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Phytochemical screening, pharmacognostics, and biological activity of Myristica fragrans

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ABSTRACT

Using drugs originating from plants is often safer and more secure than using synthetic ones. A phytochemical analysis was conducted on the n-hexane seed extract of Myristica fragrans, an ayurvedic medicinal herb, which was also used as a food additive. Of the twenty-three phytoconstituents found, elemicin (24.44%) was the most abundant. The isolated elemicin demonstrated strong antioxidant activity in lipid peroxidase, catalase, and DPPH tests. The antibacterial study revealed that elemicin showed MIC of 31.25 µg/ml. against Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhi, and 62.5 µg/mL. against Klebsiella pneumoniae and Staphylococcus aureus. Elemicin exhibited better antifungal activity against Candida tropicalis and Aspergillus flavus than Aspergillus niger, Penicillium chrysogenum and Trichophyton rabrum. The study implies that the clemicin isolated from Myristica fragrans possess promising bioactive properties and can be crucially utilized in the development of therapeutic agents and food preservatives.

Keyword: phytochemical, Myristica fragrans, phytoconstituents, antioxidant, Pseudomonas aeruginosa, Penicillium chrysogenum

I. INTRODUCTION

In addition to being our main food source, plants also offer a wealth of medicinal uses. Phytochemicals, or plant metabolites, are utilized to treat a variety of human illnesses. Because of their medicinal significance, secondary metabolites of plants have long piqued human curiosity (Arora, Kaur & Kaur, 2003). Recently, there has been interest in analyzing natural products to identify dynamic chemicals from plant sources that have antibacterial, antioxidant, and anticancer activities with potential use in the food industry (Kazemi, 2015).Despite the use of different preservation and conservation techniques, food contamination and food poisoning continue to be concerns for the food business and consumers. Both antibacterial and antioxidant qualities are important for extending the shelf life of a particular food product (Singh et al., 2005). The food industry can greatly benefit from essential oils' alleged biological qualities (Piaru et al., 2012). Traditional uses of it include reducing inflammation, preventing liver ailments, calming nervous and digestive issues, and relaxing muscles.

The tree Myristica fragrans is a member of the Myristicaceae family, which has its origins in Indonesia. 78% of the tree's fruit is meat, 4% is mace, 5% is shell, and 13% is seed. This seed's kernel is processed to make the spice known as nutmeg. Since seeds contain more oil than any other portion of the plant, the essential oil that was collected from the seeds was used for the study. Generally speaking, seeds have around 60% oil (Kuete, 2017). The essential oil that is derived from M. fragrans is frequently utilized in the culinary sector as a flavoring ingredient and in cosmetics as a fragrance-incorporating agent. Steam distillation yields 80–90% monoterpene hydrocarbons and 4%–16% essential oil. It possesses strong antitumor, antibacterial, antifungal, anticancer, and hepatoprotective properties. Major bioactive chemicals including myristicin, eugenol, elemicin, and safrole are what provide M. fragrans its therapeutic properties (Kuete, 2017, Asgarpanah, 2012).

Individual components were evaluated for their biological characteristics in order to harness this ayurvedic plant's therapeutic potential. Myristicin is a colorless, water-insoluble derivative of phenyl propane that is volatile. Its primary application is as a pest and insect repellant, with anticholinergic and neurotoxic properties. In terms of medicine, it stops tumor development and liver damage. An over-the-counter analgesic for toothaches, eugenol is a phenolic molecule with antifungal and anti-inflammatory effects (Niu et al.

2012, Azir Uddin et al., 2017). Cloves are the primary source of its active component. Found in Sassafras roo, safrole is a colorless chemical molecule that belongs to the benzodiaoxole family. The FDA outlawed its usage as a flavoring additive in 1960 because to its genotoxic properties. Elemicin was selected as the compound of interest for this investigation since all of these compounds had already been investigated.

In addition to M. fragrans, Petroselinum sativum, Melaleuca bracteata, P. crispum, and Canarium commune also contain the elemicin complex. The ability to transform into a hallucinogenic derivative of trimethoxy amphetamine has demonstrated psychotomimetic properties. Elemicin has very little use in medicine, and the body of study on the topic is still extremely young. If used as essential oil, elemicin has a limited bioavailability. Reports discussing this compound's harmful properties are few (De Vincenzi et al., 2004). Therefore, in order to prevent health risks, it is necessary to investigate the therapeutic efficacy of elemicin and establish the lowest safeconcentration.

