"Quantification of amino acids, fatty acids and nutritional elements of *Myristica fragrans* Houtt. and its pharmaceutical elucidation"

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENT OF THE DEGREE
OF
MASTER OF TECHNOLOGY
IN
INDUSTRIAL BIOTECHNOLOGY

Submitted by
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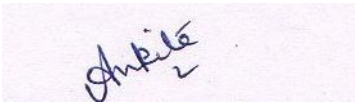
CANDIDATE'S DECLARATION

I, Ankita Chakravarty, 2K18/IBT/01, student of M.Tech (Industrial Biotechnology), hereby declare that the project dissertation titled "Quantification of amino acids, fatty acids and nutritional elements of *Myristica fragrans* Houtt. and its pharmaceutical elucidation" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any degree, diploma associateship, fellowship or other similar title or recognition.



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CERTIFICATE

I hereby certify that the project dissertation titled "Quantification of amino acids, fatty acids and nutritional elements of *Myristica fragrans* Houtt. and its pharmaceutical elucidation" which is submitted by Ankita Chakravarty, 2K18/IBT/01, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any degree or diploma to this university or elsewhere.

Jai Gopal 31/06/20

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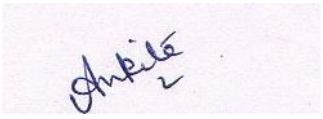

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ABSTRACT

Jaiphal (*Myristica fragrans* Houtt. or true nutmeg) is classified botanically as a member of the family 'Myristicaceae' which is a dark-leaved tree and it is cultivated for two spices which are derived from its fruit: nutmeg from its seed and mace together forms the seed covering. It is also a marketable basis of an essential oil and butter. It belongs natively to Banda Islands in the Moluccas of Indonesia and is cultivated in Southern India especially in Kerala. The essential oil is manufactured by the process of steam distillation of ground nutmeg which is especially in the perfumery and pharmaceutical industries. Since ages, traditional knowledge of using jaiphal is commonly used for treating of various diseases such as cold and coughs, anti inflammatory anti diabetic etc. In today's world, like pharmaceutical industry, food industries are also focused on development of substances that naturally contain some health benefits. Thus, the present study was done to investigate the nutritional elements, biochemical compound profiling from the fruit extract of *Myristica fragrans* Houtt. so that it can be used for various therapeutic effects in pharmaceutical industries. Proximate analysis include moisture content which is 9.2 % ± 2.6 (done in triplicates),total protein content which is estimated to be 4.82 ± 0.26 with total nitrogen content 0.772 %, total amino acid analysis confirmed the presence of nine essential, nine non essential amino acids and seven free amino acids. 30 elements with categories of five macro, eight micro or trace and seventeen ultra trace elements were analyzed which directly or indirectly relates to different disease curing capacities. Surface methodology (SEM) showed smooth and bounded morphology at different angles and resolution and X-ray diffraction (XRD) studies revealed that surface methodology was pure and crystalline to be used for further process. Various functional groups with the range of

the spectrum from 1016-3460 cm^{-1} such as phenol with O-H bending at 139.83 cm^{-}, C=N stretch bond of imine, some peaks are also present in the range of 400- 930 cm^{-1} which could be benzene derivatives, etc. were confirmed from Fourier - transform infra red analysis (FTIR). Further, total fatty acid content analysis reveals the utility of jaiphal that includes various saturated and unsaturated fatty acid. Myristic acid, the major component of jaiphal is highly present is useful in various human purposes too.

Keywords - *Myristica fragrans* Houtt., jaiphal, amino acid, fatty acids, Surface methodology (SEM), X ray diffraction (XRD), elemental analysis, functional group (FTIR), proximate analysis.

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LIST OF ABBREVIATIONS AND SYMBOLS

℃ - Degree Celsius

ICPMS - Inductively Coupled Plasma Mass Spectrometry

ICP - Inductively Coupled Plasma

SEM - Scanning Electron Microscopy

XRD - X Ray Diffraction

FTIR - Fourier Transform Infrared Spectroscopy

RCF - Relative Centrifugal Force

FAME - Fatty Acid Methyl Ester

GC - Gas Chromatography

nm – nanometer

KBR- Potassium Bromide

CHAPTER 1 - INTRODUCTION

Spices and herbs are the major identity of India since ancient times and nutmeg also commonly known as jaiphal is used majorly for its aromatic, flavoring and health benefits. Spices are referred to as the building blocks of any food substance as it accelerates appetite, add flavor and texture to food. Additionally spices are enriched of various medicinal properties too such as anti microbial, anti inflammatory etc. [9]. Ayurvedic treatment of any disease is common in India and some other country since ages and hence modern day medicine are also diverting their focus toward the utilization of plant derived compounds for treatment as biomolecules from plant plays a crucial and important and a positive role in human metabolism [4]. Like food industry, pharmaceutical industries are also focused on development of substances that naturally contain some health benefits and have less or no side effects. All human being requires various organic/and inorganic compounds in their diet to meet the daily requirements as a balanced diet to enhance the immune system. Jaiphal has been used as a flavor in many varieties of sauces, vegetables, baked goods, confections, potatoes, meats, etc. The fleshy aril surrounding the nutmeg seed forms the basis of the spice mace. The important portion of the plant with respect to its pharmacological activity and in health benefits is the dried kernel (seed) also called as the nutmeg. It also contains myristin (a toxin).

Figure 1.1: Physical visual view of jaiphal (*Myristica fragrans* Houtt.) fruit extract surface

Myristica fragrans Houtt. is a member of the family Myristicaceae which is the largest genus, with 18 genera and approximately 300 species. Myristica is the largest genera with 72 species in total. It was initially introduced in 11th century in Europe but was industrially and commercially available from 16 th century onwards. It belongs natively to Banda Islands in the Moluccas of Indonesia and is cultivated in Southern India especially in Kerala. It is distributed to many regions such as North Australia, Pacific Island also. It is a scattering aromatic evergreen tree with brown-red bark usually rising to 5 to 13 meters high, sometimes up to 20 meters [24]. It grows at an altitude of 700 - 4500m with an average temperature of 25 – 30 ℃. It can grow in any kind of soil which has higher water quantity but prefers a soil with pH in the range of 6.5 – 7.5 with a huge amount of organic matter [16]. At the age of 5 years, flowering initiates and the sex is determined at this stage. Females are culled to a ratio of 1:10 males. Commercial production initiates around 7th or 8th year and by the age of 15 years the tree will reach its maximum productivity and can attain a height of 40-50 feet. Fruiting continues for further 40 to 80 years. Flowers are generally single sexed; occasionally female and male flowers are found on the same tree and are pale yellow, bell shaped and waxy in structure. Leaf veins are pinnate and leaf blades are densely pubescent. Plants are dioecious, with the exception of a few monoecious. The nutmeg fruit is a fleshy drupe and is variable in size of about 6.25 cm in length and the shape varying from oval to round. When ripened, fleshy husk called pericarp which is around 1.25 cm in thickness splits into two halves, enlightening the seed. The kernel (nutmeg of commerce) is surrounded by a shell which is deep brown in color. Adjacent to this seed coat lays a striking network called mace, an outgrowth from the bottom of the seed.

Figure 1.2 & 1.3: Morphology of different parts of jaiphal (*Myristica fragrans* Houtt.) fruit extract

Young nutmeg saplings require 50 percent of shade as a geographical condition for its cultivation but once they reach the age of 6 or 7 years, they can fully grow in shady condition too. Superficial feeders such as goat droppings, dried cow dung's and calcium are provided to without disturbing its roots. The suitable and preferred harvesting season is from june to august [16]. It contains approximately 4 – 10% of essential oil.

There are various commercial uses of jaiphal which are reported in various studies. There are various non medicinal uses of jaiphal too. In its ground form, it is used as a spice in food industries. There are various components present in its essential oil, one of which is camphene that is used in preparation and manufacturing of camphene and its related products. Myristic acid is used in preparation of various flavors in food industries, also is used in the manufacturing of liquid detergents, various shampoos, shaving cream and soaps.

Toxicity from jaiphal is a rare condition but is reported to affect lungs, liver and spleen. It has some symptoms such as nausea; headache and can also cause hallucinations in some patients too. These conditions are caused due to Myristicin also called as 1-allyl-3, 4-methylenedioxy5-methoxybenzene which is reported to be a neurotoxin in nature and a psychoactive substance [20].

