Starch grains, Paranchymatous cells, Glandular trichomes and Endosperm **Fig. 2**.

Physicochemical Parameters: Ash of any organic material is composed of their non-volatile inorganic components[15]. The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. It is useful for the estimation of specific constituents, soluble in that particular solvent used for extraction[16]. Physicochemical parameters of *Myristica fragrans* such as total ash, water soluble ash, acid in-soluble ash, loss on drying, ethanol soluble extractive value, and water soluble extractive value were carried out and are summarized on **Table 2**.

Preliminary Phytochemical Analysis:
The seed powder was extracted with water and ethanol. These extracts were tested for presence of different phytoconstituents. The results of phytochemical analysis are tabulated in **Table 3**. Preliminary phytochemical studies revealed the presence of saponin, resin, carbohydrate, terpenoids, steroids and volatile oils.

HPTLC Fingerprint Profile:
The TLC profile of methanol extracts of *Myristica fragrans* (sample JF_1 & JF_2) along with *Tolune: ethyl acetate* (9:1) as mobile phase resolved major spots at R_f 0.05, 0.10, 0.20, 0.41, 0.54, 0.72, 0.82 (all black) at 254 nm; After spraying with Anisaldehyde-sulphuric acid major spots at R_f 0.05, 0.10 , 0.19 (all black), 0.22 (pink), 0.37 (brown), 0.41 (violet), 0.54 (pink), 0.65 (brown), 0.70 (grey) and 0.82 (red) at 366nm; and at R_f 0.05, 0.10, 0.17(all red), 0.28 , 0.38 (both brown), 0.41 (violet), 0.54 (brick red), 0.75 (brown), 0.82 (red) at visible light **Table 4, Fig. 3**. HPTLC studies of the extracts of *Myristica fragrens* in comparison with the myristicine standard revealed that corresponding values at R_f 0.82 is identical to myristicine at 254 nm [17]. Here, similar corresponding spots at 0.82 were observed which indicate the presence of myristicine in the sample as main active principle.

Microbial limits, Aflatoxins and Heavy Metals:
The results of the microorganisms, aflatoxins and heavy metals were given in **Table 5.** The drug was subjected to microbiological evaluation namely for *E.coli, Staphylococcus aureus, Salmonella spp., Pseudomonas aeruginosa*. All microbial species were found to absent. Total aerobic microbial count and total yeast and mould were found to be within range as per API limits **Fig. 4**.

The substance known as aflatoxin, is a product of the microbial strain of *aspergillus flavus*, will produce serious side effects if consumed along with the crude drugs[18]. In the present study the presence of aflatoxins were determined by chromatographic methods using standard aflatoxins B_1, B_2, G_1, and G_2 mixtures. The four application of the aflatoxin solution appeared as four clearly separated blue fluorescent spots at R_f 0.55, 0.44, 0.49 and 0.39 for B_1, G_1, B_2 and G_2 respectively **Fig. 5**. The results showed absence of aflatoxins in the studied samples.

Heavy metal if present in formulations will have a deleterious effect on different organs of body in particular kidneys and leads to renal toxicity [19].

Heavy metals include Arsenic, Lead and Mercury. In the present study these metals were evaluated by means of limit tests where the allowed maximum limits were 3 ppm, 10 ppm, 1 ppm respectively and were found to be absent. The presence of mercury was determined qualitatively and found to be absent. The safe level of heavy metals in the drug suggests it is safe for consumption.

TABLE 1: ORGANOLEPTIC PROPERTIES OF *MYRISTICA FRAGRENS*

SN.	Sample	Appearance	Color	Odor	Taste	Texture	Particle size
1.	(JF1)	powder	light brown	strong	pungent	fine	80#
2.	(JF2)	powder	light brown	strong	pungent	fine	80#

TABLE 2: PHYSICO-CHEMICAL PARAMETERS OF *MYRISTICA FRAGRENS*

SN.	Parameters	Unit	Samples		Standard limits (API)
			JF1	JF2	
1	pH		4.8*	4.9*	
2	Loss on drying	(% w/w)	6.9*	6.6*	
3	Total Ash	(% w/w)	2.2*	2.2*	NMT 3
4	Acid-Insoluble	(% w/w)	0.22*	0.23*	NMT 0.5

5	Alcohol Soluble Extractive	(% w/w)	22.0*	21.0*	NLT 11
6	Water Soluble Extractive	(% w/w)	9.9*	11.0*	NLT 7
7	Volatile oil	(% w/w)	11.22*	11.20*	6-16

*Values are mean of three determinations.

