Phytochemical Evaluation of Elemicin from Myristica fragrans

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***Abstract*—The present research study investigates the phytochemical composition and biological activities of phytochemical elemicin isolated from the n-hexane seed extract of *Myristica fragrans* (nutmeg). GC-MS analysis confirmed the presence of elemicin as a major constituent in *Myristica fragrans*. The isolated compound was evaluated for antioxidant properties using lipid peroxidation, catalase activity, and DPPH radical scavenging assays, all of which demonstrated dose-dependent activity. Antimicrobial efficacy was assessed through microdilution methods against various bacterial and fungal strains, revealing significant inhibitory effects. These findings highlight the potential of elemicin as a natural antioxidant and antimicrobial agent, supporting its further exploration for pharmaceutical and food preservation applications.**

Keywords: **Nutmeg, GC-MS analysis.**

I. INTRODUCTION

The n-hexane seed extract of Myristica fragrans, commonly known as nutmeg, is a topic of increasing interest within both culinary and pharmacological fields due to its rich phytochemical profile and potential therapeutic benefits. Nutmeg is primarily cultivated in tropical regions such as India and Indonesia and is recognized not only for its flavor-enhancing properties in various cuisines but also for its diverse bioactive compounds, including elemicin, which play a crucial role in its medicinal applications.[1][2] The notable presence of compounds like myristicin and elemicin underscores nutmeg's potential as a natural remedy for various health conditions, including mood disorders and microbial infections. Research has shown that the n-hexane extract of nutmeg seeds exhibits significant bioactivity, with studies revealing its antidepressant, antimicrobial, antioxidant, and anti-inflammatory properties.[3][4]. Elemicin has been highlighted for its potent antifungal effects against strains such as Candida tropicalis and Aspergillus flavus, establishing its relevance in the development of natural antimicrobial agents.[5]
Furthermore, the extract's neuropharmacological effects indicate its potential in treating anxiety and depression, aligning with traditional uses in Ayurvedic medicine. Despite its promising benefits, the consumption of nutmeg and its extracts, including elemicin, raises concerns regarding safety and toxicity, particularly at high doses.

The application of elemicin in medicinal field is very limited and the research data is still in its infancy. The bioavailability of elemicin is low if it is taken in the form of essential oil. There are few reports that discusses the toxic nature of this compound. Hence, there is a need to explore the medicinal value of elemicin and to determine the minimum safe concentration to avoid health risk [6][7].

II. MATERIAL AND METHODOLOGY

A. Plant Material Collection and Preparation

Seeds of *Myristica fragrans* were air-dried at 50 °C and grounded to get a coarse powder using an electric blender. About 100 g of powdered seed material was then extracted with 70% hexane in a shaker for 3 hours. The extract was filtered using filter paper, and the crude extract was then subjected to solvent evaporation.

B. Essential Oil Extraction

Essential oil (EO) was isolated from the dried powdered sample through hydro-distillation using a Clevenger apparatus and stored at room temperature.

C. Phytochemical Screening

Standard procedures described were followed for preliminary phytochemical analysis to detect major chemical classes of phytoconstituents [8].

D. GC-MS Analysis

Agilent mass selective detector in electron impact mode (70 eV) was used for analysis of the *n*-hexane extract. The injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. The identification of constituents was based on retention times and spectral matching with NIST11 library standards.

E. Isolation and Purification of Elemicin

Dissolved Essential oil of elemicin was partitioned with dilute NaOH solution in separating funnel in chloroform. The crude phase was chromatographed over a column in hexane and then ethylether as eluent. The ethereal fractions were combined and concentrated in vacuo (155 degrees C) and purified by chromatography over isooctane/chloroform/ methanol (70:29:1). Confirmation of the presence of elemicin was based on UV spectrophotometric analysis.[9]

F. Lipid Peroxidation Assay

Lipid peroxidase assayed using 1 milliliter of isolated elemicin was taken and diluted with 9mL of hexane. Different concentrations (100–500µg/mL) were evaluated. The aliquots of the resulting supernatant mixed with 200µL SDS, 1.5mL acetic acid and 1.5mL of 0.8% TBA, were swirled, and distilled water was added to a final volume of 10mL. The mixture was then boiled at 95°C, after the addition of glass beads. [10]

A mixture of water and n-butanol/pyridine in 1:5 ratio was added and centrifuged at 4000 rpm for 10 min. The absorbance was measured at 532 nm, and the inhibition percentage was calculated using the formula

[(Ac - Ab)/Ac] × 100.

G. Catalase Activity Assay

A diluted sample (200 µL) of elemicin was mixed with Tris-NaOH buffer, EDTA, Triton, and PVP, followed by centrifugation at 22,000rpm for 10min at 4 °C; the supernatant was added to a reaction solution made of 50mM potassium phosphate buffer, enzyme extract (250Î¼L), and 60mM H2O2. The absorption at 240nm was measured for 3 min and degradation of H_2O_2 was calculated as $\Delta A/\varepsilon$, $\varepsilon = 43.6 M^{-1} cm^{-1}$. [11]

H. DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of elemicin was measured [12]. Different concentrations of elemicin (100–500µg/mL) were added to 0.1mM DPPH in methanol. The absorbance was read at 570nm, at 30min.

