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Preliminary phytochemical screening, antioxidant and antimicrobial activity of *Myristica fragrans* family myristicaceae

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Abstract
The present investigation were performed the phytochemicals and *in-vitro* antioxidant, antimicrobial and GC-MS activity of *Myristica fragrans*. In preliminary phytochemicals screening results confirmed the presence of alkaloids, phenols, saponin, tannins and steroids. The *in-vitro* antioxidant activity of nutmeg was investigated by DPPH and FRAP method. In both method, plant extract possess high antioxidant activity when compared with standard ascorbic acid due to presence of high content of various phytochemicals. Antimicrobial activity of nutmeg was investigated by DPPH and FRAP method. In both method, plant extract possess high antioxidant activity when compared with standard ascorbic acid due to presence of high content of various phytochemicals. The research showed that nutmeg seed extracts have high antioxidant, antimicrobial activities due to presence of their bioactive phytoconstituents.

Key words: Antioxidant activity, antimicrobial activity, nutmeg, phytochemicals screening

Introduction
From thousands of years, we studied that plants have always played an important role in maintaining human health and also improve the quality of human life. *Myristica fragrans* Family Myristicaceae known as Nutmeg. The synonyms of nutmeg is *Myristica aromatica*, *Myristica moschata* etc. *M. fragrans* is the spice which is large tropical evergreen plant (Okiki *et al.*, 2023) [16] that thrives well under tropical climates. It is the main source of three commercial goods i.e., nutmeg, mace (fleshy aril), and essential oil. These all are broadly used for spices, flavoring purposes and found valuable health effects (Weerakoon *et al.*, 2021) [11]. Nutmeg is dried kernel of broadly ovoid seed of *Myristica fragrans*, is a tropical evergreen tree disturbed widely in America, Africa, and Asia continents (Saputri et. al., 2024) [13]. Nutmeg is widely used in alternative medicine as it has been reported to have many traditional activity i.e., aphrodisiac (Tajuddin *et al.*, 2003) [5], memory enhancer, antidiarrheal (Grover *et al.*, 2022) [3], anti-inflammatory and anti-cancer activities (Olajide *et al.*, 1999) [4] etc. Nutmeg butter is fatty material obtained from seeds and mainly used as freshner in manufacturing of perfumes, tobacco and toothpaste etc. It has been used for the treatment of rheumatism and it also improved digestion (Shinohara *et al.*, 1999) [12]. Plants served humans well as valuable in medicines (Veerendrakumar *et al.*, 2021) [10]. Many studies have identified nutmeg is a potential source of antioxidants and their bioactive compounds found in its mace, kernel, and pericarp parts (Perera *et al.*, 2025) [9]. A fully-grown tree height were found about 50-60 feet. The first harvest of nutmeg trees takes place 7-9 years after planting and total life span of trees about 20 years. Traditionally this plant is used as a medicine for stomachaches, headaches, diarrhea, nausea, vomiting, fever, stimulating appetite, as well as treating flatulence (Kuete, 2017; Saputri et. al., 2024) [13, 14].

Materials and Methods
Chemicals: Chloroform, Methanol, Ethanol, acetone, petroleum ether, Mayer's reagent, Dragendorff's reagent, Magnesium powder, $FeCl_3$, anhydrous acetic acid, HCl, DPPH (2, 2-dyphenyl-1- picrylhydrazyl) (Sigma) and Ascorbic acid. The solvents and chemicals used were of analytical grade.

Morphological characterization of plant
Morphology of fresh and dried *Myristica fragrans* plant were studied. The foreign organic matter and moisture content of pulverized nutmeg samples were observed accordance the

standard methods of the Association of Official Analytical Chemists (AOAC) procedures (AOAC 2015) [17].

Extraction from plant material
The plant *Myristica fragrans* Family Myristicaceae were collected from the local area Rohtak Haryana India. The Collected plant material were shade dried. The seeds were then hulled manually and stored at room temperature in an air tight container. Thoroughly washed nutmegs were dried in hot air oven at 35-40 °C till air dried, then seeds were grounded to a fine powder which was kept in an air-tight container until further use. For the aqueous extraction, 50 g of fine powder of sample was extracted with 500 mL cold water. 50 grams of powdered sample was kept into soxhlet apparatus and successive soxhletion were performed for 8 hours using organic solvent of petroleum ether, acetone, and ethanol. Then solution were filtered through Whatman filter paper and the organic solvent were separated by rotary dryer. The obtained extract/s were dried by lyophilization and then calculated the percentage yield. The obtained extracts were kept in dessicator at room temperature for further use.