II. LITERATURE REVIEW

- **Kumar et al. (2021)** conducted a comprehensive investigation of Myristica fragrans' phytochemical screening, with a particular emphasis on identifying its secondary metabolites. They extracted the phytochemicals from the plant's seed and aril using a variety of solvents. According to their findings, there were significant amounts of myristicin and eugenol among the alkaloids, flavonoids, tannins, terpenoids, and essential oils. According to the study, eugenol and other bioactive chemicals are significantly present in the plant and may contribute to its antibacterial, analgesic, and anti-inflammatory effects. Furthermore, Kumar et al. (2021) highlighted that Myristica fragrans may be a useful source for pharmacological applications, particularly given its possible use in the treatment of illnesses linked to inflammation and digestive problems.
- **Rathore et al. (2021)** looked at how cytotoxic Myristica fragrans were to different types of cancer cells. They evaluated the effects of seed extracts from Myristica fragrans on prostate, lung, and breast cancer cells. According to their research, the seed extract had the most efficacy against breast cancer cells and showed notable cytotoxic effects. The presence of myristicin, which was discovered to be significantly concentrated in the seed extract, was ascribed to the cytotoxicity. Myristica fragrans may have anticancer potential, according to Rathore et al. (2021), who used HPLC to measure the concentration of myristicin and found a dose-dependent cytotoxic impact.
- **Jayaraman et al. (2020)** Investigated Myristica fragrans's antioxidant qualities in detail by looking at both its methanolic and ethanolic extracts. To evaluate the plant extracts' overall antioxidant capability and capacity to scavenge free radicals, they used the DPPH and FRAP tests. The findings showed that Myristica fragrans has strong, dose-dependent antioxidant activity. In their quantitative examination of the main antioxidant chemicals, Jayaraman et al. (2020)

found that myristicin and eugenol were the main contributors. According to their research, these substances may be essential in avoiding oxidative stress-related illnesses including cancer and heart disease because of their antioxidant qualities.

- **Vasudevan et al. (2020)**examined how growing Myristica fragrans for both commercial and medical uses affected the environment. Their research addressed the ideal growing conditions for Myristica fragrans, including tropical regions with consistent rainfall, and emphasized sustainable farming methods. They underlined how crucial it is to preserve Myristica fragrans' native environment, particularly in areas where deforestation threatens to impede its growth. Vasudevan et al. (2020) examined the effect of culture on the availability of active phytochemicals in Myristien fragrans, despite the study's greater emphasis on agricultural practices. They proposed that appropriate cultivation methods might increase the concentration of bioactive components.
- **Chandran et al. (2019)** performed a comprehensive review of the pharmacological activities of Myristica fragrans and its phytochemical constituents. They highlighted the significant role of myristicin, a compound that has been shown to have neuroprotective properties, and eugenol, which has anti-inflammatory and analgesic effects. According to their review, the seeds and essential oils of Myristica fragrans exhibit a range of biological activities, including antimicrobial, antifungal, and anticancer effects. Chandran et al. (2019) also discussed the potential health benefits of Myristica fragrans, noting that its bioactive compounds could be explored further for the development of novel pharmaceutical drugs.
- **Ramachandran et al. (2019)**centered on Myristica fragrans' anticancer qualities and possible application in chemotherapy. Their research demonstrated the cytotoxic effects of extracts from Myristica fragrans seeds on a range of cancer cell lines, including prostate, lung, and breast cancer cells. Ramachandran et al. (2019) proposed the use of Myristica fragrans' phytochemical constituents, especially myristicin, as a natural adjuvant in the treatment of cancer. The quantitative analysis of these compounds showed that they had the potential for future therapeutic usage since the doses needed to exhibit substantial anticancer action were quite low.
- **Singh et al. (2019)**investigated Myristica fragrans's antibacterial qualities as well as those of its phytochemical components. They assessed the essential oils extracted from the seed and aril's effectiveness against a variety of microbiological diseases, including as viruses, fungus, and bacteria. Both the seed and aril oils had potent antimicrobial activity, according to the study, with the seed's essential oil displaying the most antibacterial and antifungal effects. The presence of eugenol and myristicin, which were determined to be the main components, was responsible for the antibacterial action, according to Singh et al. (2019). The study's quantitative analysis revealed that the antimicrobial effectiveness vaned with the concentration of these compounds, underlining the importance of precise quantification for clinical applications.
- **Krishnan et al. (2019)**examined Myristica fragrans' pharmacological characteristics, including as its neuroprotective, anti-inflammatory, and analgesic activities. They talked about how Myristica fragrans' traditional use line up with current scientific discoveries, such how well it works to reduce inflammation and discomfort. The scientists underlined that in order to confirm the therapeutic properties of these plants, more thorough clinical research are required. Future research, according to Krishnan et al. (2019), should concentrate on improving extraction techniques in order to increase the quantity of bioactive substances like myristicin and zugenol. They also emphasized how crucial quantitative research is in figuring out these drugs' therapeutic dosages.
- **Sharma et al. (2018)**examined how Myristica fragrans affected gastrointestinal health, specifically in relation to treating nausea, bloating, and indigestion. According to their research, Myristica fragrans seeds have chemicals that have both carminative and digestive stimulating qualities, which makes them a good treatment for a number of gastrointestinal disorders. In addition, the researchers quantitatively analyzed the seed extract and performed a phytochemical screening, finding the main active components as safrole, myristicin, and cugenol. Additionally, Sharma et al. (2018) emphasized the significance of quantitative research for

therapeutic applications by showing a clear correlation between the concentration of these chemicals and the plant's efficacy in treating digestive diseases.

