

Antimicrobial activities and phytochemical analyses of *Myristica fragrans* Seed extracts on Some Clinical Microbial Isolates

¹Imoniye, F. E., ¹Igeleke, C. L. and ^{2*}Enerijiofi, K. E.

Page | 5225 ¹Department of Biological Sciences, Faculty of Science, Benson Idahosa University, Benin City, Edo State, Nigeria

²Department of Biological Sciences, College of Basic, Applied and Health Sciences, Glorious Vision University, Ogwa, Edo State, Nigeria

Date Received: 01-02-2025

Date Accepted: 14-04-2025

DOI: <https://doi.org/10.48198/NJPAS/25.A06>

ABSTRACT

The global public health challenge resulting from antimicrobial resistance (AMR) reaching about 5 million annually necessitates the search for viable alternatives to synthetic drugs for treating infectious diseases. In this study, the phytochemical and antimicrobial activities of the *Myristica fragrans* seed extract was evaluated against some selected clinical isolates. The phytochemical screening was performed using standard methods, while the antimicrobial assay, and the minimum inhibitory/ bactericidal concentration tests of the extracts were conducted using agar well and broth dilution methods respectively. Antibiotics susceptibility test was used to determine the sensitivity of the isolates to the seed extracts. The phytochemical analysis yielded some extracts with Quinones, alkaloids, saponins in higher quantities. The results from the antimicrobial assay showed that methanolic extracts had higher inhibitory zones of 20mm against *Escherichia coli* and least in *Staphylococcus aureus* with 9mm. The minimum inhibitory concentration and minimum bactericidal concentration values of 31.25 mg/ml and 62.5mg/ml were recorded for the methanolic and aqueous extracts. Conventional antibiotics like streptomycin, tarvid and gentamycin showed higher zones of inhibition than the seed extracts while others such as ampiclox, erythromycin and pefloxacin failed to inhibit the growth of some isolates. This study showed that *Myristica fragrans* seed extracts possess the potentials to treat antibiotic-resistant infections caused by the tested isolates in this study. However, there is need for further studies on optimization and standardization of the extraction method, mechanisms of actions of the bioactive compounds and toxicity of the *Myristica fragrans* seeds extract.

Keywords: Antimicrobial activities, extracts, *Myristica fragrans*, pathogens, public health

Introduction

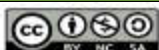
In recent times, researchers have reported that antimicrobial resistance may account for over 10 million annual deaths by 2050 (Ikokwu *et al.*, 2023). This estimation suggests a 100% increase in

the 5 million antimicrobial resistance-related deaths recorded each year (Salam *et al.*, 2023). Several studies have ascribed the steadily advancing global burden of antimicrobial resistance to the indiscriminate usage of

Corresponding Author: Enerijiofi, K. E.

Department of Biological Sciences, College of Basic, Applied and Health Sciences, Glorious Vision University, Ogwa, Edo State, Nigeria.

Email: kingsleyenerijiofi@gmail.com



antibiotics in clinical treatment and animal healthcare (Akortha *et al.*, 2010 Erhabor *et al.*, 2017). At present, humans are also at a heightened risk of contracting antibiotic-resistant pathogens due to the overuse of antibiotics in livestock rearing (Allel *et al.*, 2023). Another prominent issue arising from the upswing of antimicrobial resistance includes economic disadvantages such as delayed hospital stays, and financial difficulties (Chandra *et al.*, 2021; Pulingam *et al.*, 2022). In consideration of these grievous effects and the burgeoning shortage of new therapies, scientists have reignited the exploration of medicinal plants as alternate sources of disease treatment (Anand *et al.*, 2019; Ovuru *et al.*, 2024).