I. Fruiting twig of *Myristica fragrens,* II. Seeds of *Myristica fragrans,* III. Powder form of *Myristica fragrans*

FIG. 1: MACROSCOPY OF *MYRISTICA FRAGRANS* (JAIFAL) – FRUIT

Prismatic crystals of calcium oxalate | Starch grains | Paranchymatous cells | Glandular trichomes | Endosperm

FIG 2: POWDER MICROSCOPY OF *MYRISTICA FRAGRANS* (JAIFAL) - SEED

TABLE 3: QUALITATIVE CHEMICAL ANALYSIS

S.N	Active constituents	Name of the test	JF1	JF2
1	Alkaloid	Wagner's test	-	-
2	Flavanoid	HCl+ Mg	-	-
3	Tannin	$FeCl_3$	-	-
4	Saponin	$NaHCO_3$	+	+
5	Resin	Acetone	+	+
6	Carbohydrate	Fehling test	+	+
7	Protein	$NaOH+CuSO_4$	-	-
8	Terpenoid	$CHCl_3$ + Con. H_2SO_4	+	+
9	Steroid	Aceticanhydrite + H_2SO_4	+	+

(+) Presence of phyto-constituents
(-) Absence of phyto-constituents

TABLE 4: TLC PROFILE OF METHANOLIC EXTRACTS OF *MYRISTICA FRAGRENS* IN TOLUNE: ETHYL ACETATE (9:1V/V)

Rfs	Visualization of spots								
	At 254nm			At 366nm			At visible light (AD)		
	JF_1	JF_2	Colors	JF_1	JF_2	Colors	JF_1	JF_2	Colors
R_{f1}	0.05	0.05	dark grey	0.5	0.5	black	0.05	0.05	cherry red
R_{f2}	0.10	0.10	dark grey	0.10	0.10	black	0.10	0.10	red
R_{f3}	-	-		0.17	0.17	black	0.17	0.17	cherry red
R_{f4}	0.20	0.20	dark grey	0.20	0.20	pink	0.28	0.28	brown
R_{f5}	0.41	0.41	dark grey	0.40	0.40	violet	0.38	0.38	Brown
R_{f6}	0.54	0.54	dark grey	0.54	0.54	pink	0.41	0.41	Violet
R_{f7}	0.72	0.72	dark grey	0.65	0.65	brown	0.54	0.54	Brick red
R_{f8}	0.82	0.82	dark grey	0.70	0.70	gray	0.75	0.75	Brown
R_{f9}	-	-		0.82	0.82	red	0.83	0.82	Red

TABLE 5: MICROBIAL SCREENING, AFLATOXINS AND HEAVY METALS

Safety evaluation	Parameters	Results	Permissible limits
Microbial contamination	*Staphylococcus aureus\g*	Nil	Nil
	Salmonella spp.\g	Nil	Nil
	Pseudomonas aeruginosa\g	Nil	Nil
	E.coli.	Nil	Nil
	Total Aerobic Microbial count (AMC)	68 cfu\g	10^5\g
	Total Yeast and Moulds	24 cfu\g	10^3\g
Aflotoxins	B_1	Nil	NMT 0.50 ppm
	B_2	Nil	NMT 0.10 ppm
	G_1	Nil	NMT 0.50 ppm
	G_2	Nil	NMT 0.10 ppm
Heavy metals	Arsenic	Nil	NMT 3.0 ppm
	Lead	Nil	NMT 10.0 ppm
	Mercury	Nil	NMT 1.0 ppm