- **Rao and Raghavendra (2018)**centered on Myristica fragrans's bioactive components and their analgesic and anti-inflammatory qualities. its research examined the seed's ethanolic extracts and assessed its impact on animal models of inflammation and discomfort. According to the findings, extracts from Myristica fragrans considerably decreased pain and inflammation, and their effects were on par with those of common analgesic medications. According to the study, the presence of myristicin, cugenol, and other flavonoid molecules was responsible for these benefits. Using high-performance liquid chromatography (HPLC), Rao and Raghavendra (2018) also carried out a quantitative investigation of these substances, showing the presence of myristicin and eugenol in significant amounts. According to their research, Myristica fragrans shows promise as a natural substitute for synthetic analgesics.
- **Chouhan et al. (2018)**conducted a phytochemical investigation of Myristica fragrans and found that it contained a number of essential oils, including as eugenol, safrole, and myristicin. They underlined that a key component of the plant's therapeutic qualities, including its intoxicating effects, is myristicin. Using high-performance liquid chromatography (HPLC), Chouhan et al. (2018) carried out quantitative investigation to ascertain the myristicin content in different plant extracts. The findings showed that the myristicin concentration varied according to the extraction process, indicating that the best extraction methods are required to obtain the maximum amount of active chemicals.
- **Nishimura et al. (2018)**centered on the quantitative examination of Myristica fragrans essential oil components, identifying and quantifying the active ingredients using gas chromatography-mass spectrometry (GC-MS). Myristicin and safrole were the next most common compounds in the essential oil, according to their analysis, after eugenol. The study also showed that the geographical location and the techniques employed for oil extraction might affect the concentration of these substances. According to Nishimura et al. (2018), quantitative research on the chemical makeup of essential oils from Myristica fragrans is critical to guaranteeing both their safety and effectiveness in therapeutic applications.
- **Prabhu et al. (2017)**analyzed the phytochemical components of Myristica fragrans seeds in detail using both qualitative and quantitative methods. According to their findings, Myristica fragrans seeds contain high concentrations of volatile oils, such as myristicin, which gives them their distinctive scent and many of their therapeutic qualities. The quantitative analysis showed that myristicin was more abundant in the seed than other volatile oils like eugenol and safrole. Additionally, the researchers observed that the essential oil of the sced had strong antioxidant qualities that may help prevent disorders linked to oxidative stress.
- **Mohan et al. (2017)**investigated Myristica fragrans' potential for treating neurodegenerative illnesses and its neuroprotective benefits. Their study used in vitro models of Parkinson's and Alzheimer's illnesses to investigate the impact of extracts from Myristica fragrans on neuronal health. According to the study, extracts from Myristica fragrans, especially the seed, displayed strong neuroprotective properties that improved neuronal survival and decreased oxidative damage. It was determined that one of the main contributors to these effects was the active ingredient myristicin. Using gas chromatography, Mohan et al. (2017) also conducted a quantitative investigation of myristicin and found that the concentration of this compound in the seed extracts was sufficient to provide these protective effects. According to the study, Myristica fragrans may be a useful natural treatment for neurodegenerative diseases..
- **Sathya et al. (2016)**investigated the phytochemical components and pharmacological effects of Myristica fragrans. They emphasized in their study the importance of essential oils and the many components that comprise them, such as safrole, eugenol, and myristicin. These substances are well-known for their antibacterial, anti-inflammatory, and antioxidant qualities. Myristica fragrans has long been used in traditional medicine, especially to treat respiratory diseases, digestive difficulties, and discomfort,

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according to Sathya et al. (2016). They underlined the significance of quantitative analysis and phytochemical screening in order to fully comprehend the plant's therapeutic potential.

- **Nair et al. (2016)**have out research on Myristica fragrans's potential to prevent diabetes and control blood sugar levels. Their study concentrated on how extracts from Myristica fragrans affected the way that diabetic rats metabolized glucose. The plant's extracts considerably lowered blood glucose levels, according to the study, with the seed extract outperforming the aril extract. In order to ascertain the quantity of active molecules that provide the anti-diabetic effect, Nair et al. (2016) also conducted a quantitative study, revealing myristicim as the primary bioactive component. According to the study's findings, Myristica fragrans may turn into a diabetes management supplementary medication.
- **Sankari et al. (2015)**investigated Myristica fragrants' anti-inflammatory and antioxidant qualities utilizing both in vitro and in animal methods. Their research verified that the plant's phytochemicals, especially myristicin and eugenol, had substantial anti-inflammatory and antioxidant properties, which support the plant's possible medical use in ailments including arthritis and heart disease. Using tests such as DPPII and FRAP, the scientists also quantitatively evaluated the antioxidant potential of Myristica fragrans extracts. The findings indicated that antioxidant activity increased in a dose-dependent manner.
- **Kumar et al. (2013)**We out a study on Myristica fragrans phytochemical screening, concentrating on the presence of several secondary metabolites in the plant's seeds and essential oils. Alkaloids, flavonoids, phenolic compounds, saponins, and tannins are among the bioactive substances found in Myristica fragrans seeds, according to the research. Numerous phytochemicals provide the plant its therapeutic qualities, including antibacterial, antioxidant, and anti-inflammatory effects. According to Karnar et al. (2013), the phytochemicals found in Myristica fragrans seeds may help treat a number of illnesses. They also said that in order to determine the precise amounts of these bioactive substances, a more thorough quantitative analysis would be necessary.
- **Ravi et al. (2012)**reviewed Myristica fragrans's many pharmacological characteristics and its potential for treating conditions including depression, Parkinson's disease, and Alzheimer's. The phytochemicals found in Myristica fragrans, particularly myristicin, have demonstrated neuroprotective properties in a number of animal models and might potentially cure neurodegenerative illnesses, according to the study. According to Ravı et al. (2012), comprehensive quantitative research is required to assess the safety of these substances when administered over an extended period of time and to identify the optimal dosages for clinical application.