Phytochemical Analysis
The phytoconstituents in the seeds were extracted using different solvents, namely, petroleum ether, acetone, chloroform ethanol and water. Phytochemical screening of *Myristica fragrans* to identify the presence of flavonoids, tannins, phenols, terpenoids, saponins, glycosides, alkaloids and steroids were done using standard methods (Krishnamachari and Nithyalakshmi 2017) [6].

Test for alkaloids
1. **Mayer's reagent:** In the sample extract solution add Mayer's reagent (potassium mercuric iodide) and formation of a creamy white precipitate indicated presence of alkaloids
2. **Dragendorff's reagent:** In the sample extract solution add Dragendorff's reagent (potassium bismuth iodide) and formation of orange-red precipitate.
3. **Saponin test:** In sample extract, add 4 mL of hot water into a test tube. Cooled and shaken the above mixture. After 10 minutes, the solution was observed, and add one drop of 2 N HCl. Observation of foam of more than 1 cm indicated the presence of saponin in sample.
4. **Flavonoid test:** Dissolved sample extract in 4 mL of ethanol then added 0.5 grams of Magnesium powder and 2 mL of 2 N HCl. Left the mixture for 1 minute and then add ten drops of concentrated HCl. The colour change to bright red after being left for a few minutes indicates the presence of flavonoids.
5. **Tannin test:** Add 15 ml hot water in sample extract and boiled on a hot plate for several minutes. After boiling, cool the mixture and filtered. To the filtrate add three drops of $FeCl_3$. Observed the colour change to greenish purple which showed the presence of tannins.

Terpenoid and steroids
1. **Liebermann-Burchard** test: Dissolved 2 mL of chloroform in sample extract and add ten drops of anhydrous acetic acid and stirred well. In above mixture, slowly added two drops of concentrated sulfuric acid. A color change from violet to blue or green indicates the presence of steroids.
2. **Salkowski test:** In the sample extract add concentrated sulfuric acid. In lower layer produced a reddish-brown color indicated the presence of a steroidal aglycone part of a glycoside.

Antioxidant Activity
Qualitative and quantitative analysis was performed by using standard method AOAC. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is based on the ability of antioxidants to scavenge the stable free radical DPPH resulting in a color change from deep violet to yellow. This change can be quantitatively measured by a decrease in absorbance at 517 nm using a spectrophotometer (Porrawatkul and Pimsen 2014; Assa *et al.*, 2014; Orabi *et al.*, 2022) [7, 18, 20].

Procedure
Preparation of DPPH Solution
1. Dissolve DPPH in methanol to obtain a 0.1 mM solution (e.g., 3.9 mg DPPH in 100 mL methanol).
2. Protect the solution from light and use freshly prepared solution for the assay.

Assay protocol
1. Mix 3.9 mL of DPPH solution with 100 µL of test sample solution.
2. For control, mix 3.9 mL of DPPH solution with 100 µL of methanol (without sample).
3. For blank, use methanol only.
4. Incubate the reaction mixtures in the dark for 30 minutes at 37 °C temperature.
5. Measure the absorbance at 517 nm against the blank using a UV-Vis spectrophotometer.

$$\text{DPPH activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Where $A_{control}$ = Absorbance of control (DPPH + methanol); A_{test} = Absorbance of DPPH + sample

The experimental results were verified by comparing them to the antioxidant activity of ascorbic acid used. Ascorbic acid was used as a positive control.

