Original Research Article

Phytochemical analysis and effect of *Myristica fragrans* extract on *Escherichia coli* and *Staphylococcus aureus* strains isolated from Automated Teller Machine, Bikaner, Rajasthan

ABSTRACT

Aims: Plants produce compounds known as secondary plant products, as they are not required for the survival of plants. Earlier it was thought that these compounds were only the waste product of metabolism in plants but later it was found that they have important functions. These phytochemicals possess the ability to combat pathogenic bacteria and lethal diseases. These phytochemicals even don't have the same side effects as modern medications. So the objective was to analyze the phytochemicals and antimicrobial activity of a traditional herb *Myristica fragrans* (nutmeg).

Study design: Quantitative analysis of raw seed powder of *M. fragrans* was done for moisture content and solubility in water and 70% ethanol. Phytochemical analysis was conducted for both water and 70% ethanolic extract. Beside this the antimicrobial activity of both water and ethanolic extract was analyzed for gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria isolated from ATM of Bikaner, Rajasthan.

Place and Duration of Study: The study was conducted in the laboratory of MN College and Research Institute, Bikaner, Rajasthan (https://www.mncollegebkn.com/) for the period of 2 months (May and June).

Methodology: Moisture content and solubility of *M. fragrans* was determined using the procedure given by B.C. O'Kelly and Elena (2012). The extraction of powdered seeds of *M. fragrans* was done following the procedure of Ozaki*et al.* (1989)procedure with slight modification. Alkaloid content of both the extracts

was determined using the method given by Harbone(1973). Tannins and phenol contents were analyzed using the method given by Tomar and Shrivastava (2016). Beside this the saponin content was analyzed using two methods (TLC and foam method) given by Ginovyan*et al.* (2020) and Mendhekar*et al*.(2017) respectively. Further the antimicrobial activity of both the extracts for *S. aureus* and *E. coli* were determined using the method given by Ibrahim *et al.* (2013).

Results: Quantitative analysis of raw seed powder showed high moisture content and solubility in water and ethanol. The extraction process in 70% ethanol and water yielded extracts containing alkaloids, saponins, tannins, and phenols in the ethanolic extract, while the water extract contained alkaloids and saponins but lacked tannins and phenols. The ethanolic extract exhibited higher alkaloid content compared to the water extract, with percentages of 13.25% and 7.14% respectively. Notably, the ethanolic extract demonstrated significant effectiveness against both gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria, which were of human origin (isolated from ATM).

Conclusion: Our findings suggest that phytochemicals found in seeds of nutmeg have antimicrobial activity and can effectively combat pathogenic bacteria.

Key words: Antimicrobial activity, E. coli, Myristica fragrans, phytochemicals, S. aureus.

INTRODUCTION

The use of natural products to relieve illness dates back thousands of years (Christophersen *et al.,* 1991). Since herbs have healing potential, botanical medicine stands as one of the oldest practised professions by mankind (Van Wyk, and Gerike, 2000). The utilization of traditional medicinal plants can be traced back 4000 years to Mesopotamia, where seeds and plant extracts were employed for disease prevention and treatment, as documented in clay tablets (Dahham *et al.*, 2018). Approximately 25% of modern prescribed medications are derived from plant-based substances (Hamburger and Hostettmann., 1991). Some of these drugs are synthesized chemically or semi-synthetically, the rest are extracted and purified directly from plants (Farnsworth, 1990). Unlike current therapies which may have limited effectiveness and side effects (Al-Rawi *et al*., 2024), plants and seeds contain valuable compounds (secondary

metabolites and aromatic substances) for treating diseases, which can be used alone or with other medications to enhance efficacy and reduce adverse effects (Ibrahim *et al*., 2011).

Myristica fragrans (nutmeg) is a tropical, evergreen dioecious tree commonly found in Penang, Malaysia, India, Indonesia and Southeast of Asia (Orwa *et al*., 2009)**.** Nutmeg, the dried kernel of the seed of *Myristica fragrans*, is known for its pleasant aroma and warm taste, making it a popular spice in many countries. Hence it is widely used to flavor meats, vegetables, teas, baked goods and more (Al-Ravi *et al.,* 2011). The distinctive odour of nutmeg is attributed to the presence of essential oil-containing terpenes (α-pinene, p-cymene, sabinene, camphene, myrcene and γ-terpinene) terpene derivatives (terpinol, geraniol, and linalool) and phenylpropanes (myricetin, safrole, and elemicin) (Widelski *et al.,* 2017). Nutmeg is known for its various properties, including aphrodisiac, stomachic, carminative, tonic, nervous stimulant, aromatic, narcotic, astringent, hypolipidemic, antithrombotic, antifungal, antidysenteric and anti-inflammatory effects (Francis *et al*., 2014; Pashapoor *et al*., 2019). Recent studies indicate that nutmeg can potentially mitigate damage caused by gamma radiation and improve memory in mice. Furthermore, nutmeg has been found to possess anti-inflammatory properties and exhibit insulin-like biological activity. In ancient Indian medicine, nutmeg is believed to have cardio tonic properties, leading to nutmeg aqueous extract (NMAET) for treating hepatotoxicity linked to ISO (isoproterenol)-induced oxidative stress. Further studies showed the antihyperglycemic and hyperlipidemia effects of NMAET (Kareem *et al*., 2013).