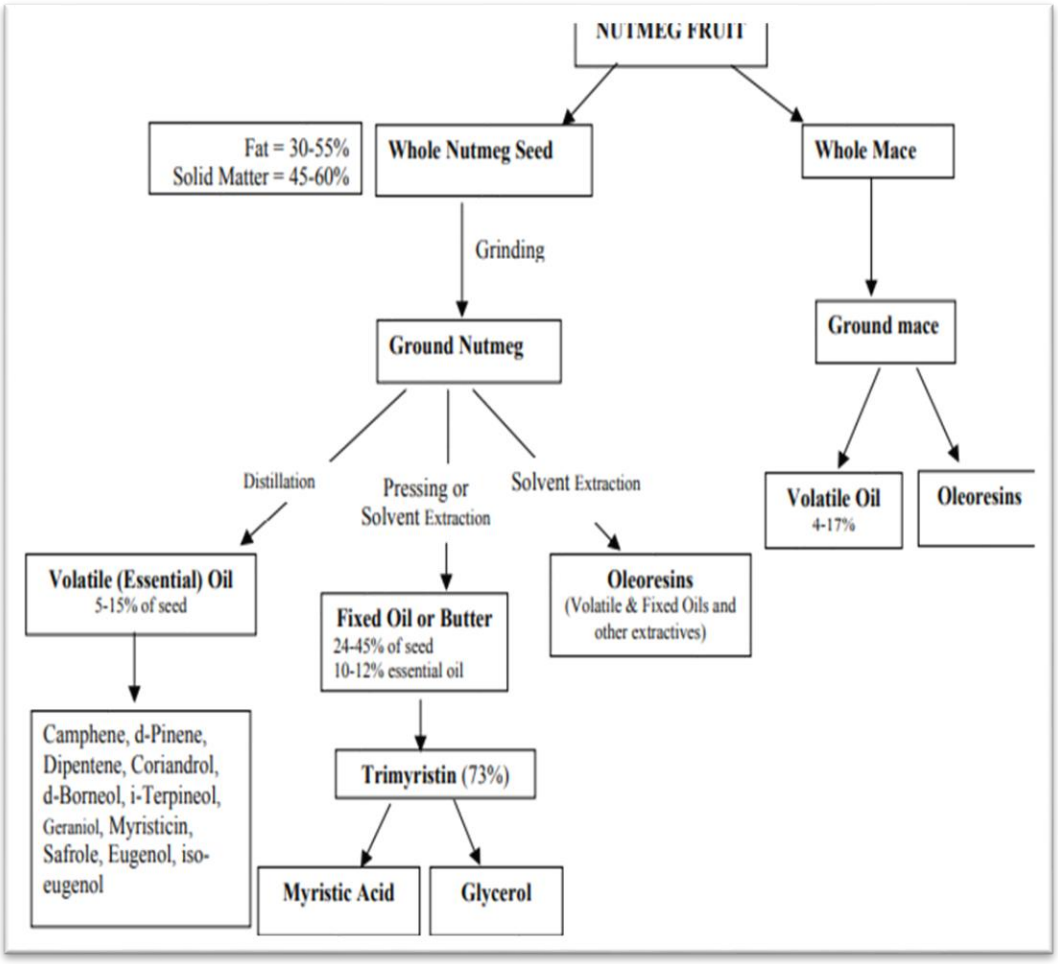


Figure 1.4: Various commercial products from *Myristica fragrans* Houtt. fruit extract

CHAPTER 2 – REVIEW OF LITERATURE

2.1 *Myristica fragrans* Houtt. – A Brief Overview

The medium sized, thick and shady, evergreen dark leaved and an aromatic dioecious *Myristica fragrans* Houtt. is found in Indonesia and some of the tropical countries like India, Malaysia**.** It has two forms or products– seed and mace of nutmeg. It is classified botanically as a member of family "Myristicaceae". The meaning of Myristica is taken from Greek philosophy in which it meant sweet liquid distilled from the plant (Everett 1981).

Taxonomical Classification:-

Kingdom: Plantae

Subkingdom: Viridiplantae

Infrakingdom: Streptophyta

Division: Tracheophyta

Subdivision: Spermatophytina

Class: Magnoliopsida

Superorder: Magnolianae

Order: Magnoliales

Family: Myristicaceae.

Genus: Myristica Gronov. nutmeg

Species: *Myristica fragrans* Houtt.

Botanical Name: *Myristica fragrans* Houtt.

The plant grows in an altitude of 700 – 4500m with required annual rainfall of 2000 – 3500 mm. It is a plant that grows in shade. 50 % shady region is required by the plant in early stage of its growth but can grow in full shade at the later stages. This spreading evergreen tree grows to height 5 – 13m. Nutmeg is a rich source of nutmeg butter and essential oils. The approximate percentages of different compositions of essential oil from nutmeg are - fixed oil 25 – 30% and volatile oil 5 – 15%. Nutmeg butter is also a rich source of fat, is used specifically preparation of perfumes, toothpaste etc. [23].

2.2 Chemical Composition of *Myristica fragrans* Houtt. –

The major chemical constituents of jaiphal are - alkyl benzene derivatives (myristicin, elemicin, safrole) myristic acid, alpha-pinene, terpenes, beta-pinene and trimyristin [31]. It also contains approx. 10% of essential oil, primarily composed of terpene hydrocarbons, myrcene, camphene, limonene, terpinene. It also yields nutmeg butter which has 25 - 30 % fixed oil and is a semi-solid reddish brown fat bearing the aroma of nutmeg. Nutmeg butter contains trimyristin, oleic acid, linoleic acid and resinous material. Both nutmeg and mace contain approx. 2% of lignans (diarylpropanoids), which are non-volatile dimers of phenylpropanoid elements of the essential oil, *e.g.* dehydrodiisoeugenol. The key glycoside is trimyristin containing anxiogenic activity. Chromatography of the nutmeg extract showed the presence of epicatechin and cyaniding. Nutmeg contains trimyristin which is a ester of myristic acid also called as tetradecanoic acid and glycerol.

It also offers various biological effects that includes as a stimulant, carminative, astringent and aphrodisiac. It is being used as a component in tonics and also forms a constituent of preparations prescribed for dysentery, stomach ache, flatulence, nausea, vomiting, malaria, rheumatism and also in early stages of leprosy (Table 2.2.1)

Table 2.2.1 - Uses and composition of *Myristica fragrans* Houtt. [24]

Name	Percentage (%)	Attribute	Therapeutic benefits
Sabinene	24.0	Spiciness	Invigorating, anti-inflammatory, antimicrobial
alpha-Pinene	16.8	Camphoraceous	Cognitive, anti-

			inflammatory, bronchodilator
beta-Pinene	11.7	Woody-green pine like smell	Anti-depressant, antibacterial, cytotoxicity and antimicrobial
Terpinen-4-ol	2.2	Mild pine aroma with a slight herbal pepper flavoring	Antibacterial, antifungal, anti-inflammatory
Limonene	5.7	Fragrance and flavor	Anti-carcinogenic, antioxidant and chemo preventive
gamma-Terpinene	5.3	Lime -like, spicy, herbal and sweet flavor and fragrance.	Refreshing, antioxidant
Elemicin	4.0	Spicy floral aroma	Antibacterial, antifungal, anti-allergic
alpha-Terpinene	3.8	Perfume and flavor that is employed in cosmetics, food and pharmaceutical	Antioxidant, antifungal, antibacterial, sedative and repellent
Myristicin	3.3	Spicy aroma	Cytotoxicity, antioxidant, hepatoprotective
delta-3-Carene	2.3	Sweet, woodsy, penetrating and pungent odor	anti-inflammatory
Terpinolene	2.2	Woody and smoky odor	Antifungal, antioxidant

Myristicin also known as (4-methoxy-6-(2-propenyl)1,3-benzodioxole according to IUPAC nomenclature is the most crucial and important component of the essential oil from mace and nutmeg which is already known for hallucination and neurotoxicity but is also a chemo-protective agent [28]. It is a chemical liquid which is not soluble in water but is soluble in organic solvent. Distillation sequencing and column chromatography are the preferred methods to extract out this clear liquid.

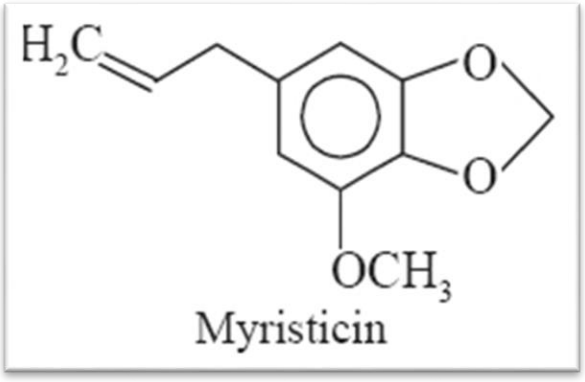


Figure 2.2.1: Molecular structure of Myristicin

Principle	Nutrient Value	Percentage of RDA
Energy	525 Kcal	26
Carbohydrates	49.29 g	38%
Protein	5.84 g	10%
Total Fat	36.31 g	180%
Cholesterol	0 mg	0%
Dietary Fiber	20.8 g	55%
Vitamins		
Folates	76 µg	19%
Niacin	1.299 mg	8%
Pyridoxine	0.160 mg	12%
Riboflavin	0.057 mg	4%
Thiamin	0.346 mg	29%
Vitamin-A	102 IU	3.5%
Vitamin C	3 mg	5%
Electrolytes		
Sodium	16 mg	1%
Potassium	350 mg	7.5%
Minerals		
Calcium	184 mg	18%
Copper	1.027 mg	114%
Iron	3.04 mg	38%
Magnesium	183 mg	46%
Manganese	2.900 mg	126%
Phosphorus	213 mg	30%
Zinc	2.15 mg	20%
Phyto-nutrients		
Carotene-ß	16 µg	--
Crypto-xanthin-ß	90 µg	--
Lutein-zeaxanthin	0 µg	--

Figure 2.2.2: Nutritional information of *Myristica fragrans* Houtt. (Value per 100g)

(Source: USDA National Nutrient data base)

2.3 Pharmacological Properties of *Myristica fragrans* Houtt.-

Recent approach of medicine is turned into traditional way of therapeutics which includes analyzing and using medicinally important plants and herbs for the ailment of various diseases. Our folk medicine literature is full of such therapeutic uses of jaiphal. It is full of various properties such as anti – diabetic, anti – obesity, anti – carcinogenic, anti – depressant etc. The extracted essential oil is used to enhance the memory skill.