Antibacterial Activity
The aqueous extract of *Myristica fragrans* plant was used for antibacterial activity against both *Pseudomonas aeruginosa,* and *Staphylococcus aureus* (Jahan *et al.* 2010; Gupta *et al.*, 2013) [8]. The Test protocol were performed at BioBridge: Research, Training and Services, Rohtak Haryana India 124001. Overnight grown culture of pure bacterial isolates (*Pseudomonas aeruginosa*, *Staphylococcus aureus*) inoculated in Luria Bertani broth (secondary culture), incubated at 37°C under shaking until turbidity matches to 0.5 MacFarland. Swab the culture back and forth on the Muller Hinton Agar plate and let the plate to dry for 10 min. Then, a hole with a diameter of 8 mm is punched aseptically with a sterile cork-borer and 80 µL of the desired concentration of the tested extracts were dissolved in DMO viz. 50 mg/mL, 25 mg/mL, 12.5 mg/mL and ampicillin (50 ug/mL) was introduced into the well. After that, agar plates were incubated under suitable conditions that is 37°C for 20 h. After incubation the plates were observed for the zone of inhibition (Ahani and Danesh 2021; Cui, *et al.*, 2021) [1, 2].

Results and Discussion
Taxonomical Classification
- **Kingdom:** Plantae
- **Order:** Magnoliales

- **Family:** Myristicaceae
- **Genus:** *Myristica*
- **Species:** *fragrans*

Morphology

The nutmeg fruit, is a drupe shape and about the size of an apricot. After ripen nutmeg splits up to reveal single centrally situated oval shaped hard kernel known as "nutmeg spice." The seed observed as crimson-red colored lacy or thread like arils known as mace, it is most important part of the plant in terms of its pharmacological activity. The nutmeg has a distinctive fragrance and a slightly sweet in taste. Nutmeg seed roughly egg-shaped and about 20 to 30 mm long and 15 to 18 mm wide, and weighing between 5 and 10 g dried. Sample photos are shown below.

Nutmeg seed (Inside)

Nutmeg seed

Nutmeg seed powder

Table 1: Other physicochemical parameters of *Myristica fragrans* plant seeds

S. No	Physicochemical parameters	Values± SEM
1	Foreign Organic Matter (FOM)	1.76+0.41
2	Moisture content	4.8±1.33
3	Bitterness value	Sweet (Not bitter)
4	Foaming Index	Less than 1
5	Total Ash Value	3.8±0.25

The Foreign Organic Matter (FOM) and Moisture content of selected sample of *Myristica fragrans* plant seeds were shown in above table 1.

Extraction from plant material

The percentage yield of powdered sample of organic solvent of petroleum ether, acetone, and ethanol were found 5.18±0.14, 1.37±0.05, 12.7±1.73. The aqueous extract percentage yield were found 10.65±2.2. The obtained extracts were kept in dessicator at room temperature for further use.

Phytochemical Screening

The results of preliminary phytochemical analysis of *Myristica fragrans* plant seed extracts are shown in Table 2.

Table 2: Preliminary phytochemical analysis of *Myristica fragrans* plant seeds extracts

S. No.	Constituents	Petroleum ether	Ethanol	Aqueous
1	Alkaloids	+	+	+
2	Saponin	+	+	+
3	Flavonoids	+	+	-
4	Tannins	+	+	+
5	Terpenoids	+	+	-
6	Steroids	+	-	-

The plant *Myristica fragrans* contained saponin, terpenoids, steroids, alkaloids, glycosides and Flavonoids. Ethanol extracts and aqueous solution of plant *Myristica fragrans* seeds showed the presence of high flavonoids, terpenoids and alkaloids concentration whereas concentrations of steroids were high only in petroleum ether solution.

Antioxidant Activity: The ability of the seed extract to scavenge DPPH ions (2, 2-dyphenyl-1-picrylhydrazyl) was measured to determine the antioxidant potential. Qualitative and quantitative analysis was performed by using standard methods. We know that a compound has very strong antioxidant activity if the IC50 value is <50 µg/mL, the general range of strong (50-100 µg/mL), medium (100-150 µg/mL) and weak (151-200 µg/mL) (Panjaitan and Yuliana, 2022; Handayani *et al*., 2014).

Table 3: Anti-oxidant activity of nutmeg seed extract using the DPPH method

S. No.	Concentration	Standard (Gallic Acid)	Nutmeg
1	50	50.44±0.6	35.21±0.04
2	100	93.5±0.2	43..61±0.2
3	200	104.3±1.3	51.07±0.5
4	300	122.7±2.1	59.53±0.3
5	400	144.5±1.6	65.21±1.1
	IC50	50±1.6	192±2.4

Data are mean ±SEM, n=3. One-way ANOVA test showed significant differences.