In addition, *M. fragrans* exhibits significant antimicrobial activity against various bacterial pathogens (*Streptococcus mutans, S. sanguis, S. salivarius*, and *Lactococcus casei*) and antioxidant properties as it is rich in many bioactive compounds (Chung *et al*., 2006; Matulyte *et al*., 2020). Recently, the disc diffusion method was used to investigate the antimicrobial activity of nutmeg against Gram-positive bacteria (*Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus*) and Gram-negative bacteria (*Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa*) (Nikolic *et al*., 2021).

Besides this, it was also shown that terpene hydrocarbons (60-80% of the oil) are the main constituents of *M. fragrans* (pinene, sabinene, camphene, p-cymene, terpinene, phellandrene, myrcene, and limonene), followed by aromatic ethers (myristicin, elemicin, safrole, elemicin,and eugenol

derivatives) representing 15% to 20% of the oil, and oxygenated terpenes (geraniol, linalool, and terpineol), which make up approximately 5% to 15% of the *M.fragrans* structure (Muchtaridi *et al*., 2010; Rashidian *et al*., 2022). Additionally, phytochemical studies of *M. fragrans* seeds have also shown that it is rich in alkaloids, glycosides, flavonoids, tannins, saponins and steroids (Cruz *et al*., 2024). So the resourcefulness of nutmeg makes it a significant ingredient in a variety of fields, including culinary arts, healthcare, and pharmaceuticals (Ramteke *et al*., 2024).

Escherichia coli is a best-known extraintestinal and clinically important pathogen that can cause a wide variety of extraintestinal infections (Russo and Johnson, 2003) and gain resistance to several antibiotics (Nayak *et al*., 2024). *Staphylococcus aureus* are the main causative agent of food-borne infections and infections caused by contaminated materials. It is also known for its zoonotic potential (Pal *et al*., 2023) just like *E.coli* (Onwumere-Idolor *et al*., 2024). Recent studies revealed the prevalence of *S. aureus* strains resistant to β-lactam antibiotics in hospitals and communities (Foster and Geoghegan 2024).

The objective of this study was to investigate the phytochemical constituents and antimicrobial activity of *M. fragrans* against gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria isolated from ATM (Automated Teller Machine) of Bikaner, Rajasthan, India.

MATERIAL AND METHODS

Collection and Preparation of *M. fragrans* Seeds Extract

Dried seeds of nutmeg collected from a local market in Bikaner, Rajasthan were washed and the extract was prepared according to Ozaki.,*et al* procedure with slight modification (Ozaki *et al*., 1989). To extract bioactive compounds from the seeds of nutmeg, 14gms of the crude powder of seeds was refluxed with 1000 ml of water and 70% ethanol, respectively in the soxhlet apparatus for 8 hours.To eliminate residual solids the solution was filtered through a filter paper (Whatman Grade No.18) and evaporated to dryness under vacuum at 40^0 C.Subsequently, the dried extract was weighed and the stock solution was prepared by dissolving one gram of dried extract in 10 ml of 70% ethanol and water respectively.

Determination of Moisture Content and Solubility

The moisture content was determined using the procedure given by B.C. O'Kelly (Kelly *et al.,* 2016) using the following formula.

Moisture content (%) $= \frac{\text{W1}-\text{W2}}{\text{Weight of sample}}$ X 100

The solubility was determined using the method given by Elena, 2012.

Phytochemical Analysis

The nutmeg extracts were screened for the presence of alkaloids, tannins, phenols and saponins using the standard procedures.

