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Phytochemical analysis and TLC Profile of *Myristica fragrans* Houtt.

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Abstract

Myristica fragrans Houtt. of Myristicaceae is used in all parts of the world for both as food flavours and for medicinal uses. The present study primarily aims to carry out preliminary phytochemical screenings ,so as to detect the major class of compounds present in the seeds of *Myristica fragrans* Houtt. and to perform TLC profiling .The extracts were prepared by using hydromethanolic, aqueous and methanol solvents. The yield of extract was calculated for all the three solvents and they were studied for qualitative analysis of phytochemical compounds. Qualitative analysis of the seed extracts confirmed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, phenols, anthraquinones, cardiac glycosides, and triterpenoids. The methanolics extract of *M. fragrans* showed highest amount of phytochemicals when compared with other solvent extracts. Thin layer chromatographic studies of the *M. fragrans* seed extracts constituted different colored phytochemical compounds with different Rf value compound. The present study provides evidence that solvent extract of *M. indica* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

Nutmeg is a tropical fruit native to Banda Island, Indonesia but also grows in India and in Asian countries[19]. Pulp, mace and seed are parts of nutmeg which have been widely used as traditional Ayurvedic, Chinese and Thai medicine[18]. It is also used as spices in India. It has many bioactive compounds which are humanly beneficial. Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs[9]. *Myristica fragrans* Houtt. of Myristicaceae yields the nut-mug and mace which are credited with high flavors and fragrance. They are used in all parts of the world for both as food flavours and for medicinal uses. The plant produces odoriferous secondary metabolites in their fruits[12]. The medicinal properties of plant

species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. Over the past few years, medicinal plants have regained a wide recognition due to an escalating faith in herbal medicine. In view of its lesser side effects compared to allopathic medicine in addition, the necessity of meeting the requirements of medicine for an increasing human population[13]. Medically, nutmeg has strong antibacterial properties. It is effective in killing a number of cavity-causing bacteria in the mouth. Nutmeg oil is used to treat toothaches. Drops of essential oil are put on cotton swab and applied to the gums around an aching tooth, sometimes also used to control bad breath. Drops of nutmeg oil can also be mixed with honey to treat nausea, gastroenteritis, chronic, diarrhea and indigestion[6].

Extraction is the separation of medicinally active portions of plant tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use.

The chemical constituents that are of medicinal importance are mainly the secondary metabolites, and the examination of the chemical constituents of the plant can only reveal those compounds that have accumulated to some extent at a specific organ of a given plant. The presence or absence of such compounds depends largely on the extent of accumulation, the amount of plant material used and the analytical method employed[3].

The major aim of this study seeks to investigate the presence of compounds responsible for the medicinal activities in the seed of the plant *M. Fragrance* by carrying out the phytochemical screening and TLC profiling

Sample collection and preparation :

The fresh, dried seeds of *Myristica fragrans* Houtt were purchased from the local market of Bhopal, India. The *Myristica fragrans* seeds were weighed, ground into small pieces, and kept away from heat, moisture, and sunlight.

Extraction :

The dried samples of *Myristica fragrans* were extracted using a Soxhlet extraction method with methanol, ethanol and water: methanol (50:50) solvents. The extraction processes were carried out for 3 days for each of the samples to obtain crude extracts which were concentrated by using water bath at 70°C then the resulting pellet was finally collected. The crude extracts were then stored at room temperature.

Phytochemical analysis :

Preliminary phytochemical analysis was done for the detection of the presence of different Phyto constituents in the plant extracts[14]. The tests for carbohydrates, alkaloids, flavanoids, tannin, saponins and phenols were carried out. Phytochemical analysis of plant extract was done to detect the bioactive compounds using qualitative test[16].

Test for Qualitative Estimation of Bioactive Compounds of *M. fragrans.*

1) **Test for Alkaloids:** Methanolic extract was warmed with 2% H_2SO_4 for two minutes. It is filtered and few drops of reagents were added and indicated the presence of alkaloids.
(a) Dragendroff's reagent-A red precipitation indicates the positive.
(b) Mayer's reagent-A creamy- white colored precipitation positive.
(c) Wagner's reagent-A reddish-brown precipitation positive.
(d) Picric Acid (1%)-A yellow precipitation positive.

2) **Test for Flavonoids:** A small quantity of the extracts is heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture is filtered differently and the filtrates are used for the following test.
(a) Ammonium Test- The filtrate was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was observed at ammonia layer. This indicates the presence of the flavonoid.
(b) Aluminum Chloride Test- The filtrates were shaken with 1 ml of 1% aluminum chloride solution and observed for light yellow color. It indicated the presence of flavonoid and diluted NaOH and HCl was added. A yellow solution that turns colorless indicated positive.