The various components and its associated therapeutic actions are well documented (table 2.3.1)

Table 2.3.1 - Active composition and its associated therapeutic activities of *Myristica fragrans* Houtt. [16]

Therapeutic activity	Active constituent	Chief investigator
Antioxidant	Myristicin	Yadav and Bhatnagar., 2007
Antibacterial	Malbaricone	Orabi*et al*., 1991
Antidiabetic	Macelignan	Han *et al*., 2008
Cytotoxicity	Dihydroguaiaretic acid	Park *et al*.,1998
Hepatoprotective	Macelignan	Morita *et al*., 2003
Insecticidal	Myristicin + pyrethrum	Park *et al*., 2008
Molluscidal	Myristicin , Trimyristin	Singh & Agarwal., 1981; Singh & Agarwal., 1983
Memory enhancing	Myristicin	Parle*et al*., 2004
Hypolipidaemic	Myristicin	Sharma &Mathur., 1995; Ram *et al*., 1996; Capasso *et al*.,2000
Aphrodisiac	Myristicin	Tajuddin*et al*., 2005

Jaiphal is well known for having various types of therapeutic effects as illustrated by Grover et. al, 2002 and Jan et. al, 2005 [15] where it is documented for the various calcium channel which reduces the volume of total acidity of gastric secretions by the extracts of jaiphal. These extracts are also reported to be a low analgesic effect and no harmful effect on blood pressure upon intake. Thus it is stated it can be effectively used in the cure of peptic ulcer. It has the same effects as of verapamil as observed by various experiment in fasting rabbit [24].

It has also shown to have antagonistic activity for bacteria, microbes and fungus [23]. The antibacterial activity was observed in volatile oil which was extracted from the seed.

During the experimental process 25 different bacterial strains were included (equally gram negative and gram positive) and volatile oil was effective against the majority. It is also known to exhibit antimicrobial activity against plant and animal pathogen, *E.coli*, *saccharomyces cerevisiae*, multi-drug resistant strains like *Salmonella typhi* and *Helicobacter pylori.* Alcoholic extract of jaiphal revealed antibacterial activity against *Micrococcus pyogenes.* Essential oil extracted from the seed of Jaiphal is reported to possess inhibition activity for growth and survival of *Listeria monocytogenes* in broth culture. And inhibit bacterial spore and can be used as food preservative**.**

Anti-inflammatory activity of seed extract was evaluated in acetic acid induced vascular permeability in mice which depicted the same result as of indomethacin and thus the result obtained because of the anti-inflammatory action of myristicin. Myristicin inhibits the action of chemokines, cytokines and nitrous oxide via calcium pathway

Parle et. al, 2004 in his experiment demonstrated the effect of jaiphal on the learning capability and memory level in mice. Elevated plus maze and passive-avoidance apparatus was utilized to assess the various learning and memory parameters. n-Hexane extract at its lowest dose for three days showed the positive result. Hydro alcoholic extract of jaiphal showed 50% inhibition of acetylcholinesterase and thus was used for the treatment Alzheimer's disease [25].

Aril parts of jaiphal possess strong antioxidant activity which is due to the activity of inhibition of lipid peroxidation and superoxide radical scavenging activity (Yadav and Bhatnagar 2007). The scavenging capacity of jaiphal is more with respect to the normal antioxidants like propyl gallate, butylated hydroxytoluene (BHT) using Trolox equivalent antioxidant capacity assay (TEAC) or by DPPH [21].

Jaiphal is also a major anti diabetic which is used as a treatment for type - 2 diabetes as it contains macelignan, a natural compound which plays an important role in enhancing the insulin sensitivity and improved lipid metabolic disorders through activating peroxisome proliferator receptor as reported by Han *et al*., 2008.

In human it is also reported to show some clinical effects such as nutmeg intoxication due to trimyristin is similar to intoxication effect due to extreme intake of anticholinergic agents. It then shows some resultant effects such as profuse sweating, flushed face, dry throat etc. It is also being reported that it shows an altered state of mind, confusion and a sense of doom. These symptoms may be opposing depending on various factors like the length of time lapsed after ingesting the toxin, dose taken and the variability between different samples of nutmegs. Higher dose of 4g/kg results into such abnormal condition [20]. It may also induce cytotoxicity in human neuroblastoma SK-N-SH cells by an apoptotic means [24].

Anti depressant activity of jaipahl is observed in n-hexane extract using Forced Swim Test (FST) and Tail Suspension Test (TST) in different mice with three different doses. This effect is due to the reaction and interaction with dopaminergic, adrenergic and serotonergic complex systems [11].

Hexane soluble fraction of methanolic extract of jaiphal is reported to show insecticidal activity. This fraction is used to inhibit the growth of adult female *Blatella germanica*. Similarly, essential oil from nutmeg is used to inhibit the growth of various larvae and insects [23].

Despite various utilities of jaiphal, prolonged use of high dose (400 – 500 mg/kg) can thus result in low sperm count and affect the sexuality of the individual [20]. It is also used in treating bad breath, and asthma related disorders [4].

Jaiphal has also various non industrial uses such as its utility in food industry in the form of grounded powder. It is used in formula preparation of shampoo, detergent, liquid soaps, perfume bases etc.

2.4 Various biochemical tests utility for *Myristica fragrans* Houtt.-

Bioactive compounds help to proceed with the knowledge of functionality and thus mineral estimation was done by Inductively Coupled Plasma Mass Spectrometry (ICPMS). It is an analytical technique used for determination of trace elements and metalloids. The instrument detection limit is in the range of 1 – 100 ng/l. Minor category of elements play a

very important role as it is assumed to be a key component of proteins such as haem protein and hemoglobin. Quantitative determination of minerals is crucial for determining the quality of the food. Bioactive chemicals along with nutrients and fiber found in natural substances are directly or indirectly related to the mechanism of action against diseases. Studies revealed that optimal intakes of some elements like sodium, potassium, magnesium, calcium, manganese, copper etc. could reduce the individual risk factors, coupling those with cardiovascular disease for both human beings and animals. Calcium comprises as the component of bones and teeth. Its elementary function in cell membranes and on muscles is to regulate the endo - exoenzymes and blood pressure. The macro minerals forms the structural components of tissues and helps in cellular and basal metabolism and water and acid-base balance like potassium is an important component of cell and body fluids which helps to control heart rate and blood pressure, manganese and copper as used as co-factors for the antioxidant enzyme namely superoxide dismutase. Iron is required for red blood cell production and as a co-factor for cytochrome oxidases enzymes".

Jaiphal is also rich in many vital B-complex vitamins, including vitamin-C, folic acid, riboflavin, niacin, vitamin-A".

Jaiphal seeds have a superior amount of fat and adequate amount of fiber and moisture with suitable mineral element which shows the high nutritive value. This in turn indicates that it is good for younger people and anemic people.

Functional group analysis by Fourier Transform Infrared Spectroscopy such as alkyl halides, alkenes, aldehyde, saturated aliphatic, aliphatic amines, primary and secondary amines etc. Presence of various functional groups such as amines, nitrites, and aldehyde leads to phytochemical screening of the sample which are responsible for various therapeutic effects such as anti inflammatory, anti cancerous, anti diabetic etc. The curative effects for curing various human diseases could be the result of presence of various bioactive constituents in them.

Total amino acid, total lipid content, total fatty acid content and total protein content preceded the process to evaluate the bioactive metabolites.

All the above mentioned nutritional elements are directly related to cure hidden hunger. The literal meaning if hidden hunger according to World Health Organization (WHO) is the

lack of vitamin and minerals especially. Hence, complete nutritional bioactive compounds profiling of jaiphal can help in curing hidden hunger too.

CHAPTER 3 – METHODOLOGY

3.1 Chemicals and Sample Procurement

- Chloroform, methanol, Potassium bromide, gold plating nano particle, concentrated sulfuric acid, copper sulfate, sodium sulfate, boric acid, sodium hydroxide, nitrogen, hydrochloric acid, performic acid, hydrobromic acid, indole, nitric acid
- Sample was collected from an authorized dealer of local market.

3.2 Sample Preparation

- Samples were thoroughly washed with water and were initially shred dried at room temperature and then kept at 35℃ for 48 hours in hot air oven.
- The dried sample was subjected to grinding and stored at 4℃ in an air tight container for future use.

3.3 Proximate Analysis:-

3.3.1 Moisture Content Estimation

- The moisture content was determined by keeping the dried and grounded sample in an oven at 105 ºC for 6 hours in triplicates [27].
- Weighed the crucible before and after drying and also with and without the sample.
- Estimation was calculated using the formula –

$$\textbf{Moisture content (\%)} = \{w_1 - w_0\}\{w_2 - w_0\}/\{w_1 - w_0\} * 100 \quad (3.3.1)$$

Where, w_o = weight of empty crucible

w_1 = weight of crucible and sample before drying

w_2 = weight of crucible and sample after drying

Figure 3.3.1: Chemtech dry oven system for moisture content (Hyderabad, India) which uses thermogravitic method.

