A preliminary phytochemical analysis of "*Jatiphala kosha*" (pericarp of *Myristica fragrans* Houtt)

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Abstract:

Background and Objective: *Jatiphala kosha* (pericarp of nutmeg tree) is used traditionally (home remedy) as anti-diabetic. No study was undertaken to evaluate preliminary phytochemical analysis of "*Jatiphala kosha"* (pericarp of *Myristica fragrans* Houtt.) The aim of the present study was to perform a preliminary phytochemical analysis of "*Jatiphala kosha"* (pericarp of *Myristica fragrans* Houtt.).

Materials and Methods: Plant Material - *Myristica fragrans* Houtt. Pericarp was collected from the Herbal garden of Arya Vaidya Sala, Anoli, Kottakkal. The pericarp of nutmeg was cleaned and shade dried at room temperature. Dried pericarp was subjected to size reduction to a coarse powder by using a grinding machine. Preliminary phytochemical analysis of study drugs was performed as per standard protocol of phytochemical analysis using chemicals - Petroleum ether, Cyclohexane, etc., apparatus - Soxhlet apparatus, Dean and Stark"s apparatus, etc. and TLC was performed.

Observations & Results: Moisture content of the shade dried drug determined by Dean and Stark appa- ratus was found to be 11%. Total Ash value of the drug was found to be 7.76%. Water insoluble ash mainly gives the percentage of organic matter present in the ash and this was found to be 1.69%. Acid insoluble ash, which mainly gives the percentage of the sand and impurities that remain insoluble in HCl, was found to be 0.15%. Fibre content of the drug was found to be 29.35. The largest percentage of extract was obtained by the extraction with acetone (14.96%) and the least with the solvent Cyclohexane (0.43%). The color of acetone extract was dark reddish brown and the remaining extracts were brown. The consistency of all the extracts was oily and sticky. Tannins and steroids are present in all the extracts. While alkaloids were absent in all extracts. Phenol and flavonoids are present in all the extracts, except Petroleum ether and Cyclohexane extracts. Red ppt was produced that shows the presence of pectin in the study drug.

Conclusion: Qualitative analysis of extracts revealed the presence of tannins, steroids, flavonoids and phenols. Dried pericarp can be preserved and used for a longer time than fresh pericarp because the former one has less moisture content in it.

Key Words: *Jatiphala Kosha, Myristica fragrans* pericarp, Phytochemicals, TLC, Pectin.

Introduction:

Jatiphala, popularly called as Nutmeg tree, is a medium size evergreen tree found in few states of India. *Jatiphala* is source of two spices i.e. Nutmeg and Aril (mace). It is stimulant, carminative, astringent and commonly prescribed for dysentery, stomachache, flatulence, nausea, vomiting, malaria, rheumatism etc. In *Ayurveda*, it is used for diarrhea, disease of mouth, heart and acne etc. Traditionally used as aphrodisiac. It is mentioned as *Mehaghna* in *Dhanwantari*[(1)] and *Raja nighantu*[(2)]. Recent studies have proved that nutmeg has anti-diabetic and anti- hyperlipidemic effect[(3)(4)(5)]. Also leaf and stem of *Jatiphala* is said to be having insulin releasing property[(6)]. These studies were done on seeds and seed kernel of *Jatiphala*, as seeds are taken by name *phala* of *Jatiphala*. No reference is found for medicinal uses of *Jatiphala* pericarp in classical *Ayurveda* texts as well as modern books. Pericarp of *Jatiphala* is a byproduct and does not have much commercial value. It is cost effective and easily available. *Jatiphala kosha* (pericarp of nutmeg tree) is used traditionally (home remedy) as anti-diabetic. No study was undertaken to evaluate preliminary phytochemical analysis of "*Jatiphala kosha"* (pericarp of *Myristica fragrans* Houtt.)

Since the dawn of civilization man has always turned to nature in order to seek remedies for his ailments. Plant remedies are empirically used from generation to generation. Phytochemistry is one part of drug research, dealing with chemistry of drugs. Procurement of authentic, quality medicinal plants is a major problem. Therapeutic efficacy of medicinal plants depends upon its quality. With the help of phytochemical study it is possible to standardize the plant, which is the need of the present era.

In the present study, coarse powder of study drug (dried pericarp of *Myristica fragrans* Houtt.) is subjected to

preliminary phytochemical screening for the detection of various plant constituents.

Review of Literature

Jatiphala Kosha

In *Bhojakruta rajamartanda, jatiphala bahya twaka lepa* is indicated for *vyanga*. According to P.V. Sharmaji, *jatiphala kosha* may have been used for this *lepa* because at that time whole fruit may be used as medicine. To support this view P.V. Sharmaji explains the practical difficulty in separating *bahya twaka* from dried seed of *jatiphala*[1]. No other references regarding *jatiphala kosha* are found in any other *Ayurvedic* literature.

