

**PHARMACOGNOSTICAL STUDIES AND CHROMATOGRAPHICAL  
EVALUATION OF DIFFERENT MARKET SAMPLES OF *MYRISTICA  
FRAGRANS* HOUTT.**

**Dr. Manjunath Dundi<sup>1\*</sup>, Dr. Sonali M. Phulsagar<sup>2</sup> and Dr. Giridhar Vedantam<sup>3</sup>**

<sup>1</sup>PG Scholar, Department of Dravya Guna, KLEU Shri BMK Ayurveda Mahavidyalaya,  
Belgaum, Karnataka.

<sup>2</sup>PG Scholar, Department of Dravya Guna, KLEU Shri BMK Ayurveda Mahavidyalaya,  
Belgaum, Karnataka.

<sup>3</sup>Asst Professor, Department of Dravya Guna, KLEU Shri BMK Ayurveda Mahavidyalaya,  
Belgaum, Karnataka.

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**\*Corresponding Author**

**Dr. Manjunath Dundi**

PG Scholar, Department of

Dravya Guna, KLEU Shri

BMK Ayurveda

Mahavidyalaya, Belgaum,

Karnataka.

**ABSTRACT**

*Myristica fragrans* Houtt. is a well known spices plant which is native of moluccas and now cultivated in many tropical regions in India. Being an potent effective herb, this plant is used richly in various formulations. To maintain quality of the herb in the formualtion, proper control of raw herb is almost essential. The first step to ensuring quality of raw herb is authentication. Despite of modern techniques, identification of the plant by pharmacognostic studies is more reliable. The quality of herb also depends upon many factors like collection, storage, form of drug. Any carelessness in this factors makes the drug less active. Hence with the above rationale, the present studyis undertaken to know the effect of collection of same sample of Jatiphala from different regions by performing different qualitative and

quantitative analysis. Here pharmacognostic and phytochemical evaluation is done on four different samples of *Myristica fragrans* taken from four different regions of India that is Kerala, Mumbai, Kolkata and Delhi. These four samples are analyzed for different extractive values and chromatographic technique. This study provides referential information for identification of this important crude drug from different areas.

**KEYWORDS:** *Myristica fragrans* Houtt., Pharmacognostic evaluation, TLC.

## INTRODUCTION

The herbal medicinal science is an ancient system of medicine that is gaining a prominent importance in the global market. Due to increasing demand in the field of herbal medicine it has become necessary to explore the area of synthetic knowledge about herbal drugs. *Jatiphala* is a well known spice of Ayurvedic medicine which is biologically termed as *Myristica fragrans* Houtt. It is commonly known as nutmeg belongs to the family Myristicaceae, with about 18 genera and 300 species.<sup>[1]</sup> It is an evergreen tree, native of the E. Moluccas and cultivated in many tropical regions of India like tamilnadu, kerala, assam throughout Malaya. It is found only as a specimen tree in Botanical gardens. The seed of the plant is known as “nutmeg” and the aril of the seed is called “mace”. Both nutmeg and mace contain many volatile oils.<sup>[2]</sup>

Apart from its use as a spice the plant has several proven actions like Aphrodisiac, anti-inflammatory, anodyne, antipyretic, anthelmintic, deodorant, digestive, carminative, stomachic, expectorant, diuretics, emmenagogue, antispasmodic, febrifuge, narcotic, stimulant, ophthalmic, anticonvulsant, antiseptic, constipating and tonic. Antihepatotoxic, hypolipidemic and antifungal etc.<sup>[3]</sup> Due to this properties it is taken as an important ingredient in many of the herbal formulations. The components of the *Myristica fragrans* such trimyristin, safrole, myristicin, volatile & fixed oil are responsible for the various pharmacological activities.

So the present study was aimed to see the effect of different regions on seeds of *Jatiphala* by performing extractive values (with various solvents like water, alcohol, Chloroform and ether) and TLC method.

## MATERIALS AND METHODS

Plant materials collection and identification.

Collection of *Jatiphala* from different areas.

### Plant material

The seeds of *Jatiphala* were collected from local drug store of for main different states of India.

The botanical identification of the seeds were done at AYUSH approved Central Research facility, at Shri B M K Ayurveda Mahavidyalaya, Shahapur, Belgaum and voucher number is given in Central Research Facility for each sample mentioned below.