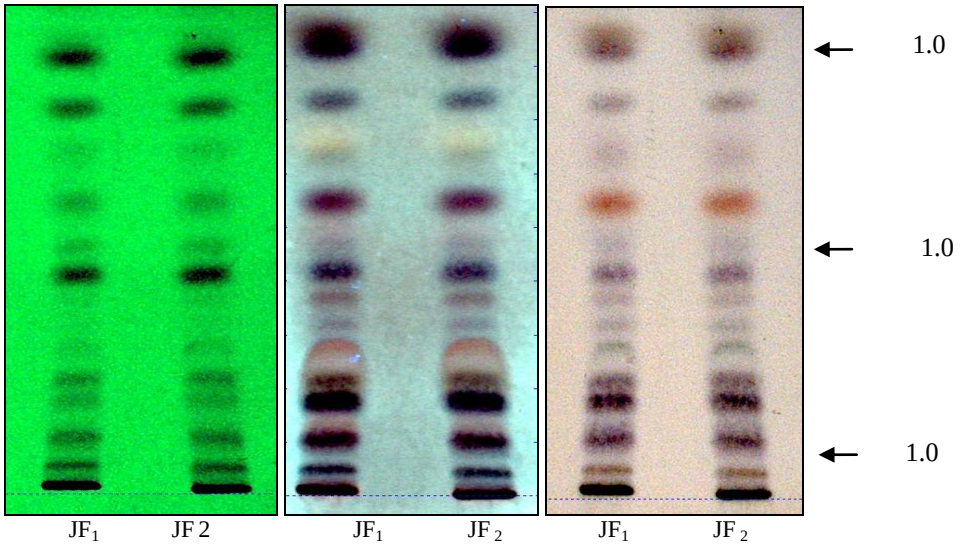


FIG 3: HPTLC FINGERPRINTS PROFILE OF *MYRISTICA FRAGRANS*

Chromatographic condition

Standards : Standard aflatoxins solutions (B_1 G_1 B_2 & G_2)

Model : Cammag HPTLC, Switzerland

SP : HPTLC silica gel 60 plate, (10×5cm) Merck Pvt. Ltd., of 0.2 mm thickness

MP : Chloroform: acetone: isopropyl alcohol (85:10:5 :)

Volume of test solution applied: 4µl

Distance traveled by SS : 8c.m.

Development chamber : Twin trough chamber with SS lid (10×10cm)

Visualization : Under ultraviolet light at 366 nm

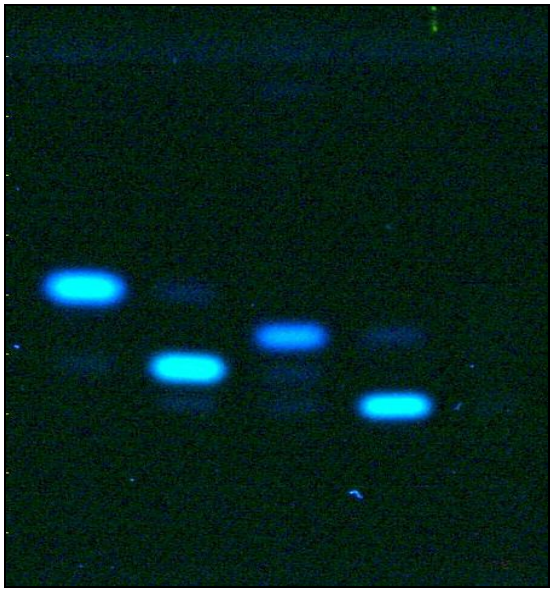
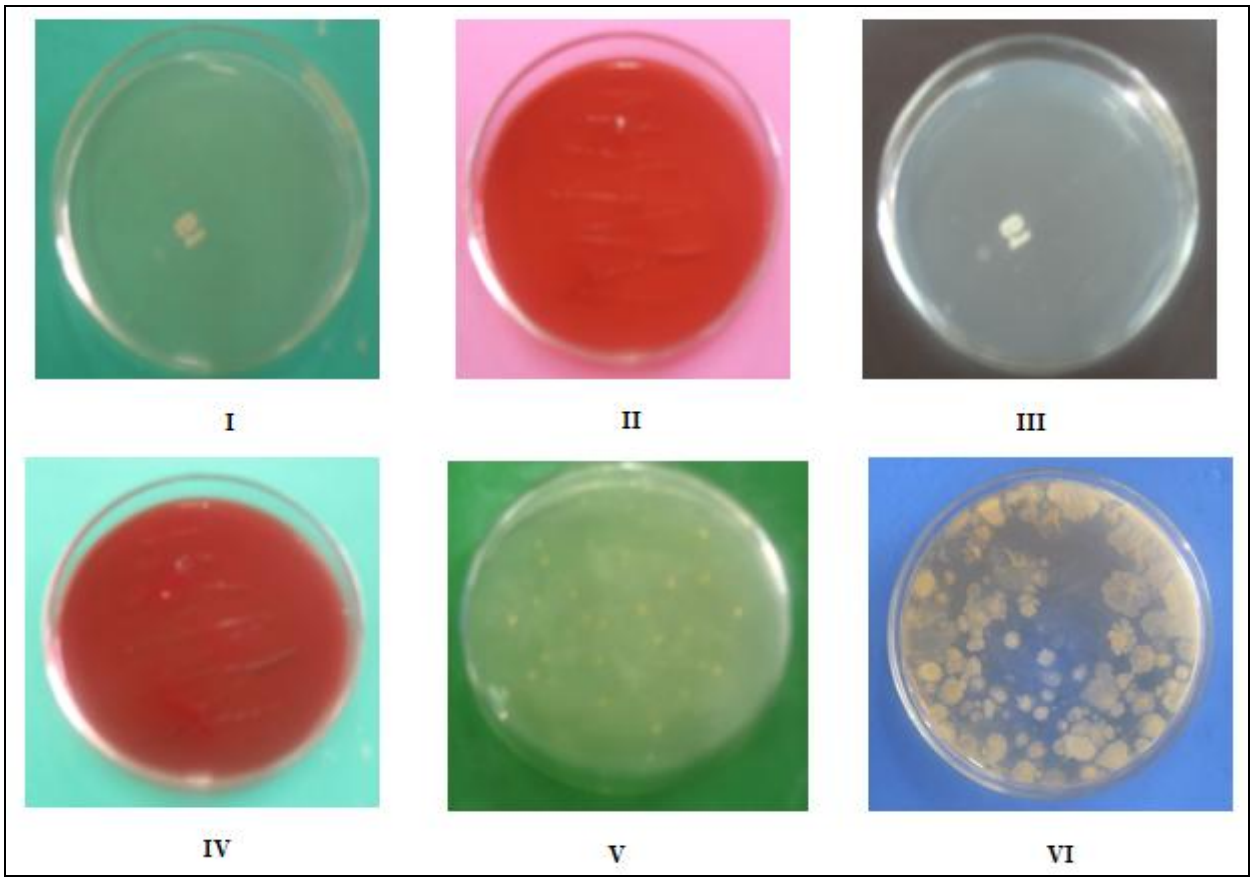