3.3.2 Total Protein Content Estimation

- Kjeldahl method using Tecator Kjeltec Auto 1030 Analyser (Foss, Warrington, UK) was used.
- Sample was digested with concentrated sulphuric acid with the addition of copper sulphate.
- For distillation, excess of sodium hydroxide solution treatment was given.
- Volumetric titration by sodium hydroxide was done [17].

$$\textbf{Protein concentration (\%) = N x 6.25} \qquad (3.3.2)$$

Where, N = Total Nitrogen (Titration value)

3.4 Total Amino Acid Profile

- Powdered sample was subjected to hydrolysis by 6N HCL at 11℃ for 22 hours.
- The hydrolyzed sample was then subjected to Pci Analytics EV Plus 08 Nitrogen Evaporator (Maharashtra, India).
- Auto injection volume of 20 µl was taken for the sample analysis.
- With this reaction methionine, cysteine and tryptophan (if in the sample) gets hydrolysed with interaction of some specific reagents such as performic acid and hydrobromic acid(48%) and for tryptophan (if present), was hydrolyzed using indole.
- For all the remaining amino acids, procedure was same.
- Cation exchange resin for separation of different amino acid components was used. are detected by a detector at 570 nm for other amino acids other than proline which was analyzed at 470 nm.
- Quantification was done by comparing with the standards (Wako Pure Ltd, Japan)
- Reaction temperature was 35 ℃ with different flow rates [17].

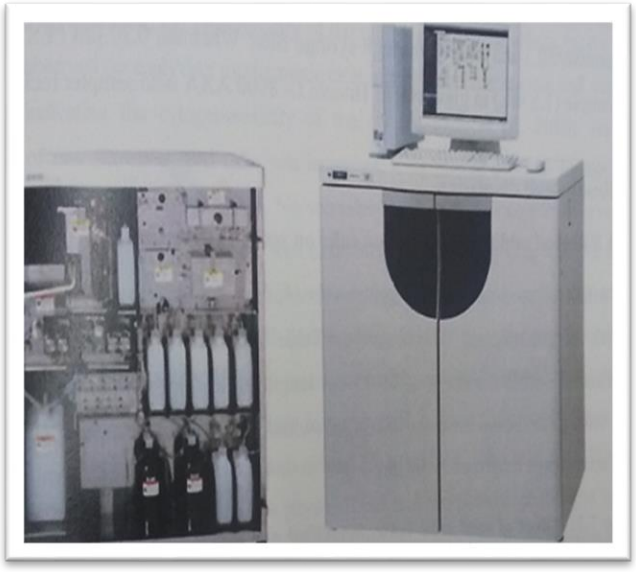


Figure 3.4: Amino acid analyzer Hitachi L-8900 (Tokyo, Japan)

3.5 Scanning Electron Microscopy Analysis

- 1 mg of powdered sample was taken and was subjected to gold plating.
- Sample was placed to the auto sampler plate and then was subjected to different resolutions and angles for scanning the surface.
- A focused beam of electrons and five segment detector is used to scan the sample.
- Best resolution and angle was captured through the camera.

Figure 3.5: SEM system EVO18 Zeiss for determining surface morphology (New York, USA)

3.6 X-Ray Diffraction Analysis

- 300 mg of powdered sample was subjected to XRD system.
- With the help of origin software, graph was plotted.

$$\text{Bragg's Law} \Rightarrow \lambda = 2\, d \sin\Theta \qquad (3.6.1)$$

Where, λ = wavelength in nm (y axis)

d = Interplanar space

Sin Θ = degree (x axis)

Figure 3.1: Bruker D8 advanced XRD system for sample purity

3.7 Elemental Analysis

- ICPMS is used for mineral estimation especially rare earth minerals. It combines ICP detector source with a mass spectroscopy. The ICP ionizes the atoms of the sample which are then separated by mass spectroscopy.
- 200 mg of the powdered sample were analyzed for 35 elements.
- Standard solution of ultra high purity grade was used – nitric acid which was added with different concentrations (v/v) such as 2% and 1% and reagent water that contains metal traces which is used as blank.
- Single or multi elements stock solutions of all elements were preferred freshly (1000mg/l).
- Calibration curve was prepared in the range of 0 to 100µl.
- All standards and blank were prepared in the 2% nitric acid matrix.
- Data was recorded with respect to laboratory blanks and instrument detection limit.

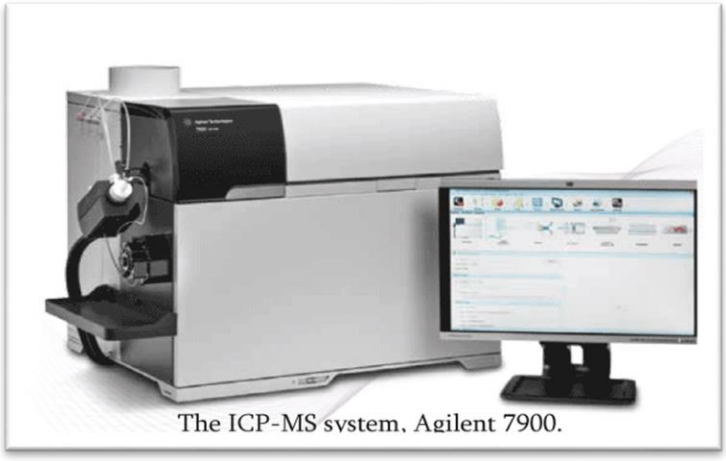


Figure 3.7: Agilent 7900 ICPMS system for elemental analysis (Santa Clara, USA)

3.8 FTIR Analysis

- 300 mg KBR (FTIR grade) and 1 mg of sample were weighed and subjected to grinding together by mortar pestle, after cleaning of the pellet holder was done by acetone.
- Transferred the mixture to a butter paper and then to the middle of the dye set.
- 10 psi pressure was set in the hydraulic pump after placing the sample in the middle of the apparatus.
- Pressure was applied both upwards and downwards.
- Transparent pellet was obtained which was the subjected to FTIR system for getting the peaks.

Figure 3.2.1: Perkin Elmer Spectrum 2 FTIR system for estimation of functional groups (USA)

Figure 3.8.2: Hydraulic pump system for pellet preparation

3.9 Crude Lipid Estimation

- 500 mg of sample was mixed with (1: 2) methanol: chloroform and subjected to homogenization using a Ultra Torrex Tissue Disrupter (Fischer Scientific, Loughborough, UK) or by subjecting it to centrifuge at 2060 RCF for 15 minutes at room temperature.
- Supernatant was transferred to a fresh tube and repeated the above step for 2 – 3 times.
- Poured the supernatants to a petri plate.
- Weighed the petri plates before and after sample.
- Calculated the lipid content after drying of the liquid from the petri plate [17].

3.10 Total Fatty Acid Profile

- Fatty acid methyl esters (FAME) were prepared by the reaction of acid catalyzed transesterification at 50℃ for 16 hours.
- The extracted FAME was then subjected to purification and quantification by Fissons GC 8160 gas – liquid chromatography (Thermo Scientific, Milan, Italy).
- Column size of 30m x 032 mm x 0.25µm with a FID (Flame Ionization detector) was used inside the system to separate the different fatty acid composition.
- Data analysis was done by chromcard software version 2.0

CHAPTER 4 – RESULTS AND DICUSSION

4.1 Powdered sample was prepared from gravimetric method using hot air oven and followed by grinding.

Figure 4.1.1: Sample (*Myristica fragrans* Houtt.) from authorized dealer of Delhi market

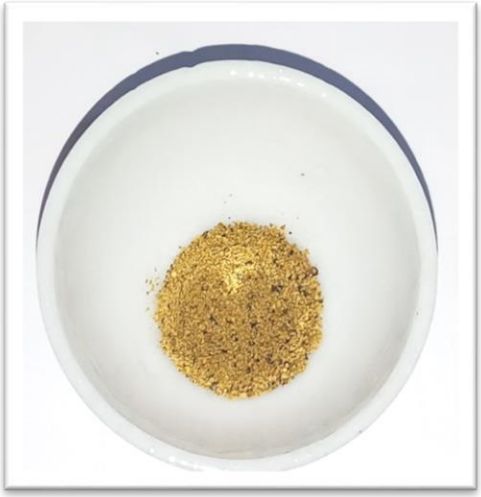


Figure 4.1.2: Powdered *Myristica fragrans* Houtt. for further biochemical tests to evaluate their physiologic functions

4.2 Proximate Analysis

4.2.1 Moisture Content

Moisture content in *Myristica fragrans* Houtt. (%) = 9.2 % ± 2.6 (Values are expressed by mean ± SD of triplicates).

It depends majorly on the various environmental conditions such as humidity, temperature, harvest time, and climate as well as storage conditions. All the geographical conditions play a crucial role and thus it is important to be able to reliably measure moisture content. Also, low moisture content is a direct indicator that the food sample in the form of powder can be stored for a higher time with less or negligible reduction of the quality.

Due to different regions, moisture content varies as reported by Chibuzor Okonkwo et al. the content reported is 11.72% in Nigerian variety and 14.91 ± 0.3 by Rancy Ann Thomas et al. in South Indian region.