Synonyms of *Jatiphala kosha*[2]

Jatiphala paristara, *Jatiphala phalabhiti.*

***Myristica fragrans* Houtt.**

Nutmeg and mace, both are derived from the fruit of *Myristica fragrans* Houtt. Nutmeg is the dried ripe seed and mace the dried arillode, which envelops the shell containing the seed or nutmeg. Both are very important spices, which have been used for a long time in the flavoring of savory dishes, baked foods and other food products. The pericarp of fruit rind is used for making candies, jellies and pickles, especially in South India.

Taxonomical Description

Kingdom: Plantae; Planta, plantes, plants, Vegetal

Sub-kingdom: Tracheobionta; Vascular plants

Division:Magnoliophyta; Angiospermes, angiosperms, Flowering plants, phanerogams.

Class: Magnoliopsida; Dicots, dicotyledones, dicotyledons

Sub-class: Magnoliidae

Order: Magnoliales

Family: Myristicaceae

Genus: *Myristica*

Species: *fragrans* Houtt.Nutmeg

Morphological Characters[3]

Distribution and Habitat

The tree is considered indigenous to Moluccas but is now found under cultivation in a number of localities in India such as Karnataka, Kerala and Tamil- nadu. It requires a well sheltered sufficiently hot and moist climate and well drained alluvial (rich loamy) soil for its growth. Nutmeg grows from sea level to 150-200 meters elevation.

Habit and General Features

Myristica fragrans Houtt. is a medium sized to large dioecious dense foliage evergreen glabrous tree exuding a reddish juice from its bark when cut, growing 15 to 20 meters high, with short trunk and several branches arising at short distances from ground level and bearing a dense glossy green foliage of simple alternate petiolate elliptic leaves and small unisexual monochlamydeous yellowish flowers either solitary or in a few flowered cymes or fasicles and one seeded fairly large follicular type of thick rinded fleshy fruits ; each seed with a hard testa (shell) covered with bright red (scarlet) laciniate fragrant aril and a ruminate endosperm inside. The plant flowers most part of the year.

Materials And Methods[1]

Plant Material

Myristica fragrans Houtt. pericarp was collected from the Herbal garden of Arya Vaidya Sala, Anoli, Kottakkal. The pericarp of nutmeg was cleaned and shade dried at room temperature. Dried pericarp was subjected to size reduction to a coarse powder by using a grinding machine.

Chemicals

Petroleum ether, Cyclohexane, Acetone, Alcohol, Xylene, Conc. Hydrochloric acid, Hydroxylamine, Ferric chloride, Conc. Sulphuric acid, dilute HCL, NaOH, Sodium bicarbonate, Lead acetate, Sodium oxalate, Magnesium ribbon, Mayer‟s reagent, Dragendroff's reagent, neutral ferric chloride etc.

Apparatus

Silica crucible, Soxhlet apparatus, Dean and Stark‟s apparatus, Clavenger apparatus, Buchner funnel, round bottom flask, beakers, measuring jars, conical flasks, funnel, watch glass, glass rods etc.

Determination of Physicochemical parameters

1. Total ash

An ash value is the criteria to judge the identity or purity of the crude drugs. In most of the cases, the crude drugs are mixed with various mineral substances like phosphates, carbonates, potassium, magnesium and calcium. The residue after incineration is the ash content of the drug, which represents the inorganic salts naturally occurring in drugs or deliberately added to it as a form of adulteration.

2 gm accurately weighed and powdered drug was incinerated in a previously ignited, cooled and weighed silica crucible until free from carbon up to a constant weight. The percentage of ash was calculated with reference to the air dried drug.

2. Water insoluble ash

The ash obtained from the previous experiment was dissolved in 25 ml of distilled water and boiled for five minutes. It was filtered using ash less Whatmann's No.1 filter paper and residue was collected. It was then washed with warm distilled water several times and ignited to get a constant weight. The

percentage of water insoluble ash was calculated with reference to the air dried drug.

3. Acid insoluble ash

Total ash obtained by incinerating 2 gm of the sample by above method was dissolved in 25 ml 2N hydrochloric acid in a 100 ml beaker and boiled for five minutes. It was then filtered through an ash less Whatmann's No.1 filter paper and the residue was collected. The residue was then washed with warm distilled water for several times until it was free from the acid. The filter paper along with the residue was then placed in a crucible and ignited to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

4. Moisture content

The moisture content is determined by using Dean and Stark apparatus. Moisture content of a particular drug is the amount of moisture the drug absorbs on exposure to atmosphere after drying. 10 gm of the air dried powdered drug was taken in a round bottom flask and xylene was added to cover the drug. A few pieces of porous porcelain chips were also added. It was connected to the Dean and Stark apparatus, which was then connected to a water condenser. It was heated with an electric mantle and allowed to boil for about one hour. The heating continued till the level of the water remained constant. It was then kept undisturbed till it at- tained room temperature. The moisture in the drug got evaporated, condensed and collected in the graduated tube of the apparatus. The level of water content in the graduated tube was noted through the lower meniscus. The percentage of moisture content in the drug was calculated by dividing the reading of water content by the weight of the original sample taken and multiplying it by 100.