AIM AND OBJECTIVE

The current study's objective is to assess Myristica fragrans' phytochemical screening.

RESEARCH ENVISAGED AND PLAN OF WORK

PLAN OF WORK

A. Exhaustive Literature survey

B. Identification, collection and drying of Myristica fragrans

C. Procurement of reagents

D. Phytochemical Evaluation

- Extraction of the chosen herbs with hydroalcoholic solvent
- Qualitative chemical evaluation

E. Compilation of work

PLANT PROFILE

` The nutmeg tree, or Myristica fragrans, is a tropical evergreen tree that is native to Indonesia's Moluccas, also referred to as the Spice Islands. Most famously, it produces mace (the dried aril around the seed) and nutmeg (the seed), both of which are fragrant seeds. Nutmeg has been a highly prized spice since antiquity, sought after for its culinary, medicinal, and fragrant characteristics. The tree flourishes in humid tropical regions and may reach a height of 20 meters. Myristica fragrans has been thoroughly investigated for its possible medicinal qualities in addition to its culinary applications.

Botanical Name: Myristica fragrans

Family: Myristica

Common Names: Nutmeg, Mace, Myristica

Plant Parts Used: Seed (Nutmeg), seed covering (Mace)

Active Constituents
1. Myristicin: A substance known to cause hallucinations.
2. Elemicin: A natural anti-inflammatory and analgesic.
3. Safrole: A first step in the production of MDMA.
4. Lignans: Anti-inflammatory and antioxidant substances.
5. Flavonoids: Compounds that are anti-inflammatory and antioxidant

Pharmacological Effects
1. Central Nervous System (CNS): Both myristicin and elemicin have sedative, analgesic, and hallucinogenic properties.
2. Antioxidant and Anti-inflammatory: Flavonoids and lignans have anti-inflammatory and antioxidant properties.
3. Antimicrobial: Against a variety of bacteria, Myristica fragrans demonstrates antimicrobial qualities.

Therapeutic Uses
1. Digestive issues: used historically to treat digestive issues including nausea and diarrhea.
2. Pain relief: used as a natural anti-inflammatory and analgesic.
3. Anxiety and insomnia: Elemicin and myristicin may help reduce anxiety and sleeplessness.
4. Antimicrobial applications. used to treat a variety of illnesses in traditional medicine.

Adverse Effects
1. Toxicity: Myristicin overdose can result in nausea, vomiting, and hallucinations.
2. Allergic reactions: Allergy responses can cause breathing problems or skin discomfort in certain people.
3. Interactions: Certain drugs, including blood thinners and antidepressants, may interact with Myristica fragrans.

Contraindications
1. Pregnancy and breastfeeding: Due to the possibility that myristicin is toxic.
2. Children and infants: Due to the possibility that myristicin is toxic.
3. Allergic individuals: those who have a history of allergies to Myristica fragrans or any of its ingredients.

Dosage and Administration
1. Traditional use: 1/4 to 1/2 teaspoon of ground nutmeg per day.
2. Essential oil: When used topically, it is diluted with a carrier oil for aromatherapy.

Before utilizing Myristica fragrans for medicinal purposes, please get medical advice.
1. **Antioxidant Activity:**The plant's strong antioxidant activity helps to counteract free radicals and lower oxidative stress, which is especially useful in the management and prevention of chronic illnesses including cancer and heart disease.
2. **Anti-inflammatory Effects:** Numerous research have shown that Myristica fragrans has anti-inflammatory properties. Its phenolic components, including eugenol and myristicin, and essential oil are mostly responsible for the anti-inflammatory properties.
3. **Analgesic and Antipyretic Effects:**Traditional medicine has utilized nutmeg to relieve fever and discomfort. The bioactive components of Myristica fragrans, especially eugenol, which is also present in clove oil, are responsible for the plant's analgesic and antipyretic effects.
4. **Neuroprotective Effects:**According to certain research, myristicin, one of the plant's constituents, may have neuroprotective properties. Because it may shield brain cells from harm and enhance cognitive function, research is being done to find out how effective it is in treating diseases like Parkinson's and Alzheimer's.
5. **Antimicrobial Activity:**Research has demonstrated that the essential oil of Myristica fragrans possesses antibacterial qualities that make it efficient against a range of bacteria and fungi. This makes it an effective food preservative and topical therapeutic agent.
6. **Digestive Health:**The traditional usage of nutmeg is to alleviate digestive issues like diarrhea, gas, and bloating. The herb might ease stomach pain due to its modest carminative properties.

MORPHOLOGICAL CHARACTERISTICS

The evergreen tree Myristica fragrans may reach a height of 10 to 20 meters. The leaves have a leathery feel, are big, glossy, and oblong. The plant produces clusters of tiny, unisexual blooms. Fruit production requires cross-pollination since the male and female flowers grow on different trees.

Nutmeg comes from the drupe fruit of Myristica fragrans, which, when fully grown, splits apart to reveal a single seed. The crimson, meaty aril that surrounds the seed is collected separately

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and dried to create mace, a spice that resembles nutmeg but has a milder flavour.
Illustrates the Morphologic Characteristics of Myristica Fragrans Houtt., Commonly Known as Nutmeg, Showcasing Various Parts of the Plant:

Habit:
Depicts the overall appearance of the nutmeg tree, which is an evergreen tree that can reach up to 50-60 feet in height.