4.2.2 Total protein Content Estimation

Protein concentration in *Myristica fragrans* Houtt.(%) = 4.82 ± 0.26 with total nitrogen content 0.772 % (Values are expressed by mean ± SD of triplicates).

Normal range of protein content in *Myristica fragrans* Houtt. is 5.84 g per 100g. 10% is the Recommended Daily Allowance (RDA) of protein is approved by USFDA.

4.3 Total Amino Acid Profile

Table 4.3.1 - Amino acid composition of *Myristica fragrans* Houtt. (mg/100g)

Data are given as means ±SEM (n = 3).

Amino acids	Concentration (mg/100g)	± SE
Essential amino acids		
Histidine (His)	0.120	0.002
Isoleucine (Ile)	0.310	0.001

Leucine (Lue)	0.503	0.002
Lysine (Lys)	0.313	0.001
Methionine (Met)	0.074	0.001
Phenylalanine (Phe)	0.368	0.006
Threonine (Thr)	0.261	0.002
Tryptophan (Trp)	0.042	0.001
Valine (Val)	0.418	0.007
Non-essential amino acids		
Arginine(Arg)	0.763	0.013
Alanine (Ala)	0.319	0.002
Asparatate (Asp)	0.664	0.010
Cysteine (Cys)	0.044	0.003
Glutamic Acid (Glu)	0.722	0.006
Glycine (Gly)	0.325	0.010
Proline (Pro)	0.382	0.006
Serine (Ser)	0.318	0.005
Tyrosine (Tyr)	0.190	0.010
Free amino acids		
Phosphoserine (p- Ser)	0.058	0.001
Taurine (Tau)	0.022	0.001
Cystathionine (Cysth)	0.007	0.001
β -Amino isobutyric acid (β -AiBA)	0.026	0.001
1 Methyl Histidine (1 Mehis)	0.054	0.001
Hydroxy proline (Hypro)	0.083	0.002
ϒ- Amino isobutyric acid (ϒ -AiBA)	0.144	0.005

In the sample three categories of amino acids are present including 9 essential amino acids with total concentration of 2.409 mg/100g, 9 non essential amino acids with total concentration of 3.727 mg/100g and 7 free amino acids with total concentration of 0.394 mg/100g.

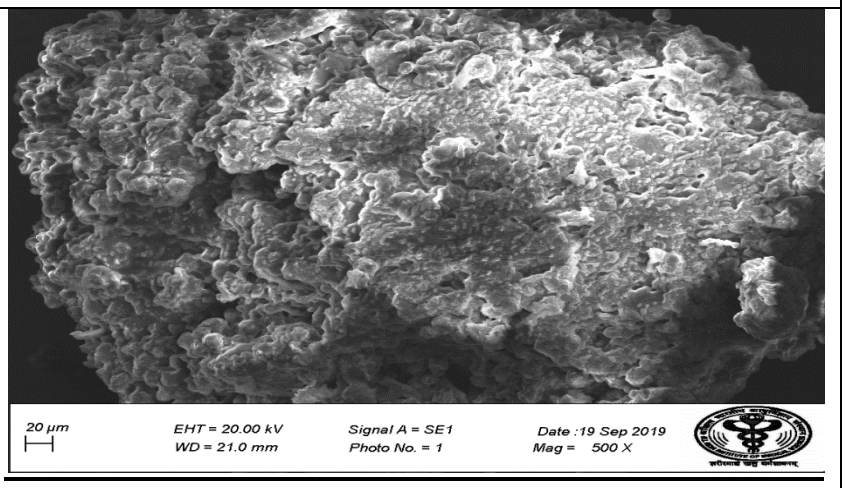
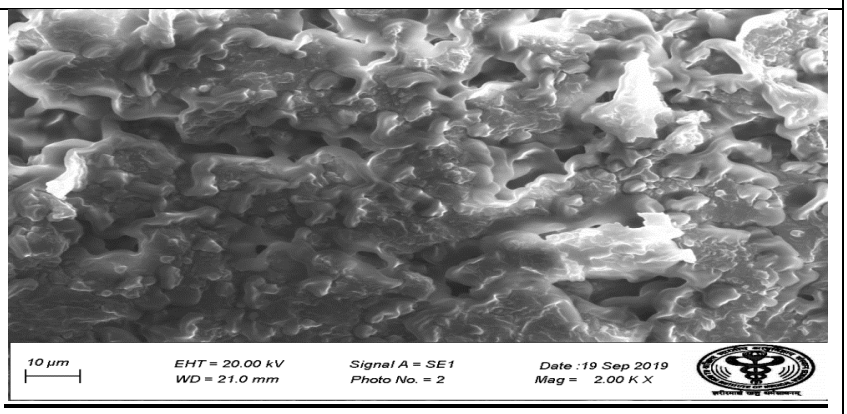
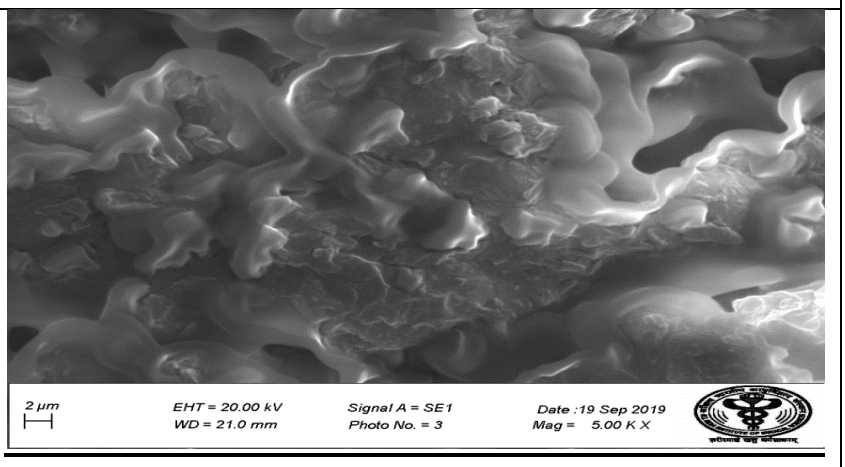
There are certain associated functions of different categories of amino acids. Essential amino acids play a crucial role in our body metabolism but can be taken through diet and jaiphal is moderately good in providing this category of amino acids. Higher amount of leucine (05.03mg/100g) in jaiphal helps in muscle repair, maintaining blood sugar level and produce growth hormones. Valine (0.418 mg/100g) is a branched amino acid that is also helpful in muscle growth. Phenylalanine (0.368 mg/100g) is an important amino acid as it serves as the precursor of various neurotransmitters dopamine, tyrosine etc. Lysine (0.313 mg/100g) is another essential amino acid present in the sample that helps in absorption of calcium thus reducing peptic ulcer. Isoleucine (0.310 mg/100g) is yet another amino acid of this category that plays a crucial role in haemoglobin production. Other amino acids in this category such as histidine (0.120 mg/100g) is vital for sexual cycle of human beings; methionine (0.074 mg/100g) is important for absorption of various essential minerals; threonine (0.261 mg/100g) is important for hormone production that are important for epidermal layer of skin and tryptophan (0.042 mg/100g).

Various non essential amino acids are present in jaiphal. Most of them are produced from glucose. Arginine (0.763 mg/100g) which is present in higher concentration and is thus important for relaxation of blood vessels, alanine (0.319 mg/100g) is important for removal of various toxic elements present in body after the breakdown and metabolism of protein. Proline (0.382 mg/100g) is a vital non essential amino acid that helps in intracellular signal production. Various other amino acids present in this category such as serine (0.318 mg/100g), is important in regulation of brain function which is related to memory enhancing; cysteine (0.044 mg/100g) helps in toxin formation.

All seven free amino acids present in the sample helps in enhancing the role of essential and non essential amino acids.