5. Volatile oil content

The volatile oil content was determined by distilling the drug with distilled water using Clevenger apparatus. The distillate was collected in a graduated tube in which the aqueous portion of the distillate was automatically separated and returned to the distilling flask. Then the volume of oil was measured. 10 gm of powdered drug was taken and mixed with 100 ml of water and pieces of porous porcelain chips were added to it. This was connected to a graduated glass tube over which a condenser was fixed. It was then boiled for one hour and allowed to cool. The volatile oil evaporated and got condensed in the graduated tube. The content of the volatile oil was noted and percentage was calculated.

6. Fibre content

Accurately weighed 3 gm of air dried powdered sample was transferred into a thimble and extracted in a Soxhlet apparatus with petroleum ether until it became colorless. The extracted sample was then transferred to a dry 500 ml beaker. Then 200 ml of 0.255 N $H_{(2)}SO_{(4)}$ solution was added to the beaker. The beaker was then boiled gently for 30 minutes by placing a glass rod and covering with a watch glass. After boiling, the solution was filtered with an ordinary filter paper into another 500 ml beaker. The residue was washed with hot distilled water until it was free from sulphate. Then the residue was transferred into a 500 ml beaker. Then 200 ml of 0.313 N NaOH solution was added into this beaker and boiled for 30 minutes by placing a glass rod and covering with a watch glass. The content was filtered through a Buchner funnel with suction by using previously weighed ash less Whatmann's No. 1 filter paper. It was washed with hot distilled water until it was freed from alkali. The residue was dried at 100°C by placing the funnel in a hot air oven. The filter paper with the residue was then transferred to a previously weighed silica crucible and the weight was taken. Then the crucible was incinerated until it was freed from carbon and the weight was taken. The difference between the weight of the dried material and that of ash obtained was calculated.

The weight of residue was found and from that the percentage of fibre content was calculated by using the formula,

$$\text{Percentage of fibre} = \frac{\text{(Wt. of residue 100)}}{\text{(Wt. of the drug)}}$$

7. Water soluble extractives

The extracts obtained by exhausting drugs are indicative of approximate measures of the chemical constituents. This method is applied to drugs that contain active constituents such as tannins, sugars, plant acids, mucilage, glycosides etc.

One gram of coarsely powdered sample was taken in a round-bottom flask and 100 ml distilled water was added to it. It was connected with a water condenser and boiled for one hour in a heating mantle, allowed to stand for some time and then filtered rapidly, taking precautions against loss of solvent. The filtrate was evaporated to dryness in a previously weighed 100 ml beaker, dried at 100°C and was again weighed. Heating and weighing continued to get a constant weight. The percentage of water soluble extractives was calculated with reference to the air-dried drug.

8. Alcohol soluble extract

5 gm of accurately weighed sample was taken in a round-bottom flask. 100 ml of 99% alcohol was added to it and closed. The content was shaken occasionally for about 24 hours, filtered rapidly, evaporated to dryness in a previously weighed 100 ml beaker, dried at 100° C and was again weighed. The percentage of alcohol soluble extract was calculated with reference to the air-dried drug.

9. Hot alcohol soluble extract

1 g of coarsely powdered sample was taken in a round-bottom flask 100 ml ethyl alcohol was added to it. It was then connected with a water condenser and boiled for one hour in a heating mantle, allowed to stand for some time and then filtered rapidly, taking precautions against loss of solvent. The filtrate was evaporated to dryness in a previously weighed 100 ml beaker, dried at 100° C and was again weighed. Heating and weighing continued to get a constant weight. The percentage of hot alcohol soluble extractive was calculated with reference to the air dried drug.

10. Successive solvent extraction

The commonly employed technique for the removal of active substance from crude drug is called extraction, which involves the use of different solvents. Extraction itself may be performed by repeated maceration with agitation, percolation or by continuous extraction using Soxhlet apparatus. Successive solvent extraction is one such technique by which the drug is successively extracted with various solvents according to the polarity, i.e. from a non- polar solvent to a more polar solvent. The dried powdered plant material is commonly used for extraction. The process of extraction is used in making fixtures, fluid extracts and solid extracts.

The solvents used for the successive extraction of the drug was

I Petroleum ether

ii. Cyclohexane

iii. Acetone

iv. Ethanol

10 gm of accurately weighed air-dried powder of drug sample was taken in a Soxhlet apparatus with a condenser. Because the drug was in powder form, it was packed into a special thimble made of thick filter paper before placing it in the Soxhlet apparatus. The Soxhlet extractor was placed on top of a well-supported round-bottom flask containing the organic solvent petroleum ether. The flask was heated in a water bath. The extraction was continued till the solvent in the siphon tube became completely colorless. The system was then allowed to cool to room temperature. The extract was concentrated by distilling off the solvent using a simple distillation apparatus and was collected in a previously weighed 100 ml beaker. It was then evaporated to dryness in a water bath and dried in desiccators.