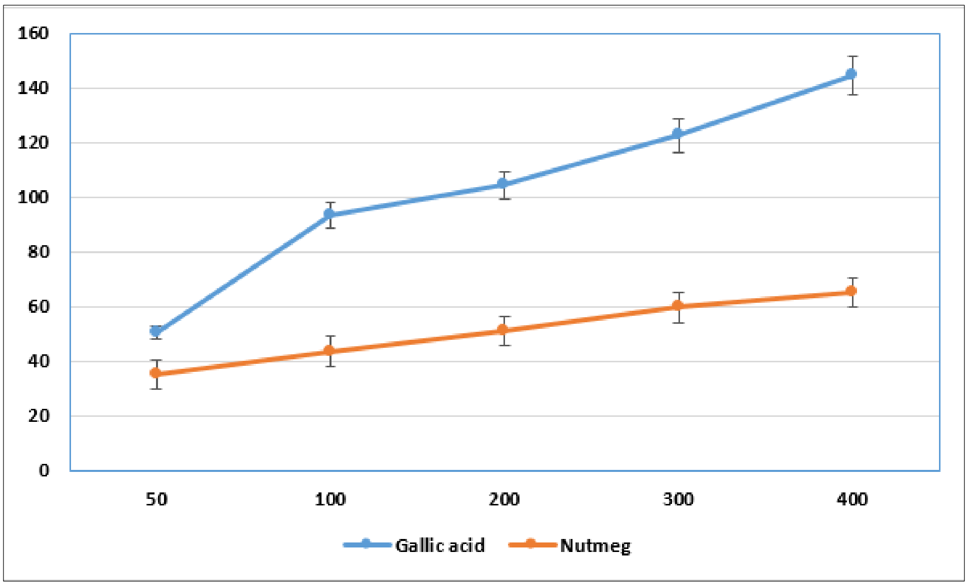

Fig 1: Anti-oxidant activity of nutmeg seed extract using the DPPH method

In addition, the ethanol extract of nutmeg leaves showed potential antioxidant activity with DPPH 65.21±1.1 mg of Ascorbic acid /g at 400 µg/mL and ascorbic acid showed IC50 at 50 µg/mL. These results also show that the ethanol extract of nutmeg leaves has great potential to be developed. In addition to ethanol extract from nutmeg leaves was also reported to have an IC50 value of 192 µg/mL using the DPPH method.

Antibacterial Activity

The aqueous extract of *Myristica fragrans* plant was used for Antibacterial activity against both *Pseudomonas aeruginosa,* and *Staphylococcus aureus*. The experiment showed significant results against different bacterial strains as shoen in table 4 and figure 2.

Table 4: Observation of *P. aeruginosa* and *S. aureus* with plant *Myristica fragrans* seeds including their zones of inhibition (diameter in mm)

S. No.	Samples	Zones of inhibition (diameter in mm)	
		P. aeruginosa	*S. aureus*
1	Ampicillin	22	28
2	Control (DMSO)	-	-
3	Nutmeg 12.5mg/ml 25mg/ml 50mg/ml	- 17 19	- 20 22

Fig 2: Observation of *P. aeruginosa* and *S. aureus* with plant *Myristica fragrans* seeds including their zones of inhibition (diameter in mm)

Nutmeg seeds Ethanol extract were tested for their antimicrobial activity. Preliminary screening against *P. aeruginosa* and S. aureus was performed. Ethanol extract at the concentration of 50 mg/ml showed good effect against all tested micro-organisms. From the above experiment carried out *Myristica fragrans* seeds ethanol extract in a concentration of 50 mg/ml was recorded active against strains of *P. aeruginosa* and *S. aureus.*

Conclusion

In present study the plant *Myristica fragrans* is found to presence of alkaloids, saponins, tannins, terpenoids and steroids which have therapeutic uses in treating various ailments. Traditionally this plants is also used in spices and medicinal values. The Antibacterial study was performed against two bacteria viz., *Staphylococcus aureus* and *Pseudomonas aeruginosa* indicated that had powerful activity against these microorganisms. More research work were also performed in standardization.

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