3) **Test for Terpenoids:**
(a) Salkowski Test- The extract was mixed with 2ml of chloroform and concentrate H_2SO_4 (3ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of the presence of terprnoids.

4) **Test for Tannins/ Phenol**: A small quantity of the extract is boiled with 5 ml of 45% solution ethanol for 5 minutes. Each of the mixture is cooled and filtered. The different filtrates were used to the following test:
(a) Lead Sub Acetate Test- 1ml of the different filtrate was added with three drops of lead sub acetate solution. A cream gelatinous precipitation indicates positive test for Tannins.
(b) Ferric Chloride Test- 1ml each of filtrate is diluted with distilled water and added with two drops of ferric chloride. A transient greenish to black color indicates the presence of Tannins.

5) **Test for Steroids:** Acetic anhydride (2ml) was added to 2ml extract of H_2SO_4. The colour changed from violet to blue or green in some samples indicated the presence of steroids.

6) **Test for Saponins:**
(a) Frothing Test- A small quantity of different extract was diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed on standing for stable brake.

7) **Test for Carbohydrates**: The extract was shaken vigorously with water and then filtered. To the aqueous filtrate was added few drops of Molisch's reagents. Followed by vigorous shaking again, concentrated H_2SO_4 1ml was carefully added to form a layer below the aqueous solution. A brown ring at the interface indicated the positive.

Thin Layer Chromatography :

TLC plates were prepared by using silica gel G mixed with distilled water to make slurry. After preparing the slurry is poured into the glass slides, then allowed to air dry for half an hour and fixed by drying in oven at 110°C for half an hour. The methanol extract of *Myristica fragrans* was loaded gradually with capillary tube over the TLC plates and air dried. The plates were developed with petroleum ether: Ethyl acetate: n-Hexane: Methanol (20:5:5:5), hydromethanolic extract of *Myristica fragrans* was loaded on TLC plate and developed with Ethyl acetate: Methanol (4:6) and the aqueous extract of *Myristica fragrans* was loaded on TLC plates and developed with Acetic acid: Ethyl acetate: Methanol: n-Hexane (10:4:3:3). All solvents showed different Rf values of plant extracts. We used UV-light to observe the chromatograms.

The preliminary phytochemical screening of the *Myristica fragrans* (H.) seed extract using different solvents was analyzed. Data presented in the (Table-1) showed results of methanol, hydromethanolic and aqueous solvent seed extracts. Phytochemical screening of extracts revealed the presence of alkaloids, flavonoids, tannins and terpens in all extract. The methanol and hydromethanolic solvent of *Myristica fragrans* seed extract showed the presence of alkaloids. Alkaloids, comprising a large group of nitrogenous compounds are widely used as cancer chemo-therapeutic agents essential oil showed the presence of alkaloids, steroids and glycosides[10]. Joseph J and George M, (2014) also reported the similar observation. In their findings alkaloids, saponins, phytosterols, tannins, flavonoids and proteins were identified as the major compounds[11]. The aqueous seed extract of *Myristica fragrans* was found to have steroids, tannins, phenols and flavonoids.

Table 1: Phytochemical analysis of *Myristica fragrans* Houtt.

Phytochemical constituents	Methanolic extract	Hydromet-hanolic extract	Aqueous extract
Alkaloids	+	+	-
Saponins	+	+	-
Tannins	+	+	+
Flavonoids	+	+	+
Phytosterols	-	-	-
Terpenes	+	+	+
Phenols	+	+	+
Carbohydrates	+	+	+

+ = present, - = absent.

The phytochemical is a natural bioactive compound found in plants such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as a defense system against disease or more accurately, to protect against disease[12]. Many researchers evaluated the phytochemical, antioxidant, antimicrobial and antinutrients properties of *M. fragrans* [1,5,11]. Hima *et al*.,[4], has reported the extraction and screening of trimyristin in the seeds of *Myristica fragrans* and in poly herbal formulations by spectroscopic and chromatographic techniques[4].

Several thin-layer chromatographic systems designed for the analysis of bioactive compounds. for quality control of herbal products, thin-layer chromatography (TLC) is the most versatile technique for the identification of botanical raw materials[8]. TLC of all sequential extracts of *M. fragrans* obtained by sequential extraction methods. Results was carried out to confirm its nature by analyzing TLC chromatograms and to isolate active ingredients. TLC of methanolic extract of *M. fragrans* revealed the presence of 7 spots having Rf values of 0.18, 0.24, 0.28, 0.46, 0.56, 0.66 and 0.76 respectively when a solvent phase of Petroleum ether: Ethyl acetate: Hexane: methanol (20:5:5:5) solvent system was used. Whereas in hydro alcoholic and aqueous extract 5 and 4 spots were obtained having different Rf value (Table-2) and developed chromatogram shown in (fig. 1). Various phytochemicals give different Rf values in different solvent system[7]. This variation in Rf values of the phytochemicals provides a very important clue in understanding the polarity and also helps in selection of appropriate solvent system for further separation of pure compounds by column chromatography[2].