Each time before extracting with the next solvent, the extracted material was dried in hot air oven below 500°C. The whole procedure was repeated with each solvent, i.e. Cyclohexane, acetone and ethanol respectively. The extract obtained with each solvent was weighed and the percentage of the extract was calculated with reference to the air-dried drug. The color and consistency of the extracts were noted.

The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents.

Observations And Results:

Physico-chemical analysis of *Myristica fragrans* Houtt. Pericarp are tabulated in Table No.1.

Physico - chemical parameters are given in the table. Moisture content of the shade dried drug determined by Dean and Stark apparatus was found to be 11%. Total ash of any drug is the residue obtained on its complete incineration in an electric Bunsen burner. This mainly represents the inorganic salts present in the drug, if the **drug is pure and any impurities like sand, soil etc. adhering to the drug will also** remain as ash, thus increasing the ash value several fold. Ash value is the general criterion to ascertain the purity of the drug. Total Ash value of the drug was found to be 7.76%. Water insoluble ash mainly gives the percentage of organic matter present in the ash and this was found to be 1.69%. Acid insoluble ash, which mainly gives the percentage of the sand and impurities that remain insoluble in HCl, was found to be 0.15%. Fibre content of the drug was found to be 29.35%.

Water soluble extracts of the drug mainly represent the percentage of organic constituents such as tannins, sugars, plant acids, mucilage and glycosides. Alcohol soluble extracts mainly represent the percentage of organic constituents such as alkaloids, phenols, flavonoids, steroids, sugars etc. present in the drug. Successive solvent extraction, which is the extraction of a drug with organic solvents of increasing polarity, was applied for the isolation of active constituents from the crude drug. The largest percentage of extract was obtained by the extraction with acetone (14.96%) and the least with the solvent Cyclohexane (0.43%). The color of acetone extract was dark reddish brown and the remaining extracts were brown. The consistency of all the extracts was oily and sticky.

Qualitative analysis of the extracts

The extracts obtained were subjected to qualitative tests for the identification of various plant constituents.

1. Detection of Tannins

2 ml of extract was taken in a test tube and few drops of lead acetate solution were added. A yellow or white precipitate indicates the presence of tannins.

2. Detection of Alkaloids

a) With Mayer"s reagent

2 ml of extract was taken in a test tube and dried by placing it on a heating mantle. Then added a few drops of dilute hydrochloric acid and filtered into another test tube using a

filter paper and a funnel. Then added a few drops of Mayer"s reagent to it. Turbidity indicates the presence of alkaloids.

b) With Dragendroff"s reagent

2 ml of extract was taken in a watch glass and one drop of Dragendroff"s reagent was added to it and rubbed gently with a glass rod. Formation of an orange brown precipitate indicates the presence of alkaloids.

3. Detection of Phenols

2 ml of extract was taken in a test tube and a few drops of neutral ferric chloride were added to it. A deep blue or violet color indicates the presence of phenols.

4. Detection of Flavonoids

2 ml of extract was taken in a test tube. A few drops of concentrated hydrochloric acid and a piece of magnesium ribbon were added to it. A reddish brown, magenta or pink color indicates the presence of flavonoids.

5. Detection of Steroids

1 ml of extract was taken in a clean test tube and 3 ml of chloroform was added. Then a few drops of concentrated sulphuric acid were added through the sides of the test tube. Formation of a brown ring indicates the presence of steroids.

Observations and Results:

The extracts obtained by exhausting crude drugs are indicative of approximate measure of their chemical constituents. Tannins and steroids are present in all the extracts. While alkaloids were absent in all extracts. Phenol and flavonoids are present in all the extracts, except Petroleum ether and Cyclohexane extracts.

Table No. 1: Physico-chemical analysis of *Myristica fragrans* pericarp powder

	Test	Result
1.	Total ash	7.76 %
2.	Water insoluble ash	1.69 %
3.	Acid insoluble ash	0.15 %
4.	Moisture content	11 %
5.	Volatile oil	2 %
6.	Fibre content	29.35 %
7.	Water soluble extract	80 %
8.	Alcohol soluble extract	8.6 %
9.	Hot alcohol soluble extract	20.09 %
10.	Successive solvent extraction:-	
	1) Petroleum ether	1.46 %
	2)Cyclohexane	0.43 %
	3) Acetone	14.96 %
	4) Ethanol	1.61 %

Table No. 2: Qualitative analysis of extracts

Extract	Tannins	Phenols	Flavonoids	Alkaloids With MR	Alkaloids With DDR	Steroids
Water	+	+	+	-	-	+
Alcohol	+	+	+	-	-	+
Hot Alcohol	+	+	+	-	-	+

Table No. 3: Qualitative analysis of Successive solvent extracts

Extract	Tannins	Phenols	Flavonoids	Alkaloids With MR	Alkaloids With DDR	Steroids
Water	+	+	+	-	-	+
Petroleum ether	+	-	-	-	-	+
Cyclohexane	+	-	-	-	-	+
Acetone	+	+	+	-	-	+
Alcohol	+	+	+	-	-	+

Detection of Pectin (Hydroxylamine – ferric chloride)[2]

Reaction for pectin

15 gm of pericarp powder was weighed and taken in a 150 ml flask , to it 30 ml dilute acid 0.5 N HCL was added . Extraction was done by boiling the above mixture at 100º C for 60 min. The marc (precipitate) was separated and extraction was repeated. To the extract acetone was added to precipitate pectin.