Leaves:
Shows the elliptic or elliptic-lanceolate leaves, typically 4-8 cm long, with a nearly leathery texture.

Ripened fruit displaying the aril:
Displays the ripened fruit, which dehisces to reveal a single seed covered by a bright red aril. The aril is the outer covering of the seed, known as mace when dried.

Ground nutmeg:
Exhibits the ground spice derived from the seed. The seed is typically 2 to 3 cm long and 1.5 to 2 cm wide.

Flowers:
Shows the pale yellow, fleshy, and bell-shaped flowers. Male flowers are in groups of one to ten, while female flowers are in smaller groups of one to three.

Seeds:
Displays the seeds, which are oval or broadly elongated, grayish-brown with reddish-brown spots.

Fruits:
Shows the oval or pyriform yellow fruits.

Dried arils:
Presents the dried arils, commercially known as mace.

Thai massaman curry:
Illustrates a culinary application of nutmeg, specifically in Thai massaman curry.

After drying, the seed itself turns into the well-known spice nutmeg. It is necessary to grind or grate the hard, dark seed before using it in food or medicine. There are volatile oils in the plant's bark, roots, and leaves as well, but the seed and aril are the main parts of interest.

Synonym
Myristica fragrans is known by several other names, including:
- Nutmeg Tree
- Fragrant Nutmeg
- Myristica
- Java Nutmeg (in some regions)

Biological Source
The dried seed and aril are Myristica fragrans' biological source. Whereas the arıl is the source of mace, the seed is the main commercial source of nutmeg. The plant's two portions are both collected and turned into different spices. Both the seed and the aril can provide essential oil, which is then utilized in cosmetic and therapeutic products.

Myristica fragrans is grown for its possible therapeutic properties in addition to its culinary use. Compounds found in the plant's volatile oils, particularly those that are derived from the seeds, have been demonstrated to have a range of pharmacological properties, such as antibacterial, analgesic, and anti-inflammatory actions.

Cultivation & Collection
Commercial nutmeg and mace are mostly sourced from tropical parts of the world, including Indonesia, Sri Lanka, and the Caribbean, where Myristica fragrans is grown. The plant thrives on rich, well-drained soils at elevations between 600 and 1000 meters above sea level and likes humid, tropical climates with regular rainfall.

The ideal climate for the tree is warm, with 25 to 30°C temperatures and 75% to 85% humidity. With seed propagation, Myristica fragrans trees start to produce fruit after seven to nine years after planting. Harvested when yellow, the fruits spontaneously break apart to release the seed. The seeds are dried to make nutmeg, and the aril is carefully separated from the seed to be dried and processed into mace.

Microscopy
Upon microscopic analysis, Myristica fragrans seeds exhibit unique characteristics that aid in plant identification. There are many oil glands in the thick seed coat. Under a microscope, one can see the presence of aromatic oil cells, which are indicative of the volatile oils found in nutmeg. Usually, a thick, fibrous endosperm with an oily core makes up the seed.

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Chemical Constituents

Numerous chemical components make up Myristica fragrans, some of which are involved in its pharmacological actions. The key compounds include:

- Essential Oils: Myristicin, safrole, clemicin, and cugenol are among the substances found in Myristica fragrans essential oil. These oils give the plant its distinctive scent and many of its therapeutic qualities.
- Alkaloids: The seed contains substances including myristicine and safrole, which are believed to be responsible for some of its analgesic and psychotropic properties.
- Flavonoids: These substances support the plant's anti-inflammatory action and contain antioxidant qualities.
- Phenolic Compounds: Phenols found in Myristica fragrans have antibacterial, anti-inflammatory, and antioxidant qualities.

The many medicinal and aromatic properties of Myristica fragrans are the result of these components working in concert.

Uses

Myristica fragrans have a wide range of applications in medicine, cooking, and cosmetics.

- Culinary Uses: Both savory and sweet recipes frequently call for the use of nutmeg and mace. Desserts, baked products, sauces, and beverages like eggnog all include them.
- Medicinal Uses: Traditional uses of nutmeg include treating pain, sleeplessness, and digestive problems. Additionally, it has antibacterial and anti-inflammatory properties.
- Cosmetic Uses: Nutmeg oil is used in perfumes, soaps, and lotions due to its pleasant aroma and soothing properties.
- Aromatherapy: Aromatherapy uses nutmeg oil to reduce tension, elevate mood, and encourage sleep.

ADULTERANTS

Despite the widespread cultivation of Myristica fragrans, adulterants are now available in the market due to the spice's appeal. Typical adulterants in nutmeg include the following:

- Synthetic Nutmeg: In certain places, nutmeg powder is sometimes mixed with artificial nutmeg or other plant powders to make it heavier or less expensive.
- Mace Substitutes: To simulate the look and feel of real mace, mace (the aril) is occasionally blended with or used in place of other dried plant components.
- Low-Quality Nutmeg: The product may occasionally be low-quality seeds that may not have the fragrant qualities of real nutmeg. To guarantee quality and authenticity, consumers should be aware of these adulterants and buy mace and nutmeg from reliable suppliers.