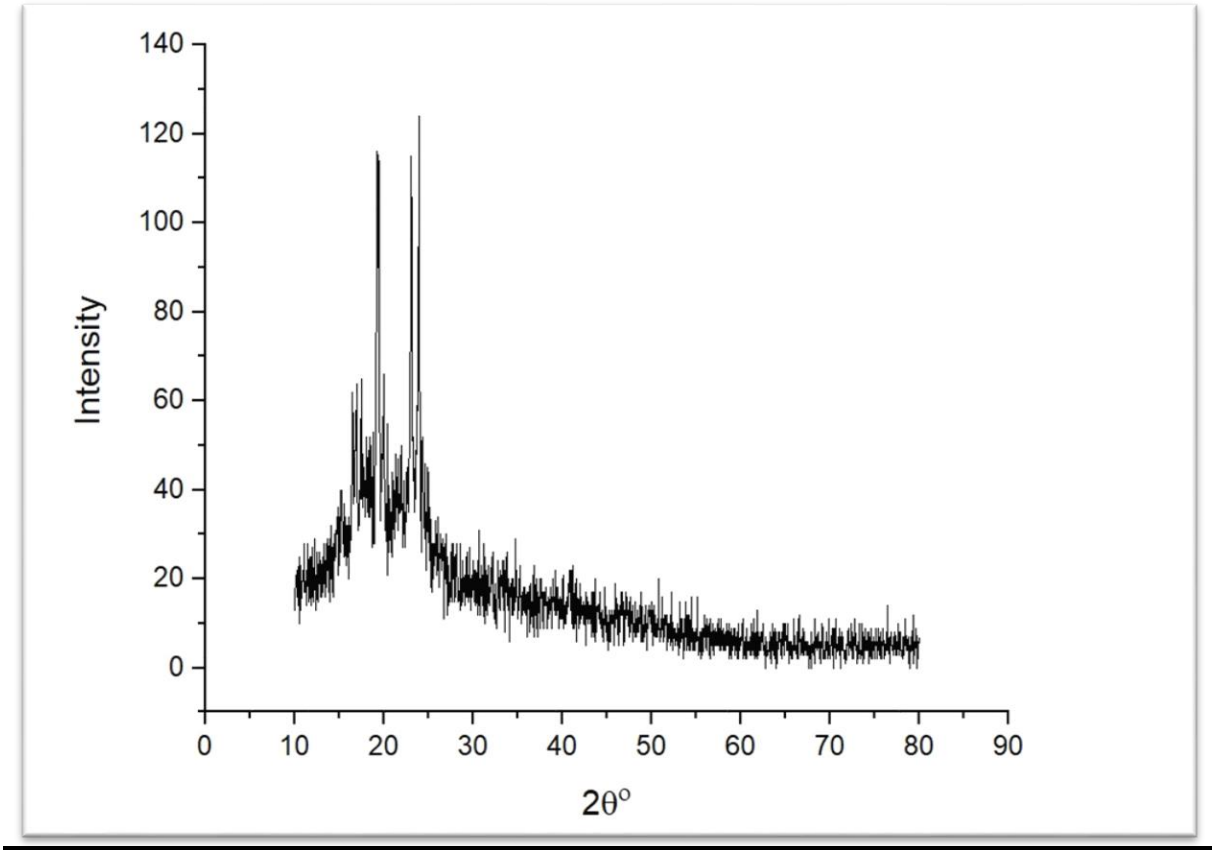
4.4 SEM Analysis

Table 4.4.1 - SEM analysis of *Myristica fragrans* Houtt.

Resolution	Image
500 x	
2kx	
5kx	

Surface methodology of jaiphal showed the bounded and smooth surface morphology (texture) that can be helpful in knowing the compounds chemical composition and its interaction when ingested to body for determining the pharmacological activities when coupled with XRD for quantification of bioactive metabolites and their solvents.

4.5 XRD Analysis



Graph 4.5.1 - XRD analysis of *Myristica fragrans* Houtt. for elucidation of the structure and crystallinity

The y axis is intensity which is measured in nanometer (nm) and x axis is $2\Theta^{o}$. The maximum intensity of 3 peaks are $2\Theta = 25^{o}, 22^{o}, 20^{o}$ with wavelength of 125 nm, 115 nm and 112 nm respectively. The other peaks are broad and joined and cannot be studied appropriately. The peaks mentioned are arrow and thus it acne be stated that the sample in

focus gas crystalline structure. Crystallization is the process of crystal formation via crystal growth which is useful in determining the purity and quality of the sample.

4.6 Elemental Analysis

Table 4.6.1 - Elemental composition (Macro and Trace elements) of *Myristica fragrans* Houtt.

Data are given as means + SEM (n = 3).

Abbreviation	Element	Amount (mg/kg)	Category
Na	Sodium	16.16	Macro
Mg	Magnesium	838.38	Macro
P	Potassium	970.15	Macro
K	Phosphorous	2037.66	Macro
Ca	Calcium	114.18	Macro
Cr	Chromium	1.12	Trace
Mn	Manganese	19.88	Trace
Fe	Iron	19.72	Trace
Cu	Copper	6.67	Trace
Zn	Zinc	6.82	Trace
Se	Selenium	0.02	Trace
Mo	Molybdenum	0.06	Trace
Co	Cobalt	0.006	Trace

Table 4.6.2 - Elemental composition (Ultra Trace elements) of *Myristica fragrans* Houtt.

Data are given as means + SEM (n = 3).

Abbreviation	Element	Amount (µg/kg)	Category
Ni	Nickel	974.16	Ultra Trace
B	Boron	4311.05	Ultra Trace
Ti	Titanium	735.02	Ultra Trace
V	Vanadium	297.73	Ultra trace
Ge	Germanium	14.913	Ultra trace
Li	Lithium	83.35	Ultra Trace
Cd	Cadmium	2.13	Ultra Trace
Pb	Lead	422.63	Ultra Trace
Al	Aluminum	5304.39	Ultra Trace
Cs	Caesium	11.18	Ultra Trace
Ag	Silver	1144.07	Ultra Trace
Au	Gold	37.28	Ultra Trace
Ga	Gallium	3.46	Ultra Trace
Rh	Rhodium	0.26	Ultra Trace
Pd	Palladium	10.65	Ultra Trace
Te	Tellurium	5.85	Ultra Trace
Ba	Barium	1822.37	Ultra Trace
Pb	Lead	422.63	Ultra Trace

34 elements were analyzed in which 30 elements showed significant amount in the powdered sample such as Au, Ag, Mg, Cr, Fe, Mn etc. whereas Be, Pt, As and Ru showed no presence in the sample.

Total amount of five macro elements and eight micro/trace elements are 3976.53 mg/kg and 54.29 mg/kg respectively whereas seventeen ultra trace elements with total amount of 15180.50µg/kg is present in jaiphal.

Various elements are grouped according to the needs of the human body according to L. Prashanth et. al 2015 –

1. Organic elements - Carbon (c), hydrogen (H), nitrogen (N), oxygen (O)
2. Quantity elements or macro elements – Sodium (Na), magnesium (Mg), phosphorous (P), potassium (K), chloride (Cl), sulphur (S)
3. Essential trace elements - Iron (Fe), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), molybdenum (Mo), selenium (Se), Iodine (I)
4. Ultra trace elements with not much role still identified – Lithium (Li), vanadium (V), chromium (Cr), boron (B), fluorine (F), silicon (Si), Arsenic (As).

Majority of the categorized elements are present in the sample in focus that makes it a good candidate to be used in pharmaceutical industries for various therapeutic roles such as high amount of phosphorous (2037.66 mg/kg) helps in maintaining blood sugar level, normal cell growth and its repairing; potassium (970.15 mg/kg) and sodium (16.1 mg/kg) plays a crucial role in maintaining charge gradient across the cell walls; calcium (114.18 mg/kg) plays an important role in bone, blood vessels etc. It is a crucial element for normal cardiac muscle formation and blood clotting [27].

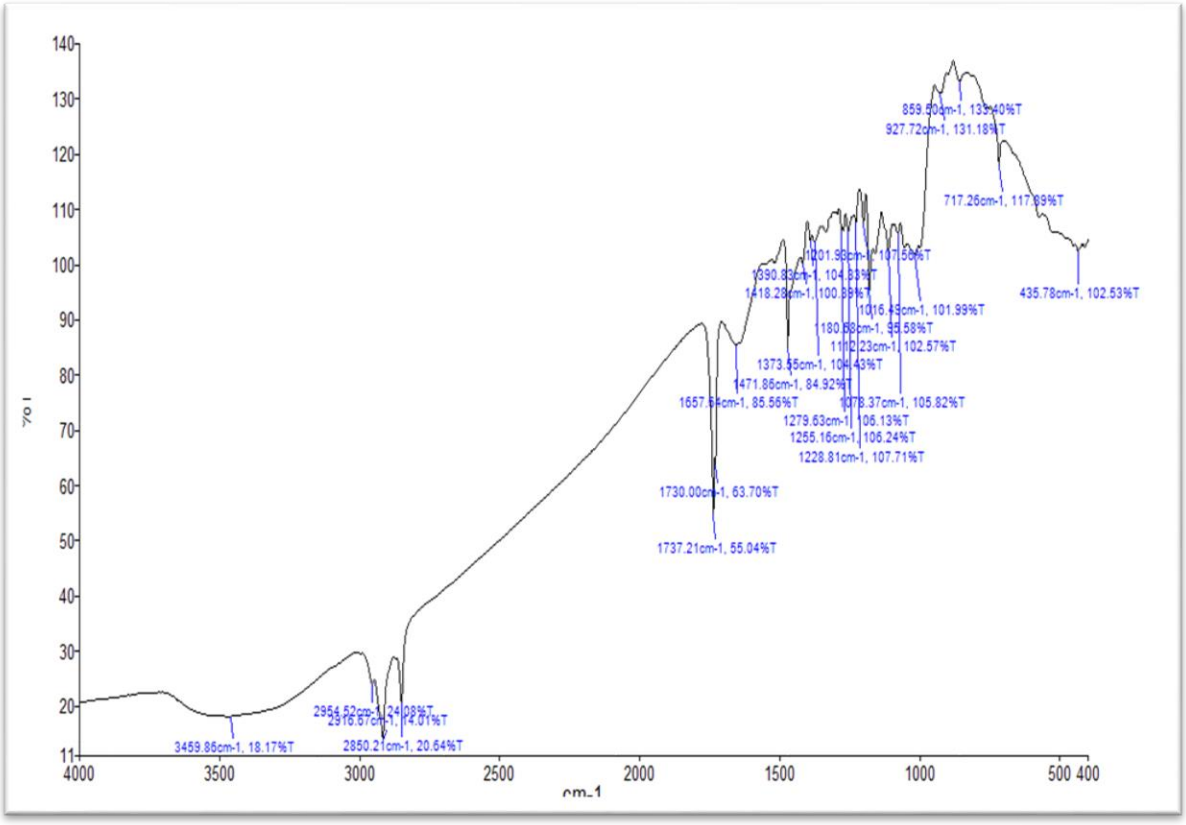
Various trace or micro elements manganese which is in higher percentage in the sample of study (19.88 mg/kg) which is important for normal working or functioning of nucleic acids, oxidative phosphorylation and cholesterol metabolism; iron (19.72 mg/kg) is required for haemogloin formation and oxidation of carbohydrates, fats etc. and zinc (6.82 mg/kg) is an amphoteric omnipotent element in nature which is invovd in nucleic acid and protein

synthesis an plays an important role in various systems of the body such as immunological, neurological, reproductive etc. All the trace and macro elements play a crucial role in regulation of the human metabolism.