Hydroxylamine reagent - Hydroxylamine reagent 13.9 gm in 100 ml water.

Sodium hydroxide solution- Sodium hydroxide 14.0 gm in 100 ml water

HCL acid 1 volume of concentrated HCL reagent (sp. gravity 1.18) diluted with 2 vol- umes of water.

$FeCl_{(3)}$ reagent 10 gm $FeCl_{(3)}$ hexahydrate in 100 ml of 0.1 N HCL acid. About 0.005 gm of the test substance is suspended or dissolved in 1 ml of water. Add 1 ml of Hydroxylamine reagent, then 1 ml of the Sodium hydroxide solution. The reactants are allowed to stand for 2 min, and then 1 ml of dilute HCL is added followed by 1 ml of $FeCl_{(3)}$ reagent. A red ppt is produced in the positive test.

Observation and Results

Red ppt was produced that shows the presence of pectin in the study drug.

Thin layer chromatography (TLC)

Thin layer chromatography (TLC) is largely an analytical technique used for determining the purity of materials and also for preliminary identification purposes. TLC is a first step towards the identification of phyto-chemicals present in the herbal extracts. It is a physico-chemical separation method in which the stationary phase, a finely divided solid, is spread as a thin layer on a rigid supporting plate and the mobile phase, a liquid, is allowed to migrate across the surface of the plate by capillary action.

Materials:

TLC plates precoated plate with Silica gel 60 $F_{(254)}$ of 0.2mm thickness, Toluene, Ethyl acetate, Formic acid, Anisaldehyde - Sulphuric acid reagent and development chamber.

Test solutions: Extracts dissolved in 5 ml respective solvents used for extraction.

A. TLC conditions for petroleum ether extract (1); Cyclohexane extract (2) and acetone extract (3):

Stationary phase: TLC precoated plate with Silica gel 60 $F_{(254)}$ of 0.2mm thickness

Solvent system: Toluene: Ethyl acetate (9: 1)

Volume of test solution applied : 10 (l)

Distance travelled by solvent system: 8 cm

Development chamber: Twin trough chamber (10 x10 cm) with SS lid.

Visualization: under 254 nm; under 366 nm; after derivatization with Anisaldehyde- Sulphuric acid reagent

B.TLC conditions for acetone extract (3) ; ethanol extract (4) and water extract (5) :

Stationary phase: TLC precoated plate with Silica gel 60 $F_{(254)}$ of 0.2mm thickness

Solvent system: Toluene: Ethyl acetate: Formic acid (5: 5: 1)

Volume of test solution applied : 10 ⌈1

Distance travelled by solvent system: 8 cm

Development chamber: Twin trough chamber (10 x10 cm) with SS lid.

Visualization: under 254 nm; under 366 nm; after derivatization with Anisaldehyde- Sulphuric acid reagent

Observations, Analysis and Results:

As solutes never travel the full length of the stationary phase in TLC, hence all the $R_{(f)}$ values are less than one. The $R_{(f)}$ value depends on the amount of the stationary phase, quality, saturation of the chamber, development distance, temperature, amount of substance added and presence of impurities. $R_{(f)}$ value is the retention factor of a particular compound and is the relation between distance moved by the compound spot and the distance moved by eluting solvent.

$R_{(f)}$ = Distance travelled by component / Distance travelled by solvent

Petroleum ether extract showed 5 spots at 254 nm, 8 spots at 366 nm and 10 spots after derivatization.

Table No. 4: TLC of Petroleum ether extract

254 nm		366 nm		After derivatization	
$R_{(f)}$value	Color	$R_{(f)}$value	Color	$R_{(f)}$value	Color
0.10	Greenish black	0.07	Blue fluorescence	0.05	Light blue
0.27	Dark Green	0.15	Blue fluorescence	0.21	Purple
0.53	Dark Green	0.25	Blue	0.28	Purple
0.66	Dark Green	0.32	Blue	0.35	Dark blue
0.77	Dark Green	0.43	Blue	0.42	Dark blue
		0.48	Blue	0.53	Brownish red
		0.61	Blue	0.59	Brownish light red
		0.68	Blue	0.65	Purple
				0.76	Dark blue
				0.86	Violet

Cyclohexane extract showed 1, 9 and 5 spots respectively at 254 nm, 366 nm and after derivatization.