Conclusion

Myristica fragrans is a very useful herb for cooking and medicine. It is spice. Nutmeg is utilized all over the world as a flavoring, and its bioactive components and essential oils have a number of medicinal advantages. Research on Myristica fragrans' potential medical uses is very fascinating, ranging from its anti-inflammatory and antioxidant properties to its possible neuroprotective benefits. Notwithstanding the difficulties caused by adulteration, the plant remains an important resource for many other sectors, solidifying its status as one of the most recognizable spices in the world.

QUANTITATIVE ANALYSIS

The value of Ash Nutmeg (Myristica fragrans) usually has an ash value of 2–5%. Depending on the nutmeg's quality, source, and processing, its value may change.

Here's a breakdown of the ash value of nutmeg: - Total Ash: 2-5%

- Acid-Insoluble Ash: 0.5-1.5%

-Water Soluble Ash: 0.5-1.5%

When evaluating the quality and purity of nutmeg, the ash value is a crucial factor. Impurities or adulterants may be present if the ash value is greater. It is important to remember that the ash value might change based on the particular nutmeg variety, including: The ash values of whole and ground nutmeg are 2–4% and 3-5%, respectively. The ash content of nutmeg oil is usually quite low, typically less than 0.1%.

Ash Value (%) = (Weight of Ash)/ (Weight of Sample) ×100

Result- The Ash value of Myristica fragrans was found to be 3.65%.

Moisture content (%) = (Weight of wet sample-Wet of dry sample)/ (Weight of wet sample) ×100.

Result-The Moisture content of Myristica fragrans was found to be 6.3%.

III. MATERIAL METHOD

- In Chennai, India, the National Institute of Siddha and Hospital in Tambaram provided the

Myristica fragrans seeds. Using an electric bladder, the seeds were ground into a coarse powder after being air-dried at 50°C.

- The spice tree, Myristica fragrans, is a big, evergreen plant that does well in tropical areas. A fully mature tree may grow to a height of 50–60 feet and yields two important spices: mace and nutmeg. The nutmeg fruit is really a drupe, roughly the size of an apricot, that, when ripe, splits apart to reveal a single, hard, oval-shaped kernel in the center, called "nutmeg spice." Crimson-red lacy or thread-like arils, called "mace," surround the seed tightly. The warm, sweet, fragrant smell of both spices is comparable. Mace is the dried "lacy" reddish coating or aril of the nutmeg seed, which is generally egg-shaped and is 20 to 30 mm (0.8 to 1.2 in) in length and 15 to 18 mm (0.6 to 0.7 in) in width. The dry weight of the nutmeg seed is between 5 and 10 g (0.2 and 0.4 ounce). Nutmeg trees attain maximum output 20 years after planting, with the first harvest occurring 7–9 years later. The most common form of nutmeg is powdered.
- An electric blender was used to grind the seeds into a coarse powder after they had been air-dried at 50°C. In a shaker, 100 g of powdered material was extracted for three hours using 70% hexane. The crude extract was then allowed to evaporate and filtered using Whatman No. 1 filter paper. Clevenger's equipment was used to hydrodistribute the essential oil (EO), which was then stored at room temperature. Following conventional protocols, phytochemical analysis was performed to detect various chemical classes of phytoconstituents (Ameh and Eze, 2010, Jeff-Agboola and Awe, 2016).
- The GC-MS 5975C Agilent mass selective detector was used to perform GC-MS analysis of the n-hexane extract of M. fragrans in the electron impact mode (70 eV). Temperature settings for the injector and MS transfer line were 220 °C and 290 °C, respectively. Comparing the components' respective time of retention and mass spectra to the standards and NIST11 mass spectral library data allowed for the determination of the components.
- Chloroform was used to dissolve the EO, and a diluted NaOH solution was used to stir it. After that, it is cleaned with purified water and let to rest with a separating funnel. Hexane and ethyl ether were used as the mobile phases in a column chromatography to further profile the basic phase. After being compounded, the ether-eluted fractions were put in a vacuum evaporator with a temperature maintained at 155 °C. A rotary evaporator was used to extract the solvent from the compounded fraction, which was then left to dry overnight. It was once more put through column chromatography with the mobile phase being isooctane/chloroform/methanol (70:29:1). To check for elemicin, the eluents were characterized using a UV spectrophotometer.
- A 1 mL sample of isolated elemicin diluted with 9 mL of hexane was used for the lipid peroxidase test. One of the six test tubes was kept empty, while the other five test tubes were filled with 1 mL of the aforementioned material at different concentrations (100, 200, 300, 400, and 500 µg/mL). Next, add 200 µl SDS, 1.5 mL acetic acid, and 1.5 mL 0.8 percent TBA, and mix well. Two glass beads were then added, and distilled water was added to bring the volume up to 10 mL. The mixture was centrifuged for 10 minutes at 4000 rpm after being heated at 95 °C with 1 mL of water and 5 mL of n-butanol/pyridine (Datta and Patil, 2020, Gyawali et al., 2020). We measured the absorbance at 532 nm and used the formula, where Ab is the test absorbance and Ac is the control absorbance, to get the inhibition percentage. trials carried out in triplicate.
- The standard methodology for the catalase test was followed (Höferl et al., 2014). 200 µl of isolated elemicin was combined with solutions of Tris-NaOH (5 mL), EDTA, Triton, and polyvinylpyrrolidone to provide the sample for the catalase test. The supernatant was produced by centrifuging the mixture for 10 minutes at 22,000 rpm and 4 °C. Additionally, the supernatant was mixed with 1 mL of the reaction mixture, which included hydrogen peroxide (60 mM), potassium phosphate buffer (50 mM), and enzyme extract (250 µl). The absorbance was measured for three minutes at 240 nm using the spectrophotometer. We used the following formula to determine the H2O2 degradation.
- where & is the extinction coefficient (43.6 M-1 cm-1) and ∆A is the absorption difference. There were three duplicates of each experiment.
- According to the conventional procedure, the DPPH test was used to assess the isolated elemicin's radical scavenging activity