Sample A	Collected from local drug store of Belgaum (Kerala)	CRF/14/331
Sample B	Collected from local drug store of Delhi	CRF/14/332
Sample C	Collected from local drug store of Mumbai (Maharashtra)	CRF/14/333
Sample D	Collected from local drug store of Kolkata (West Bengal)	CRF/14/334

All the seeds were crushed and pulverized to fine powder and stored in air-tight container at room temperature.

## Analytical Study

### Organoleptic characters

All the samples of nutmeg were seen for its organoleptic characters like colour of seed powder, odour, consistency etc.

### Determination of extractive values<sup>[4]</sup>

#### 1. Alcohol soluble extractives

About 5 gm. of the powdered drug was weighed and mixed in 100 ml. 90% alcohol in 250 ml. conical flask. The flask was kept for 24 hours with shaking at the interval of 6 hours. The content of the flask was filtered in dry and weighed beaker; the beaker was kept on water bath for evaporation and cooled. The beaker was weighed again to calculate the percentage w/w of extractive is calculated with reference to the air dried drug.

#### 2. Water soluble extractives

Same as determination of alcohol soluble extractive value but instead of Alcohol, chloroform water is used.

### Phytochemical investigation

Chemical tests are performed on alcohol extracts on all four samples obtained from using non-polar and polar solvent. It helps to find out organic compounds like carbohydrates, proteins, glycosides, alkaloids, steroids, tannins and phenolic compounds, oxygenic acids enzymes, fats and oils etc.

#### A. Test for alkaloids

Wagner's test: To 2-3ml. filtrate, few drops Wagner's reagent added.

**B. Test for Glycosides**

Borntrager's test: To 3ml of extract, dil  $H_2SO_4$  added, boiled and filtered. To cold filtrate equal volume benzene was added, shaken well and the organic solvent separated. After that, ammonia was added. Ammonia layer turns pink to red.

**C. Test for reducing sugar**

Benedict's test: Equal volume of Benedict's reagent was added to test solution in the test tube. Heated in boiling water bath for 5 min. Change in solution color observed. Solution appears green, yellow or red depending upon on amount of reducing sugar present in test solution.

**D. Test for proteins and amino acids**

Million's test: 3ml test solution was mixed with 5ml of Million's reagent. White ppt appeared. Ppt dissolved giving red colour. Test for tyrosine: 3ml. test solution taken in test tube and heated on water bath, after that 3 drops Million's reagent added. Change in solution colour observed.

**E. Test for carbohydrate**

Molisch's Test (General Test): To 2 – 3 ml aq. Extract, few drops of alpha naphthol solution in alcohol was added and shaken well. After that concentrated  $H_2SO_4$  from sides of test tube was added. Violet ring is observed at the junction of 2 liquids.

**F. Test for tannins and Phenolic compounds**

Dilute Iodine solution: To 2-3 ml of extract, dilute iodine solution was added & transient red color observed.

**G. Test for flavonoids**

Shinoda test: To dry powder or extract, 5ml. 95% ethanol, few drops of conc. HCL and 0.5gm. Magnesium turnings were added. Pink color observed.

**H. Test for fat and fixed oils**

To ethanolic solution, few drops of  $CuSO_4$  and NaOH solution added. Clear blue solution was observed.

**I. Test for saponin**

Foam test: Shaken the extract vigorously with water. Persistent foam observed.

**J. Test for steroids**

Salkowski reaction: To 2ml. of extract, 2ml of chloroform and 2ml. con.H<sub>2</sub>SO<sub>4</sub> was added. Shaken well. Chloroform layer appeared red and acid layer shown greenish yellow fluorescence.

**THIN LAYER CHROMATOGRAPHY<sup>[5]</sup>**

**Test solution:** 10gms of coarsely powered drug taken in 100 ml stoppered conical flask and extracted with 100 ml of Chloroform and n-Hexane in 1:1 proportion, stirred it continuously for 6 hours and kept overnight steady.

**Stationary phase:** TLC precoated plate with silica gel 60 F254 of 0.2mm thickness.

**Solvent system:** Toluene: Ethyl Acetate (9:1).

**Volume of test solution applied:** 3ul

**Distance travelled by the solvent system:** 10 cm

**Development chamber:** Twin trough chamber (10 X 10cm) with SS lid.

**RESULTS****Botanical Information**

The seeds of all four sample were brownish in colour with oval / ellipsoid shape measuring about 20-30 mm long and about 20 mm broad, externally greenish brown sometimes marked with small irregular dark brown patches or minute dark points and lines slightly furrowed reticulately. Each seed weighed near about –mg. Difference observed in organoleptic character of churna of seeds shown in table.