Table No. 5: TLC of Cyclohexane extract

254 nm		366 nm		After derivatization	
$R_{(f)}$value	Color	$R_{(f)}$value	Color	$R_{(f)}$value	Color
0.77	Dark Green	0.06	Blue	0.05	Light Blue
		0.10	Blue	0.27	Purple
		0.20	Blue	0.40	Dark Blue
		0.26	Blue	0.51	Brownish red
		0.31	Light Blue	0.76	Dark Blue
		0.43	Light Blue		
		0.49	Light Blue		
		0.69	Yellow		
		0.43	Light Blue		

Table No. 6: TLC of Cyclohexane extract

254 nm		366 nm		After derivatization	
$R_{(f)}$value	Color	$R_{(f)}$value	Color	$R_{(f)}$value	Color
0.07	Dark Green	0.06	Blue	0.08	Dark Blue

Acetone extract showed 5 spots at 254 nm, 3 spots at 366 nm and 5 spots after derivatization using solvent Toluene: Ethyl acetate: Formic acid (5: 5: 1).

Table No. 7: TLC of Acetone extract using solvent Toluene: Ethyl acetate: Formic acid (5: 5: 1)

254 nm		366 nm		After derivatization	
$R_{(f)}$value	Color	$R_{(f)}$value	Color	$R_{(f)}$value	Color
0.09	Dark Green	0.12	Blue	0.20	Dark Blue
0.23	Dark Green	0.45	Blue	0.39	Grey
0.39	Dark Green	0.50	Light Blue	0.43	Violet
0.44	Dark Green			0.65	Violet
0.52	Dark Green			0.72	Dark Blue

Ethanol extract TLC shows - no spots at 254 nm, 1 spot at 366 nm and 1 spot after derivatization.

Table No. 8: TLC of Ethanol extract

254 nm		366 nm		After derivatization	
$R_{(f)}$value	Color	$R_{(f)}$value	Color	$R_{(f)}$value	Color
.....	....	0.09	Yellowish Fluorescence	0.12	Purple

Water extract showed 4 spots at 254 nm, 5 spots at 366 nm and 3 spots after derivatization.

Table No. 9: TLC of Water extract

254 nm		366 nm		After derivatization	
$R_{(f)}$value	Color	$R_{(f)}$value	Color	$R_{(f)}$value	Color
0.09	Dark Green	0.09	Yellow	0.09	Purple
0.28	Dark Green	0.13	Blue	0.17	Purple
0.46	Dark Green	0.20	Light Blue	0.63	Purple
0.53	Dark Green	0.47	Blue		
		0.53	Light Blue		

Discussion on Phytochemical Analysis

The preliminary physico-chemical analysis, qualitative analysis of extracts especially for detection of Tannins and Pectin and TLC of *Myristica fragrans* pericarp was carried out with standard procedures as per API. Gopalkrishnan (1992) in his work reported that proximate analysis of nutmeg and mace[1], unlike in earlier reports, has been carried out using the raw spices. The major hallucinogenic principle in Indian nutmeg/mace oils appears to bemyristicin which is present in good amounts. GC profiles of the oils compared very well with reports in literature. The rind of the nutmeg fruit contains good quality pectin up to 14%[2]. The yield of pectin was better (10%) in the dried sample when compared to the fresh sample (7%) as reported by Maya KM[3].

1. Physico-chemical Analysis

Physico-chemical parameters of *Myristica fragrans* Pericarp are tabulated in respective sections. Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant can be easily deteriorated due to contami- nation by microbes. Moisture content in fresh pericarp was reported to be 80 % by Gopalakrishnan, hence it deteriorates very fast. The moisture content was found to 11 % in dried pericarp of *Myristica fragrans* showing it can be stored for a period of time without spoilage and it will be less susceptible to microbial growth. Fibre content was found to be 29.35%, which suggests that this drug is a good source of fruit fibres. The determination of ash value was carried out which gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash and water insoluble ash was determined and results were tabulated. The analytical results showed that Total Ash value was 7.76 % and water insoluble ash was 1.69 %. Similarly; negligible amount of acid insoluble siliceous matter present in the plant (0.15 %) was observed. The total ash and acid insoluble ash values were nearly the same as reported by Gopalakrishnan in 1992. Extractive values were also determined which are primarily useful for the determination of exhausted or adulterated drugs. The water soluble and alcohol soluble (Cold and Hot) extractive values were determined. Water soluble extract was found to be 80 %, highest among all the extracts. This may be due to pectin content which is highly soluble in water and other chemical constituents which are not identified yet and they may be also highly water soluble.