Table-2. Thin Layer Chromatography of *Myristica fragrans* Houtt. extracts and their Rf values.

S.No.	Methanolic extract	Hydroalcoholic extract	Aqueous extract
	Rf value	Rf value	Rf value
1.	0.18	0.133	0.061
2.	0.24	0.216	0.122
3.	0.28	0.500	0.163
4.	0.46	0.760	0.61
5.	0.56	0.800	
6.	0.66		
7.	0.76		

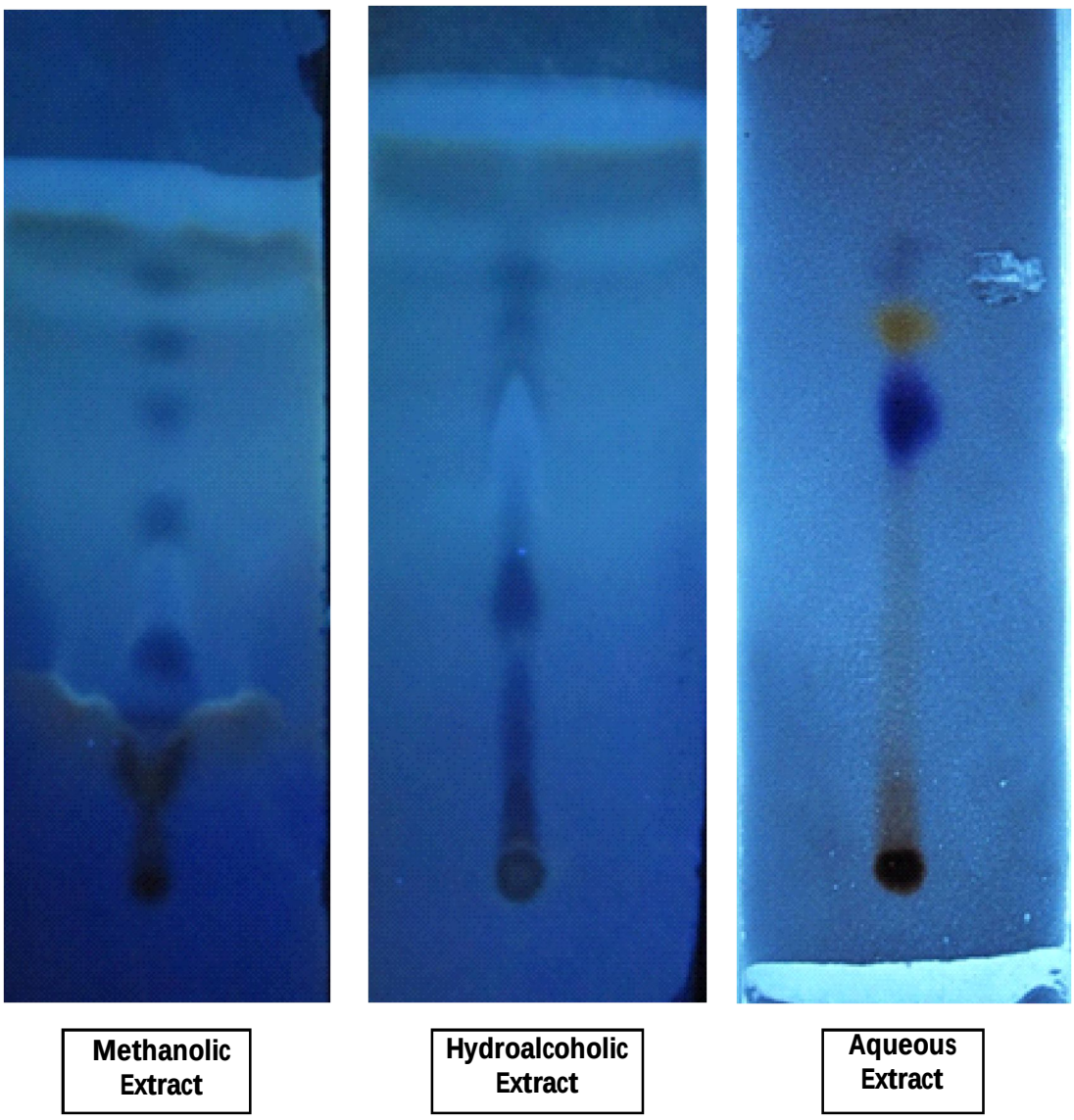

Fig. 1 TLC chromatogram of different extracts of *M. fragrans*

In the present study, preliminary phytochemical analysis and TLC shows that the methanolic extract of the seed contains more constituents. The presence of phyto constituents makes this plant useful for treating different ailments and has a potential of providing useful drugs for human use. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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