FIG.4: HPTLC CHROMATOGRAM OF AFLATOXINS AND TEST SOLUTIONS



I. Showing negative result for *staphylococcus aureus*; II. Showing negative results for *pseudomonas aeruginosa;* III. XLD agar plate showing negative results of *salmonella*; IV. Mackonkey agar plate showing negative results for *E. coli*; V. Agar plate showing aerobic microbial counts; VI. Agar plate showing yeast & mould counts

FIG 5: MICROBIAL SCREENING OF *MYRISTICA FRAGRENS* (JAIFAL) - SEED

CONCLUSION: Crude drug is the base material for manufacturing of herbal medicines. Efficacy of any drug depends on the genuineness of the raw material used for its preparation. Adulteration of the genuine raw material is the main cause for deterioration of the desired therapeutic effect of a particular drug. The present pharmacogonstic study on two different sample of *Myristica fragrans* following a series of physicochemical parameters such as total ash, water soluble ash, acid in-soluble ash, ethanol soluble extractive value, water soluble extractive value and loss on drying at 105^{o}C, preliminary phytochemical screening, HPTLC profile and Microscopic identification. These parameters indicate that the drug is potent & authentic. In the present investigation Microbial load, aflatoxins and heavy metals contamination resulted within the permissible limits of the WHO guidelines. So it can be concluded that these parameters are helpful in identification and standardization of the drug. Further Phytochemical and pharmacological studies are suggested.

ACKNOWLEDGEMENT: The authors are very grateful to Dr. Bharat Pathak, General Secretary, Deendayal Research Institute, Chitrakoot, Madhya Pradesh, India, for providing Sophisticated Analytical Instrument Facilities in their esteemed lab to carry out the study.

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How to cite this article:
Tripathi IP and Dwivedi N: Pharmacognostical Standardization of Nutmeg Seeds (*Myristica Fragrans* Houtt.) - A Traditional Medicine. Int J Pharm Sci Res 2015; 6(7): 3096-02.doi: 10.13040/IJPSR.0975-8232.6(7).3096-02.