The powdered fruit rind of *Myristica fragrans* was subjected to Successive Solvent extraction, starting with polar solvent and gradually shifting to non-polar solvents. Petroleum ether, Cyclohexane, Acetone and Ethanol were used as solvents. The extracts obtained were later kept for evaporation to remove the excessive solvents. The extracts obtained are expressed in

% in the related section. In Successive solvent extraction, Acetone yielded highest extract i.e. 14.96% and lowest extract was obtained in solvent Cyclohexane i.e. 0.43%. These extracts were stored in cool and dry places. Qualitative analysis of extracts was performed to screen for presence of phytochemicals especially tannins and pectin.

2. Qualitative Analysis of the extracts and Detection of Pectin

The secondary metabolites like tannins, phenols, flavonoids, alkaloids and steroids were assessed for their presence in dried pericarp extracts of *Myristica fragrans* as per standard procedures. Qualitative phytochemical analysis of the solvent extracts revealed the presence of tannins and steroids in all extracts and absence of alkaloids in all extracts. Phenols and flavonoids were present in all the extracts except petroleum ether and Cyclohexane extract.

Qualitative detection confirmed the presence of Pectin. This supports the previous research of Gopalakrishnan, who reported presence of 14.10% pectin from dried pericarp of nutmeg tree. Quantitative analysis of pectin was not performed and it is recommended for further study.

All the results generated from the phytochemical analysis are represented in the respective chapters under observation and results. The present study includes the preliminary phytochemical analysis of *Myristica fragrans* fruit rind powder alone and no other parts of Nutmeg tree like stem, leaves etc. Hence, further study of the different parts of the plant is recommended in order to establish the proper physico-chemical and phytochemical standards as other parts are also now reported to possess pharmacological actions such as insulin releasing property.

3. Thin Layer Chromatography

TLC is performed to detect the separation and tentative identification of components present in mixtures. For tentative identification of chemical components from TLC, standard TLC data or marker compounds are required. In this study TLC was performed using two solvent systems i.e. mobile phase, after performing a pilot TLC study. A mixture of toluene and ethyl acetate in the ratio of 9: 2 was selected as mobile phase for the petroleum ether, Cyclohexane and acetone (A) extract (TLC 1). A mixture of toluene: ethyl acetate: formic acid in the ratio of 5: 5:1 was selected as the mobile phase for acetone (B), ethanol and water extracts (TLC 2). Extracts were dissolved in 5 ml of respective solvents used for extraction and later were subjected to run through TLC plate. The resultant R_f (Retention factor) values for different spots were calculated and presented in the form

of a table in each chapter. Ethanol extract showed the least number of spots, while petroleum ether showed the highest number of spots. Marker compounds were not used in this TLC study and also there is no standard available for pericarp of *Myristica fragrans,* so identification of specific chemical constituents from TLC was not possible in this study. For standardization, TLC of *Myristica fragrans* pericarp should be performed with marker compounds.

Summary:

The phytochemical study deals with the modern pharmaceutical analysis of *Myristica fragrans* pericarp. In the preliminary phytochemical screening total ash, water insoluble ash, acid insoluble ash, moisture content, volatile oil content and fibre content were calculated. Moisture content in dried pericarp was less as compared to fresh pericarp. Fibre content was high, which supports the previous research report. Determination of water soluble extractives, alcohol soluble extractives and hot alcohol soluble extractives was carried out, with the highest yield of extract in water. Successive solvent extraction was experimentally conducted and qualitative analysis of extracts was performed. On qualitative analysis, all extracts indicated the presence of Tannins and Steroids in study drugs. Flavonoids and phenols were found to be present in all the extracts, except in petroleum ether and Cyclohexane extracts. Alkaloids were found to be absent in all extracts. Pectin was found to be present in study drugs. Thin Layer Chromatography profile of different extracts of pericarp was performed. The different spots are measured and R_f values are reported.

Conclusion:

The study was aimed to conduct preliminary phytochemical analysis of "*Jatiphala kosha"* (pericarp of *Myristica fragrans* Houtt.) of *Jatiphala Kosha* i.e. pericarp of *Myristica fragrans*. From the preliminary phytochemical analysis of study drugs, the following conclusions can be drawn.

- Qualitative analysis of extracts revealed the presence of tannins, steroids, flavonoids and phenols.
- Dried pericarp can be preserved and used for a longer time than fresh pericarp because the former one has less moisture content in it.

Source of Support: Nil

Conflict of Interest: Nil

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Figure No. 1: *Myristica fragrans* showing morphological characters