Alkaloid determination

Five grams of the sample was weighed into a 250ml beaker followed by the addition of 200ml of 10% acetic acid in ethanol. The mixture was allowed to stand for 4minutes before being filtered. The resulting extract was then concentrated on a water bath to one-quarter of the original volume. Precipitate was formed by adding concentrated ammonium hydroxide dropwise to the extract. The solution was then allowed to settle, and the precipitate was collected, washed with dilute ammonium hydroxide, and filtered. The residue obtained was alkaloid, which was subsequently dried and weighed (Harbone, 1973).

$$\%Alkaloid=\frac{W3-W2}{W1}\times 100\%$$

Where

W_1= initial weight of sample

W_2= weight of extract

W_3=final weight of the residue

Test for Tannins

The presence of tannins was assessed using the procedure given by Tomar and Shrivastava, 2016. Specifically, 2 ml of leaf extract was combined with a few drops of 5% ferric chloride. The development of brown colour indicates the presence of tannins.

Test for Phenols

The presence of phenols was determined using the procedure given by Tomar and Shrivastava, 2016. Approximately 1 ml of extract was mixed with 2 ml of distilled water and a few drops of 10% of aqueous $FeCl_3$. The presence of phenol was indicated by the formation of blue or green colour.

Test for saponin

Extracts were spotted onto a layer of silica gel-activated glass plates. The plate's bottom was immersed in the solvent, and once the solvent reached the top, the plate was taken out and the solvent was evaporated. The solvent system utilized for separation was butanol: water: acetic acid (12:2:1). Following the application of a visualisation agent (iodine vapour),brown spots of saponin was identified in the chromatogram upon examination under UV light (Ginovyan *et al.,* 2020).

Furthermore, tests for saponins were conducted using the **Foam test method.** Approximately 1 ml of the extract was diluted with 20 ml of distilled water and vigorously shaken in a test tube. The presence of foam formation in the upper part of the test tube indicates the presence of saponins (Mendhekar *et al*., 2017).

Antimicrobial Activity of M. fragrans

The agar well diffusion method was used for the antimicrobial activity of nutmeg extract using the method given by Ibrahim et al (Ibrahim *et al.,* 2013) using Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria. The microbes used in this study were previously isolated in the Department of Microbiology, MN College and Research Institute, Bikaner. The bacteria were grown in Muller Hilton agar for 18 hours at 37°C. Afterwards, a loop full of culture was added to 5 ml fresh Muller Hilton broth and adjusted to 0.5 McFarland by using a saline solution. A sterile swab was used for further seeding these bacteria on Muller Hilton agar. After drying the agar, a sterile cork borer was used to cut 4mm diameter wells. The wells were then filled with 0.1 ml of nutmeg extract and allowed to diffuse for 2 hours at room temperature. After incubating plates at 37^0 C for 24 hours, the diameters of inhibition zones (mm) were measured.

Statistical Analysis

The data collected from laboratory experiments were analyzed using descriptive statistical methods, specifically calculating simple means to determine the average value of the data points and percentages to represent the distribution of values within the dataset. This analysis helps to summarize and interpret the data clearly and concisely, providing valuable insights into the results of the experiments.

RESULTS

Moisture Content and Solubility of *M. fragrans* Seeds

Quantitative analysis of *M. fragrans* (nutmeg) extract showed that the moisture content (Loss on Drying) in powdered raw material of nutmeg was 95% and solubility of raw seeds in water was 40% and ethanol was 70%.

Phytochemical Compounds of *M. fragrans* Seeds Extract

The phytochemical analysis of water and 70% ethanolic extract of *M. fragrans* seeds were given in table 1. It was observed that ethanolic extract contains alkaloids, saponins, tannins and phenols while water extract only contains alkaloids and saponins but lacks tannins and phenols. Besides this ethanolic extract has high percentage of alkaloids in comparison to water extract, which is 13.25 and 7.14% respectively (fig 1).

Antimicrobial activity

The results of the antimicrobial activity of different extracts against chosen microorganisms are shown in table 2. In the case of *Escherichia coli*, the maximum zone of inhibition was by 70% ethanol extract of nutmeg followed by extract in water and then raw material in ethanol. Raw material in water didn't show any activity against *E coli*. Whereas, in the case of *staphylococcus aureus*, the maximum zone of inhibition was by 70% ethanol extract of nutmeg followed by extract in water, raw material in 70% ethanol and raw material in water (fig 2). The ethanolic extract showed significant effectiveness and moderate for water extract against the bacteria under study.

DISCUSSION

Medicinal plants have great significance due to the synthesis of a wide range of medicinally important chemical compounds. These compounds shield us from various pathogenic diseases (Pradhan and Huidrom, 2022). *Myristica fragrans* is one such plant that synthesizes a wide range of chemicals as described in the introduction part of this manuscript.