Ultra trace elements are categorized according to their biological role such as –

1. Essential trace elements – Boron (B), cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn)
2. Probable essential trace elements – Chromium (Cr), fluorine (F), nickel (Ni), selenium (Se), vanadium (V)
3. Physically promotive trace elements – Bromine (Br), lithium (Li), silicon (Si), tin, titanium (Ti)

4.7 FTIR Analysis



Graph 4.7 – FTIR analysis of *Myristica fragrans* Houtt.

Range of the spectrum was from 1016-3460 cm^{-1} which confirms the presence of various functional groups such as primary amine with N-H bonding at 3459.86 cm^{-1} and stretching vibrations, C-N stretching of aromatic amines at 1291.93 cm^{-1}, primary alcohol with C-O stretch bond at 1078.37 cm^{-1}, S=O sulfate stretching bond group at 1016.49 cm^{-1}, C=O aldehyde group at 1737.21 cm^{-1} etc. Some peaks are also present in the range of 400- 930 cm^{-1} which could be benzene derivatives. Finger print regions have shorter wavelength that ranges from 400 – 1000 cm^{-1} .

4.8 Crude Lipid Estimation

Crude lipid content is 16.07 % ± 0.12 (Values are expressed by mean ± SD of triplicates). Lipid extracted from here is processed by transesterification for the estimation of fatty acids.

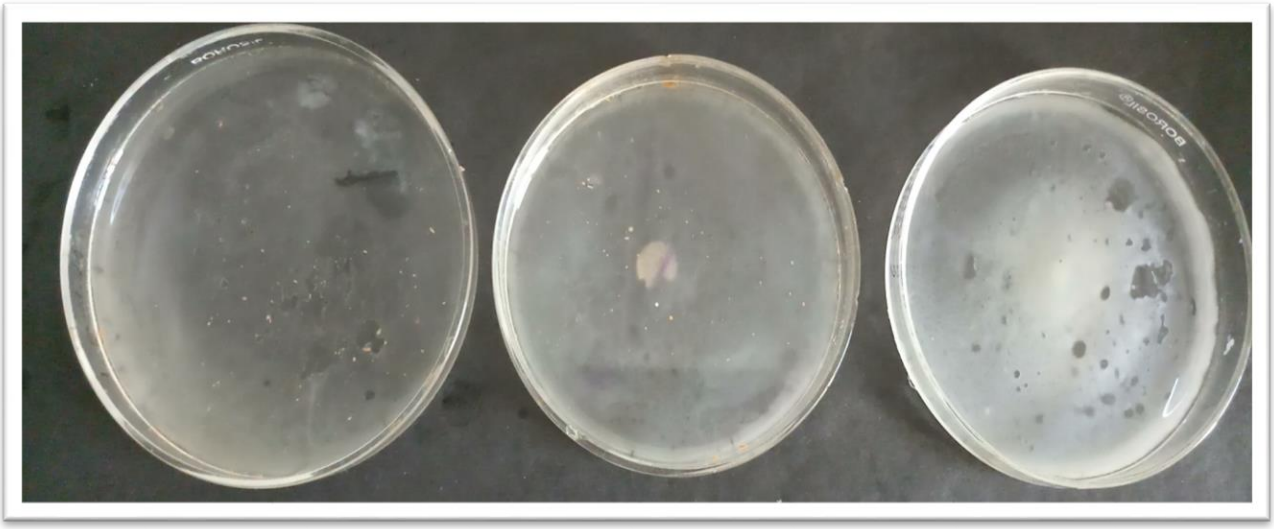


Figure 4.8.1: Chloroform: methanol extract of *Myristica fragrans* Houtt. before evaporation

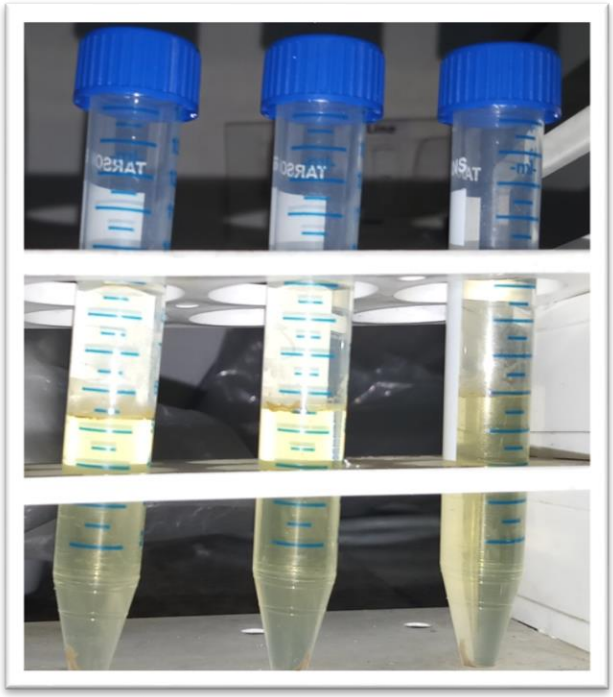


Figure 4.8.2: Extract of chloroform: methanol of *Myristica fragrans* Houtt.

Figure 4.8.3: After evaporation, crude lipid obtained from chloroform: methanol extract of *Myristica fragrans* Houtt.

4.9 Fatty Acid Profile

Table 4.9.1 - Fatty acid profile of *Myristica fragrans* Houtt. (mg/100g).

Data are given as means + SEM (n = 3).

Fatty Acid	Fatty Acid	Mean	SE
Saturated Fatty Acid			
C6:0	Caproic Acid	404.548	5.084
C8:0	Caprlyic Acid	33.782	0.823
C10:0	Capric Acid	214.084	4.626
C12:0	Lauric Acid	80.442	0.753
C14:0	Myrisic Acid	4733.434	42.779
C16:0	Palmitic Acid	125.037	10.665
C18:0	Stearic Acid	0.965	0.153
Poly Unsaturated Fatty Acid			
C18:2 n-6	Linoleic Acid	77.599	1.460
C18:3 n-3	Rumelenic Acid	22.275	0.876
C20:2 n-6	Eicosadienoic Acid	56.273	2.514
Mono Unsaturated Fatty Acid			
C18:1 n-9	Oleic Acid	498.111	7.966
C16:1 n-9	Palmitoleic acid	249.259	0.578
Total			
∑ SFA	Saturated Fatty Acid	5592.291	41.599
∑ MUFA	Mono Unsaturated Fatty Acid	747.370	8.544
∑ n-6 PUFA	Poly Unsaturated Fatty Acid	133.873	3.974
∑ n-3 PUFA	Poly Unsaturated Fatty Acid	22.275	0.876

Jaiphal is highly rich in myristic acid with chemical formula $CH_3(CH_2)_{12}COOH$ or C14:0 (4733.434 mg/100g) which is a saturated fatty acid that has been isolated in 1841 by Lyon Playfair. This acid has a significantly higher hydrophobicity to be incorporated into the fatty acyl core of the phospholipid bilayer of the plasma membrane of the eukaryotic cell and in this way, it acts as a lipid anchor in biomembranes. Reduction of this acid yields myristyl aldehyde and myristyl alcohol.

Some amounts of PUFA and MUFA make it a source of a healthy diet. Various other fatty acids are present in the sample that marks the ratio of saturated and unsaturated fatty acid. Hexanoic acid or Caproic acid and caprylic acid is used in perfume industry and as an antimicrobial pesticide.

CHAPTER 5 – CONCLUSION

Myristica fragrans Houtt. or jaiphal is used since ages to treat various diseases for example cold and cough, diabetes, memory loss etc. and thus before making it a commercialized way of treatment, it is necessary to investigate the bioactive and biochemical compounds present in it that makes it more nutritious. Therefore, through various test is now evident that presence of high amount of various minerals comprising of five macro that comprises of sodium, magnesium, calcium, potassium and phosphorous and some important trace and ultra trace elements makes it a good lead candidate to be used in pharmaceutical industries for various physiological roles. Ultra trace elements are basically termed as promotive trace elements. Fatty acids profile validates the use of myristic acid which is a psychoactive molecule that helps in relaxation of blocked vessels and thus it is used in curing of stomach aches, peptic ulcer, asthma etc. Sole and majority production of myristic acid is jaiphal, and presence of various essential and non essential amino acid makes it a good lead for treating various diseases and having smooth metabolism and free amino acids accelerates the function of both the categories of amino acids. Presence of various functional groups provides a good lead for phytochemical screening basis and vitamin estimation. XRD study gave crystalline structure of jaipahl which when used in conjugation with any drugs can help in smooth drug delivery.