**Organoleptic characters of Jatiphala churna**

Qualitative evaluation based on sensory profile by observation of colour, odour, taste and consistency was done for all four samples of *Myristica fragrans*.

**The organoleptic characters of Jatiphala churna**

Sl. No.	Parameters	Sample A (Kerala)	Sample B (Delhi)	Sample C (Kolkata)	Sample D (Mumbai)
1	<b>Colour</b>	Dark Brownish	Brown	Brown	Greenish brown
2	<b>Odour</b>	Strongly aromatic	Strongly aromatic	Strongly aromatic	Aromatic
3	<b>Taste</b>	Bitter, slightly Pungent	Bitter, Pungent	Pungent	Pungent
4	<b>Consistency</b>	Soft	Soft	Soft	Soft

Extractive value of all four samples with four different solvent is shown in table.

Extractive	Sample A (Kerala)	Sample B (Delhi)	Sample C (Kolkata)	Sample D (Mumbai)	Limits (As per API)
Water Soluble extractive	18%	18.72%	10.08%	20.32%	Not less than 7%
Alcohol soluble extractive	29.28%	30.08%	34%	22.96%	Not less than 11%
Ether soluble extractive	64.72%	62.16%	63.52%	83.84%	Not less than 25%
Chloroform soluble extractive	80.32%	88%	102.5%	95.68%	-

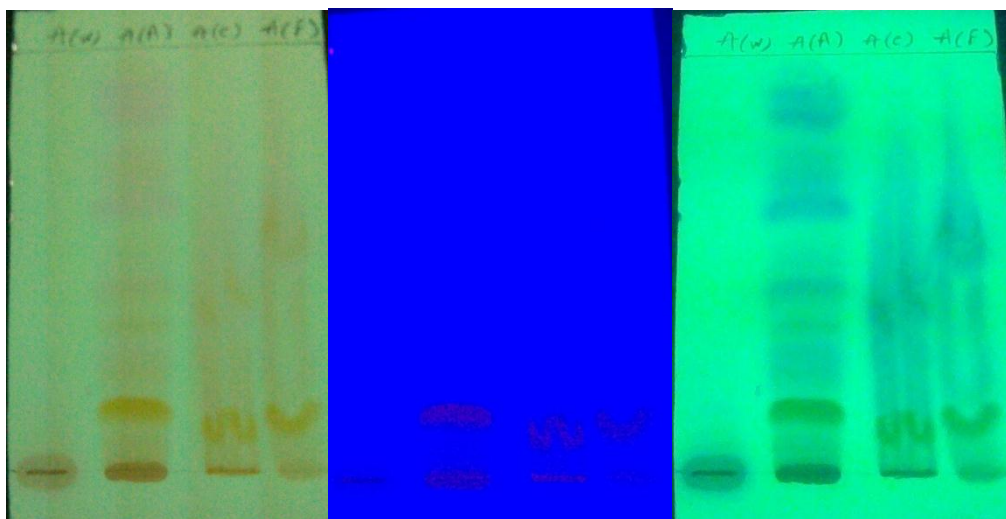
Qualitative phytochemical analysis of four different extract of four samples are shown in table.

TLC of Four samples shown in table.

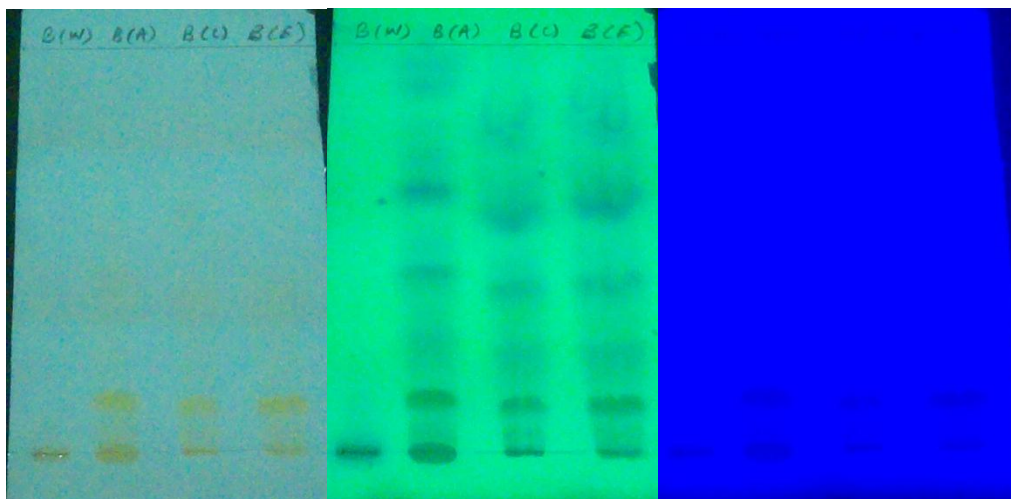
RF values calculated by the dots observed on TLC plate			
Sample A	Sample B	Sample C	Sample D
0.02	0.06	0.06	0.05
0.09	0.12	0.1	0.1
0.26	0.21	0.19	0.23
0.3	0.25	0.25	0.2
0.33	0.3	0.29	0.3
0.55	0.38	0.40	0.35
0.57	0.52	0.52	0.5
0.78	0.57	0.60	0.52
	0.66	0.72	0.67
	0.75		0.77