In the present study, phytochemical analysis was done specifically for alkaloids, phenols, saponins and tannins. Other researchers screened these components as they are the main components of medicinal plants having antimicrobial properties (Chandra, 2013). The results of the present study revealed that the ethanolic extract of *M. fragrans* contains alkaloids, saponins, Phenol and tannins while water extract only contains alkaloids and saponins but lacks tannins and phenols. Similar findings have also been reported by other workers (Gayathri and Anuradha, 2015). *M. fragrans* contains a wide variety of bioactive compounds useful as new antimicrobial remedies (Cruz *et al*., 2024). For instance, alkaloids possess many pharmacological and biological activities and are utilized in treating diabetes and cancer (Patel and Patel, 2024). Steroidal compounds are particularly significant in pharmacy due to their association with compounds like sex hormones, commonly used in the production of female contraceptive pills (Akhtar *et al.,* 2017). Tannins inhibit the growth of fungi, yeast, bacteria, and viruses, acting as antioxidants comparable to phenols (Darout *et al*., 2000). Phenols and flavonoids possess antioxidant, anti-allergic and antibacterial properties, while saponins exhibit anti-inflammatory, antiviral, and plant defence activities (Kumar, 2019). Henceforth, the presence of all these phyto-constituents contributes to the treatment of severe human infections (Olivia *et al*., 2021).

The ethanolic extract of *M. fragrans* showed the highest effectivity against both gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria while the water extract of nutmeg showed less activity for both bacteria. Similar findings were attained by other researchers (Mahady *et al*. in 2005; Shafiei *et al*., 2012; Thanoon and Kamal, 2013). The essential oil of *M. fragrans* exhibits significant inhibitory effects against a variety of bacteria, including bacteria that cause food poisoning, plant and animal pathogens, and spoilage (jose *et al*., 2024). The essential oil of *M. fragrans* has the potential to treat several hazardous diseases (Algaffar *et al*., 2024).The present study revealed the antimicrobial activity of *M. fragrans* against *S. aureus* (Al-Qahtani *et al*., 2022) and *E. coli* (Romeiro *et al*., 2024). Hence, the results

of our study also strengthened the previous studies that the antimicrobial potential of the extracts may be due to the presence of rich phytochemical compositions.

CONCLUSION

This study evaluated the antimicrobial activity of *M. fragrans* extracts against selected microorganisms isolated from local ATMs, demonstrating a wide range of antimicrobial sensitivity. The bioactive compounds extracted from *M. fragrans* using water and 70% ethanol as solvents may account for these results. The findings suggest that this spice holds promise for new formulation development. However, further investigation is required to elucidate the precise mechanism underlying the antimicrobial activity of the extract, potentially enhancing its pharmacological applications. Based on the current results, *M. frangrans* shows potential as a natural antimicrobial agent against infections caused by *E. coli* and *S. aureus* of human origin. Further research is needed to identify the principle active component and the mechanism underlying the antimicrobial activity of the seed extracts of *M. fragrans*.

CONSENT

Not applicable

ETHICAL APPROVAL

Not applicable

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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Table-1: The Result of Phytochemical Analysis of *Myristica Fragrans* Seed Extract

Phytochemicals	70% ethanolic extract of	Water extract of *M.*

	***M. fragrans* seed**	***fragrans* seed**
Alkaloid	+(13.25%)	+(7.14%)
Tannin	+	-
Saponin	+	+
Phenol	+	-

Table-2: Antimicrobial analysis of *M. Fragrans* raw material and extract in mm.

S.no	**Bacteria**	**Effectivity of raw material in water**	**Effectivity of raw material in 70% ethanol**	**Effectivity of extract in water**	**Effectivity of extract in 70% ethanol**
1	*Escherichia coli*	nil	6	9	11
2	*Staphylococcusaureus*	6	7	10	11