Thus, *Myristica fragrans* Houtt. possess all the medicinal properties that are beneficial for curing modern day problem with fewer or negligible side effects. Thus it should be a dose dependent strategy.

CHAPTER 6 – REFERNCES

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PUBLICATION

Jaiphal *Myristica fragrans* (Houtt.) -A promising seed for pharmaceutical industries

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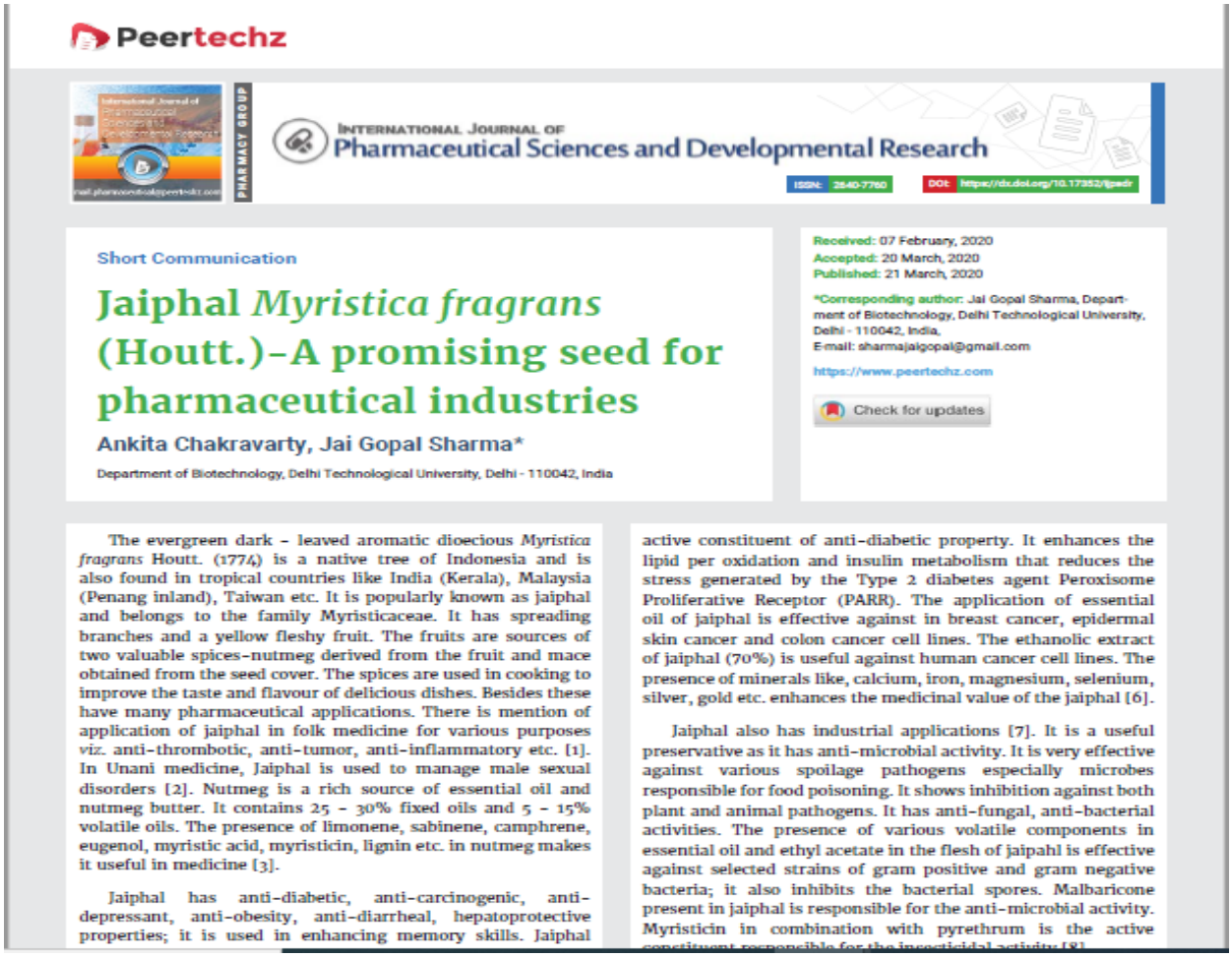

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Short Communication

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The evergreen dark – leaved aromatic dioecious *Myristica fragrans* Houtt. (1774) is a native tree of Indonesia and is also found in tropical countries like India (Kerala), Malaysia (Penang inland), Taiwan etc. It is popularly known as jaiphal and belongs to the family Myristicaceae. It has spreading branches and a yellow fleshy fruit. The fruits are sources of two valuable spices–nutmeg derived from the fruit and mace obtained from the seed cover. The spices are used in cooking to improve the taste and flavour of delicious dishes. Besides these have many pharmaceutical applications. There is mention of application of jaiphal in folk medicine for various purposes *viz.* anti–thrombotic, anti–tumor, anti–inflammatory etc. [1]. In Unani medicine, Jaiphal is used to manage male sexual disorders [2]. Nutmeg is a rich source of essential oil and nutmeg butter. It contains 25 – 30% fixed oils and 5 – 15% volatile oils. The presence of limonene, sabinene, camphrene, eugenol, myristic acid, myristicin, lignin etc. in nutmeg makes it useful in medicine [3].

Jaiphal has anti–diabetic, anti–carcinogenic, anti–depressant, anti–obesity, anti–diarrheal, hepatoprotective properties; it is used in enhancing memory skills. Jaiphal active constituent of anti–diabetic property. It enhances the lipid per oxidation and insulin metabolism that reduces the stress generated by the Type 2 diabetes agent Peroxisome Proliferative Receptor (PARR). The application of essential oil of jaiphal is effective against in breast cancer, epidermal skin cancer and colon cancer cell lines. The ethanolic extract of jaiphal (70%) is useful against human cancer cell lines. The presence of minerals like, calcium, iron, magnesium, selenium, silver, gold etc. enhances the medicinal value of the jaiphal [6].

Jaiphal also has industrial applications [7]. It is a useful preservative as it has anti–microbial activity. It is very effective against various spoilage pathogens especially microbes responsible for food poisoning. It shows inhibition against both plant and animal pathogens. It has anti–fungal, anti–bacterial activities. The presence of various volatile components in essential oil and ethyl acetate in the flesh of jaipahl is effective against selected strains of gram positive and gram negative bacteria; it also inhibits the bacterial spores. Malbaricone present in jaiphal is responsible for the anti–microbial activity. Myristicin in combination with pyrethrum is the active

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The evergreen dark - leaved aromatic dioecious ***Myristica fragrans*** **Houtt.** (1774) is a native tree of Indonesia and is also found in tropical countries like India (Kerala), Malaysia (Penang inland), Taiwan etc. It is popularly known as jaiphal and belongs to the family Myristicaceae. It has spreading branches and a yellow fleshy fruit. The fruits are sources of two valuable spices - nutmeg derived from the fruit and mace obtained from the seed cover. The spices are used in cooking to improve the taste and flavour of delicious dishes. Besides these have many pharmaceutical applications. There is mention of application of jaiphal in folk medicine for various purposes *viz.* anti-thrombotic, anti-tumor, anti-inflammatory etc. [1]. In Unani medicine, Jaiphal is used to manage male sexual disorders [2]. Nutmeg is a rich source of essential oil and nutmeg butter. It contains 25 - 30% fixed oils and 5 - 15% volatile oils. The presence of limonene, sabinene, camphrene, eugenol, myristic acid, myristicin, lignin etc. in nutmeg makes it useful in medicine [3].

Jaiphal has anti-diabetic, anti-carcinogenic, anti-depressant, anti-obesity, anti-diarrheal, hepatoprotective properties; it is used in enhancing memory skills. Jaiphal has also anti-inflammatory and anti-analgesic effects. Essential oil derived from both mace and nutmeg is used in the remedy of sprains, muscle pulls etc. The limonene is anti-carcinogenic, anti-oxidant; sabinene is anti-inflammatory and anti-microbial, quercitin is anti-inflammatory. Myristicin present in jaiphal has anti-oxidant property. Aril parts of jaiphal possess strong antioxidant activity as it has the capacity to inhibit lipid peroxidation and superoxide radical scavenging activity. Myristicin plays significant role in memory enhancing activity of jaiphal [4, 5].

Jaiphal is effective to cure peptic ulcer as it reduces the total acidity of gastric secretions without any side effect [4]. Macelignan, the lignin obtained from nutmeg is the

natural, active constituent of anti-diabetic property. It enhances the lipid per oxidation and insulin metabolism that reduces the stress generated by the Type 2 diabetes agent peroxisome proliferative receptor (PARR). The application of essential oil of jaiphal is effective against in breast cancer, epidermal skin cancer and colon cancer cell lines. The ethanolic extract of jaiphal (70%) is useful against human cancer cell lines. The presence of minerals like, calcium, iron, magnesium, selenium, silver, gold etc. enhances the medicinal value of the jaiphal [6].

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