## TLC PHOTOS

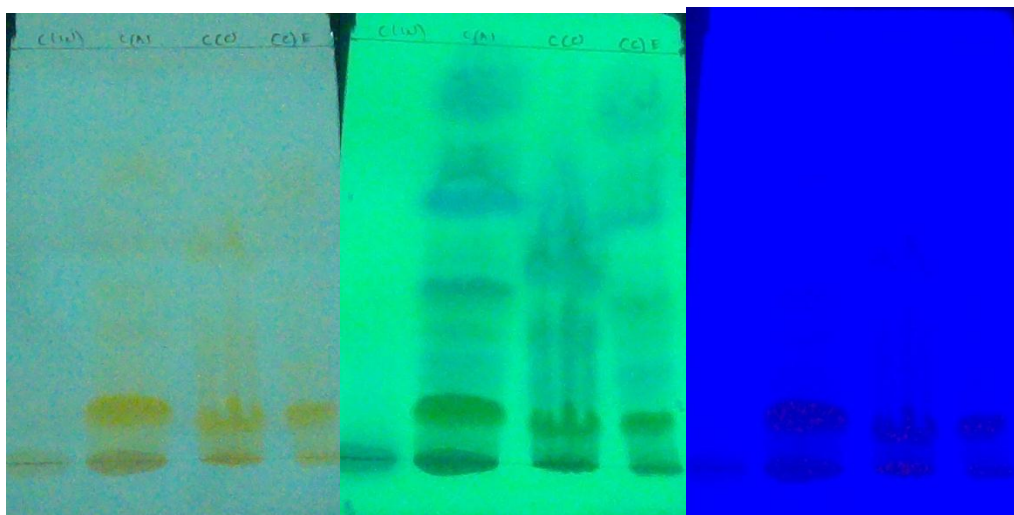
### Sample A



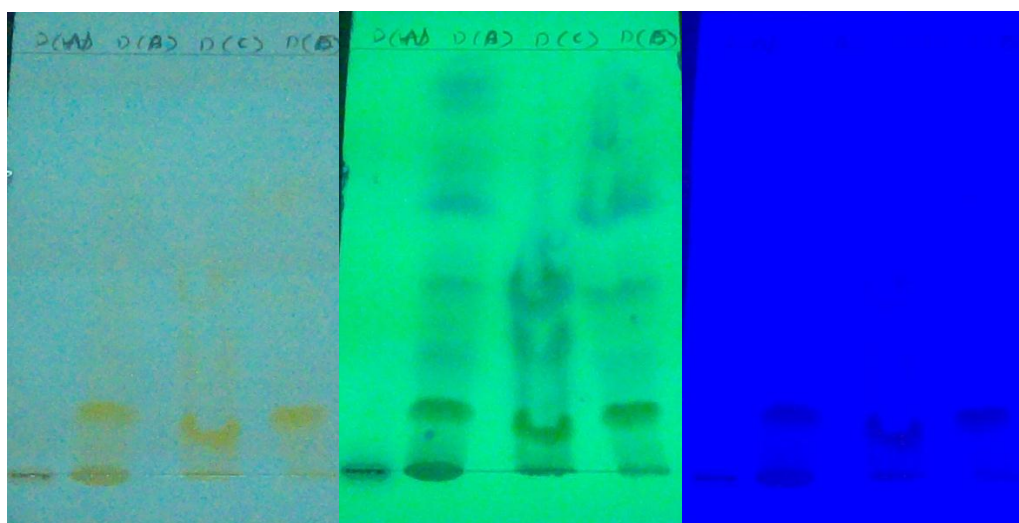
Sample B.