The scavenging activity (%) was determined as [(Abc - Abt)/Abc] × 100. The data were presented as the mean ± SD, and the experiments were repeated three times.

I. Antibacterial and Antifungal Assays

In vitro Antimicrobial activity study was done using the microdilution method [13][14]. typhi (MTCC3231) and Staph. aureus(MTCC), were obtained from MTCC. The wells of a microtiter plate were loaded with 80 µL of Mueller-Hinton agar containing 10 µL of bacterial inoculum, 10µL resazurin dye, and the elemicin extract with serial dilutions from 500µg/mL upwards. The Plates were incubated at temperature of 37 °C for 24 hours. The minimum inhibitory concentration (MIC) was determined as the lowest concentration showing no visible color change. Amoxicillin (10 µL) served as positive and DMSO (10 µL) as negative control for the study. Inhibition zones were measured.

III. RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis

The phytochemical screening of the essential oil (EO) from *Myristica fragrans* revealed the presence of key phytoconstituents including tannins, saponins, alkaloids, steroids, anthraquinones, terpenoids, and cardiac glycosides. Notably, flavonoids were absent. Among these, terpenoids were predominant, indicating a strong potential for biological activity. Table 1 summarizes the phytochemical profile of the *n*-hexane extract.

Table 1. Phytochemical analysis of the *n*-hexane extract of *M. fragrans*. (+) indicates presence; (–) indicates absence.

Phytochemicals	Presence (+) / Absence (–)
Tannin	+
Saponin	+
Flavonoids	–
Alkaloids	+
Steroid	+
Anthraquinone	+
Terpenoids	++
Cardiac Glycosides	+

GC-MS Analysis

Gas chromatography-mass spectrometry (GC-MS) identified 23 volatile compounds in the essential oil of *M. fragrans*. The predominant component was benzene, 1,2,3-trimethoxy-5-(2-propenyl) (24.44%), followed by tetradecanoic acid (22.25%). This highlights the presence of high concentrations of bioactive aromatic and fatty acid derivatives in the oil.

Antioxidant Activity

The isolated compound, elemicin, was evaluated for antioxidant activity using lipid peroxidase inhibition, catalase activity, and DPPH radical scavenging assays:

- Lipid Peroxidase Assay: Elemicin showed dose-dependent inhibition of lipid peroxidation.
- Catalase Activity: Enhanced catalase activity was observed with increasing concentrations of elemicin.
- DPPH Assay: A maximum radical scavenging activity (100%) was recorded at 300 µg/mL, indicating potent free radical neutralization capacity.

These results suggest that elemicin significantly reduces the impact of reactive oxygen species (ROS) and supports its potential as an effective antioxidant agent.

Antibacterial Activity

Elemicin exhibited substantial antibacterial activity against multiple pathogens:

- MIC (Minimum Inhibitory Concentration) of 31.25 µg/mL was observed against *E. coli*, *P. aeruginosa*, and *S. typhi*.
- A slightly higher MIC of 62.5 µg/mL was noted against *K. pneumoniae* and *S. aureus*.

Antifungal Activity

The essential oil demonstrated significant antifungal activity:

- MIC of 125 µg/mL for *Aspergillus niger*, *Trichophyton rubrum*, and *Penicillium chrysogenum*.
- MIC of 62.5 µg/mL for *Candida tropicalis* and *Aspergillus flavus*.
- Particularly strong inhibition was observed against *Aspergillus niger*.

IV. CONCLUSION

Medicinal plants have long served as a cornerstone in healthcare, offering bioactive compounds that serve as prototype for the synthesis of the new medical drugs. The current investigation demonstrates that Myristica fragrans is a plant of great pharmacological importance in traditional medicine.

Phytochemical analysis of n-hexane extract confirmed the presence of important phytoconstituents like tannins, saponins, alkaloids, steroids, anthraquinones, terpenoids, and cardiac glycosides. In particular, terpenoids were found to be rich which may contribute most to the biological activities of interest.

Analysis of the essential oil through gas chromatography-mass spectrometry (GC-MS) identified elemicin as a primary component. Elemicin was effectively isolated using a selective elution method and assessed for its antioxidant and antimicrobial properties. The compound demonstrated significant antioxidant activity, particularly in the DPPH radical scavenging test, and showed potent antibacterial and antifungal effects against various pathogenic microorganisms. These results support the traditional applications of M. fragrans and indicate that its essential oil, particularly the elemicin-rich fraction, could be a valuable source of natural antioxidants and antimicrobial substances. Consequently, M. fragrans presents potential for use in food preservation and pharmaceutical development, making it a promising subject for further research and industrial application.

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