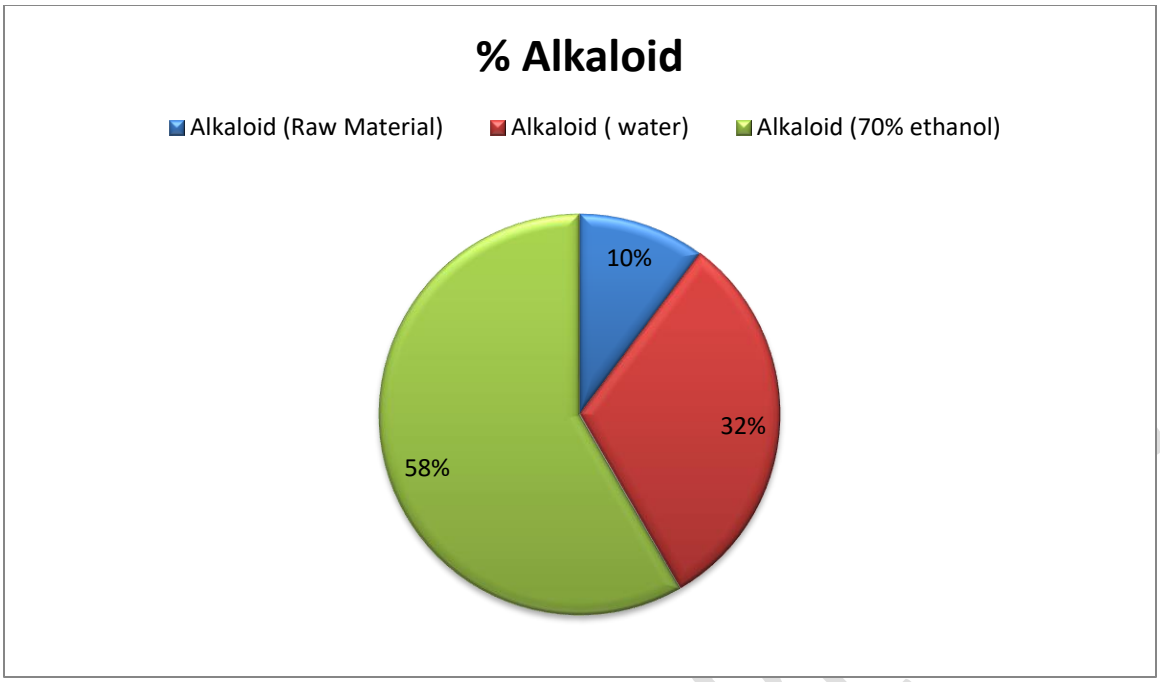

Figure-1: Showing percentage of Alkaloid in *M. fragrans* (seed) raw material, extract in water and extract in 70% ethanol

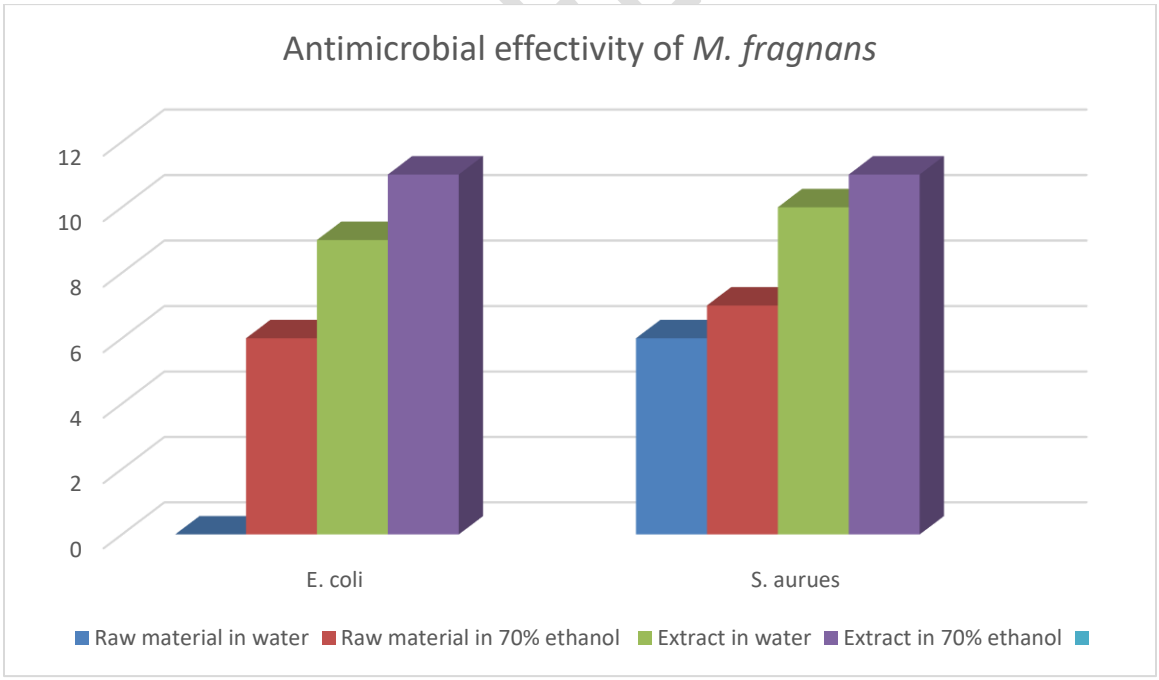

Figure-2: Antimicrobial analysis of raw material and extracts of *M. fragrans*