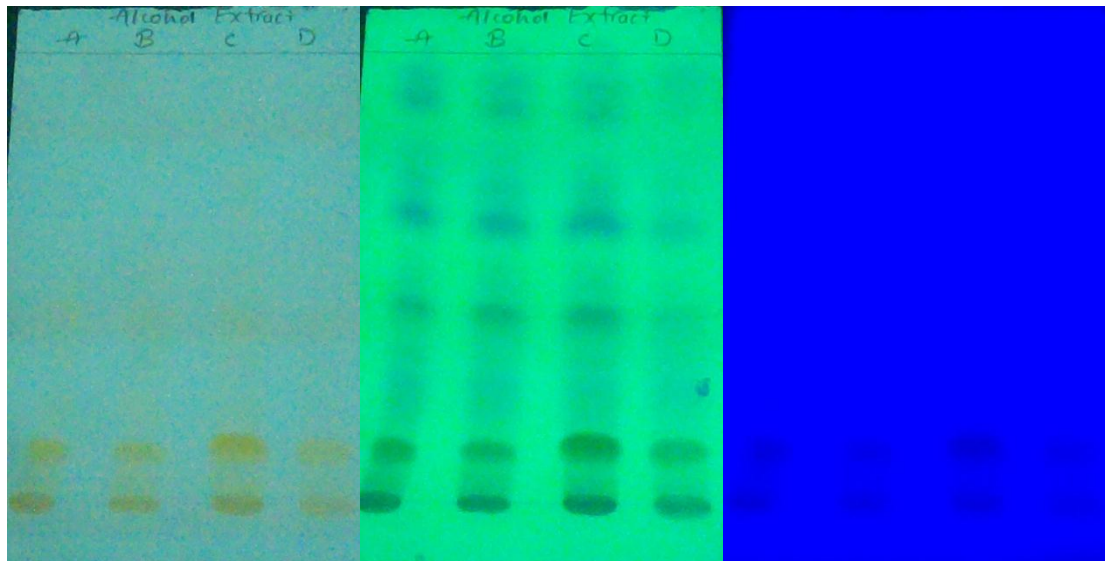
Sample C.



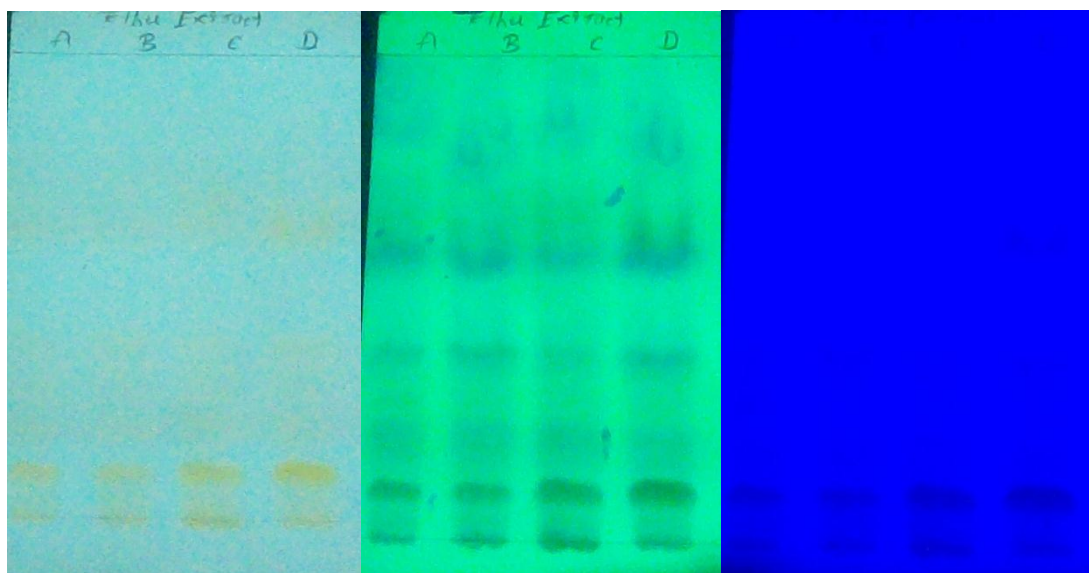
Sample D



**Alcoholic extract of all four samples**



**Ether extract of all four samples**



**All raw sample**





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