(Gayathri and Sathish, 2016). Each test tube contained 1 mL of the sample and 9 mL of hexane. A blank was placed in each of the six test tubes, and 1 mL of this sample was placed in the remaining test tubes at different concentrations (100, 200, 300, 400, and 500 µg/mL). A 0.1 mm DPPH solution in methanol was added to the aforementioned samples, and after 30 minutes, the absorbance at 570 nm was determined. We used the following formula to get the scavenging percentage:

- where Abt stands for Absorbance of Test and Abc for Absorbance of Control. Mean + SD (standard deviation) is the result. We run every experiment in triplicate. The size of the inhibitory zone on the grown layer was used to assess the antibacterial and antifungal activity.
- Elshikh et al. (2016) and Veiga et al. (2019) used the microdilution technique to assess the elemicin taken from M. fragrans for its antibacterial activity in vitro. E. coli (MTCC433), K. pneumoniae (MTCC109), P. aeruginosa (MTCC1035), S. typhi (MTCC3231), and Staph. aureus (MTCC1144) were among the five bacterial strains selected for the current investigation. The source of all the bacterial strains was the Microbial Type Culture Collection (MTCC). Each microtiter plate well contained 80 µl of Mueller-Hinton agar (MHA). Each well received 10 µl of the Resazurin dye and 10 µl of the inoculum from each species. After adding 500 µg/mL of the isolated extract to the first well, it was serially diluted in the following wells. To find the minimal concentration at which the dye changes color, plates were incubated for 24 hours at 37 °C. The positive and negative controls were 10 µl of amoxicillin and 10 µl of DMSO, respectively. MIC was defined as the lowest elemicin concentration that produced a distinct zone of inhibition. The diameter of the clear zone was used to measure the zone of inhibition, and the findings were noted. There were three duplicates of each experiment.

IV. RESULTS AND DISCUSSION

Preliminary phytochemical analysis

The phytochemical analysis of the EO from M. fragrans contain the majority of the fundamental phytoconstituents including tannin, saponin, alkaloid, protein, steroid, anthraquinone, terpenoids, and cardiac glycosides. The results on the n-hexane extract of M. fragrans are presented and the primary essential oil showed strong presence of terpenoids than the other compounds.

Phytochemical analysis of the n-hexane extract of M. fragrans. (+) represents presence and (-) represents absence.

Seed Extract	Tannin	Saponin	Flavonoids	Alkaloids	Steroid	Anthraquinone	Terpenoids	Cardiac glycosides
Myristica fragrans	+	+	–	+	+	+	++	+

1. GC-MS analysis

The essential oil of M. fragrans included 23 volatile components, according to the GC-MS analysis (Fig. 1). Tetradecanoic acid (22.25%) and 1, 2, 3-trimethoxy-5-(2-propenyl)-benzene gathered the highest amounts (24.44%) of the 23 phytochemical components found in M. fragrans essential oil.

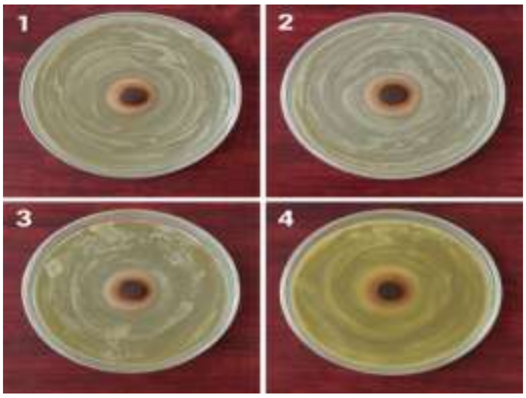

Fig: Antibacterial activity of the isolated elemicin against tested bacteria (1) E coli (2) P. aeroginosa (3) S. aureus (4) S. typhi.

The common name of benzene, 1, 2, 3-trimethoxy-5-(2-propenyl)- is elemicin. Several studies were conducted to determine the presence of various bioactive compounds in M. fragrans. The major components of its volatile extract that were identified include myristicin, eugenol, isoeugenol, elemicin, safrole, 4-terpineol and camphene (Asgarpanah, 2012, Adiani et al., 2015). Since the quantity of elemicin in the extract was more than the other compounds, attempts were made to isolate elemicin from the essential oil and study its antioxidant and antimicrobial property.

2. Isolation of elemicin

At different phases of the separation process, the solvent selection is crucial. The potential acidic component in the EO was eliminated by dissolving it in chloroform and then stirring it with diluted NaOH. The water wash distinguished between the basic bioactive chemical and the undesirable acidic molecule. Using hexane as the initial mobile phase, the selective elution method extracted all of the non-oxygenated hydrocarbons from the oil. It was the ether elution that separated the necessary oxygenated aromatic molecules. From the second cycle of chromatography, a total of six eluents were collected and put through a UV absorption spectrophotometer (Fig. 3). When compared, the sixth eluents' UV spectra showed two prominent peaks at 216 and 282 nm along with a shoulder at 220 and 240 nm that was typical of elemicin.