***Myristica fragrans* Tree**

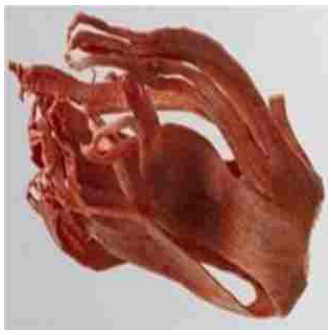
Bark of *Myristica fragrans*

Leaves

Flowers

Fruits

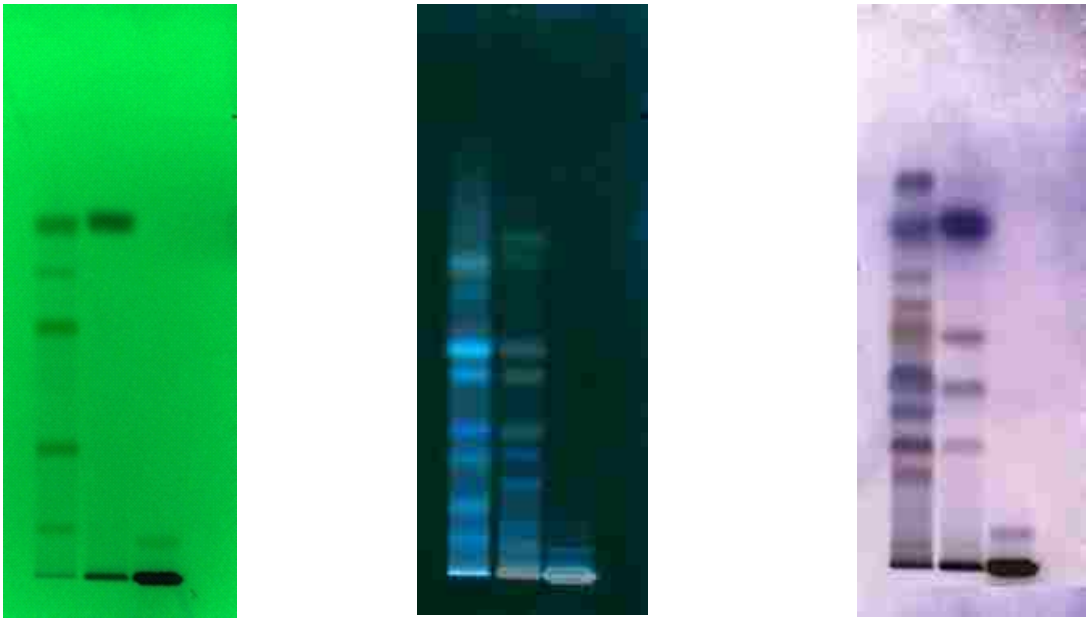
Seeds



Aril

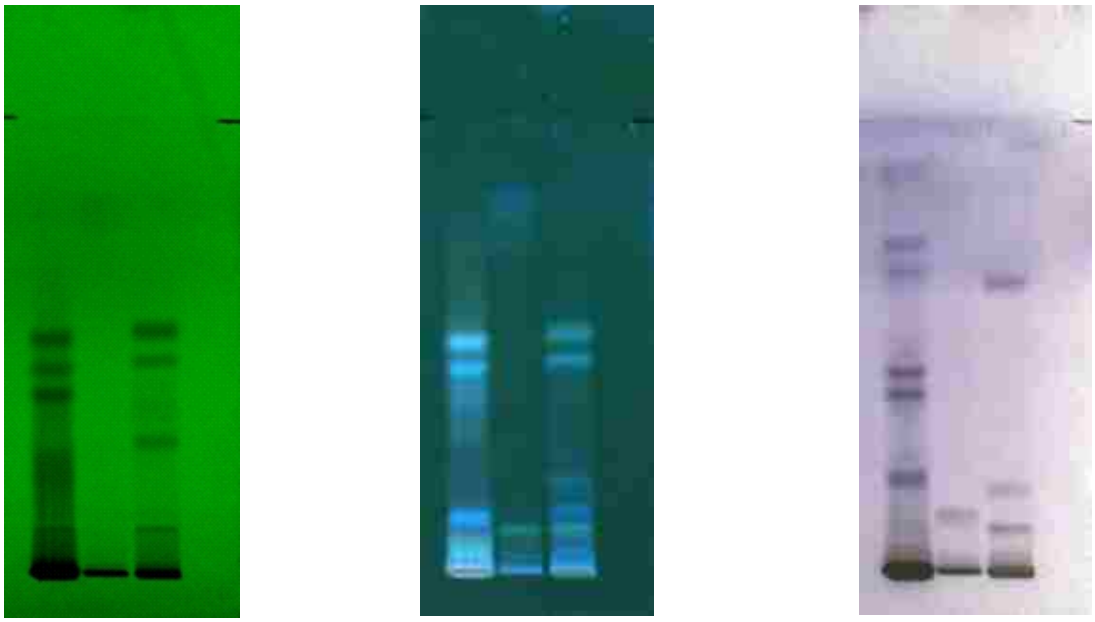
Pericarp

Figure No. 2: Plate 2A: TLC of petroleum ether extract (1); cyclohexane extract (2) and acetone extract (3) using solvent - Toluene: Ethyl acetate (9: 1)



254 nm **366 nm** **After derivatization**

Figure No. 3: Plate 2B: TLC of acetone extract (3); ethanol extract (4) and water extract (5) using solvent - Toluene: Ethyl acetate: Formic acid (5: 5: 1)



254 nm **366 nm** **After derivatization**