3. Antioxidant activity

Three methods were used to evaluate the antioxidant properties of the isolated elemicin: lipid peroxidase, catalase, and DPPH assay. The isolate demonstrated incremental antioxidant potential in both lipid peroxidase and catalase assays, and in the DPPH assay, 100% radical scavenging activity was observed at 300 µg/mL concentration. This suggests that the biological system may benefit from improved ROS (reactive oxygen species) scavenging activity (Ginting et al., 2018; Tan et al., 2013). The standard deviation of catalase activity, DPPH, and peroxidase inhibition activity was displayed, and all experiment data were conducted in triplicates, and the corresponding mean and standard deviation values were mentioned.

S. No	Bacterial species	Elemicin concentration MIC (µg/mL)	Fungal species	Elemicin concentration MIC (µg/mL)
1	E. coli	31.25	C. tropicalis	62.5
2	P. aeroginosa	31.25	A. niger	125
3	S. aureus	62.5	A. flavus	62.5
4	S. typhi	31.25	T. rubrum	125
5	K. pneumonia	62.5	P. chrysogenum	125
7	DMSO	–	DMSO	–

4. Antibacterial activity & antifungal activity

E. coli, K. pneumoniae, P. aeruginosa, S. typhi, and S. aureus were among the microorganisms that the isolated elemicin showed significant suppression against. The isolate's MIC value against S. typhi, P. aeruginosa, and E. coli was 31.25 µg/mL. The MIC value for S. aureus and K. pneumonia was determined to be 62.5 µg/mL.

With a MIC of 125 µg/mL for Aspergillus niger, Trichophyton rubrum, and Penicillium chrysogenum and 62.5 µg/mL for Candida tropicalis and Aspergillus flavus, the essential oil of M. fragrans demonstrated a notable zone of inhibition against fungus. Myristica fragrans essential oil shown strong antifungal action against Aspergillus niger.

The current study's phytochemical analysis of Myristica fragrans' n-hexane extract agrees with Jinous and Nastaran's (2012) previous findings. The UV spectrum of elemicin, however, was the subject of very few research (Victor, 1977). Moving on to the antioxidant activity, Kapoor et al. (2013) used the Clevenger equipment to extract oleoresins from M. fragrans.

Elemicin, the main constituent of the oleoresin, had potent DPPH radical scavenging properties (Kapoor et al., 2013). According to Adiani et al. (2013), the main antioxidant component in nutmeg essential oil is elemicin (17. Additionally, in a number of research, Kuete et al. (2017) talked about the antioxidant properties of nutmeg essential oil (Kuete, 2017). Elemicin was well recognized to be a significant contributing component, and the new findings align with the previous research. Regarding the antibacterial action, Staph growth was suppressed by the acetone extract of the aerial portion of M. fragrans, which contained 17.68% elemicin was useless against P. aeruginosa, although it worked against K. pneumoniae and S. aureus (Singh et al., 2005). According to Ibrahim et al. (2011), the ethanolic leaf extract of M. fragrans showed MIC values of 50 mg/mL against K. pneumoniae, Staph. aureus, and E. coli, and 100 mg/mL against P. aeruginosa.

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Compared to the EO from T. Ammi, the EO from M. fragrans, which contains elemicin (8.81%) as one of its main ingredients, shown superior antibacterial action against K. pneumoniae, Staph. aureus, and E. coli (Soni et al., 2016). An important factor was the selection of plant components, extraction technique, and solvent. All of these investigations show that the antibacterial properties of the M. fragrans extract were enhanced by the presence of elemicin, and the current study confirms this finding.

Das et al. (2021) examined the antifungal activity of elemicin as a food preservative and found that it has superior antifungal activity compared to other bioactive substances (Das et al., 2021). Elemicin-rich D. carota oil showed potent antifungal action at doses ranging from 0.16 µl/mL to 0.64 µl/mL. The EO from fresh P. carpunya leaves that contained 7.2% elemicin shown a synergistic activity against Candida sp. in another investigation. These conclusions from the previous research provide unmistakable proof of elemicin's antifungal properties.

V. SUMMARY & CONCLUSION

Additionally, plant-based medicines serve as a source of inspiration for new medicinal molecules and have significantly improved human health. It is clear from the aforementioned studies that this plant has enormous potential for application in pharmacology and as a possible source of useful medications. Its inclusion of several chemicals that are necessary for optimal health makes it useful for enhancing societal health. Since the effectiveness of plant essential oils as a traditional medicine has been demonstrated without scientific proof, they need to be given far more weight as safe and natural medicines than synthetic ones. Authenticating the existing medical potential was the design of the current study. The current investigation shows that the bulk of the essential phytoconstituents were found in the n-hexane extract of M. fragrans. GC-MS analysis of the essential oil revealed a high concentration of elemicin. A selective elution method was used to isolate Elemicin, which was discovered to have strong antioxidant properties. Additionally, the isolate showed encouraging antibacterial activity against five fungal and five bacterial species. This suggests that M. fragrans essential oil may one day be a potential source of natural antimicrobials and antioxidants for the food and pharmaceutical sectors.

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