Spanning centuries, Folk medicine proponents have used medicinal plants as natural remedies to address countless maladies around the globe. Numerous studies have also corroborated the pharmacological activities of medicinal plants due to their possession of abundant phytochemicals which can be structurally optimized and processed into new drugs (Enerijiofi and Isola, 2019; Din *et al.*, 2021; Rahu *et al.*, 2021; Aware *et al.*, 2022). *Myristica fragrans*, a verdant tree that bears peach-like fruits, is a paragon of a medicinal plant that contains a variety of bioactive compounds. It is native to countries in the South Pacific, and broadly cultivated in many countries around the world (Namra *et al.*, 2016). In Nigeria, it is referred to as *Ehuru ofia* in the Igbo language, *Guiya dan miya* in Hausa, *Ikposa* in Benin, and *ariwo* in Yoruba (Feyisayo and Oluokun, 2013; Enabulele *et al.*, 2014). Its seed, Nutmeg, is widely used to enhance the taste and flavor of foods, pastries, drinks among other things (Okiki *et al.*, 2023). Furthermore, its record of traditional applications comprises the treatment of ailments such as stomach-aches, hemorrhoids, chronic vomiting, anxiety, arthritis, insomnia and others (Nagja *et al.*, 2016).

The antimicrobial activities of nutmeg have been researched through different extraction solvents

and techniques across multiple studies. In a study by Ibrahim *et al.* (2013), 1 mg/mL of ethanol and acetone nutmeg extracts were found to have high antibacterial and antioxidant action against *Staphylococcus aureus* and *Aspergillus niger*. Similarly, a study by Ben Lagha *et al.* (2020) which explored the preservative capacity of nutmeg discovered a trend of inhibition of the eight food pathogens isolated from raw beef. There are limited studies on *Myristica fragrans* seed extracts against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* clinical isolates which necessitates the need for this study since it is commonly used in the traditional treatment of some ailments. This study aims to provide a novel and holistic perspective of *Myristica fragrans* seed extracts as a potential alternate treatment drug of choice against the aforementioned pathogenic microorganisms isolated from some hospitals in Benin City.

Materials and Methods

Collection and Preparation of Seed Samples

Samples of *Myristica fragrans* seeds obtained from Oba, and Oliha markets in Edo state, Nigeria were pulverized and dried in the oven at 60° C for 2 hours to attain a constant weight. The dried seeds were finely processed using a sterile mortar and pestle. Two hundred (200) grams of the prepared sample was discretely solubilized in 1 L of methanol, aqueous, and n-hexane solvents at 25 °C for 72 hours. The aforementioned solvents were used because of they are readily available, non-toxic, and higher extraction efficiency. Subsequently, a clean muslin cloth was used to filter the nutmeg mixtures, after which the resultant filtrates were evaporated with a rotary evaporator, and oven-dried at 45 °C for 24 hours. Thereafter, the dry extracts were reconstituted by dissolving in 10% dimethyl sulfoxide (DMSO) as diluent and prepared into a range of dilutions

(1000, 500, 250, and 125) in mg/mL (Enerijiofi *et al.*, 2021).

5227

Preparation of Inoculum/Standardization of Test Organisms

Twenty-four (24) hour broth cultures were prepared from the pure isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* supplied by the Bacteriology department of Central hospital, and the Medical microbiology department of University of Benin Teaching Hospital. The bacterial and fungal suspensions were then diluted with small volumes of distilled water to homogenize their densities with a 0.5 mL McFarland Standard. To ensure precision, the final densities of the McFarland standard and the inoculum suspensions were verified using a spectrophotometer.

Phytochemical Analysis

Phytochemical screening of *Myristica fragrans* seeds was performed following the preparation of the methanol, aqueous and n-hexane stock solutions. The extracts were examined to detect phytochemical groups of saponins, alkaloids, flavonoids, cardiac glycosides, steroids, phenols, and terpenoids (Egharevba-Ojo *et al.*, 2024).

Antimicrobial Assessment of the Extracts

The antimicrobial effects of the seed extracts were examined using the agar well diffusion method (Abdulsattar *et al.*, 2021). Mueller-Hinton agar (MHA) was selected as the culture medium and a sterile glass rod was used to evenly distribute 0.1 mL of the standardized test inoculums across the solidified agar. After aseptically drying the lawn plates, a sterile corkborer was used to bore 4 equally spaced wells on each plate. Varied concentrations (1000, 500, 250, and 125 mg/mL) of the seed extracts were then transferred into the wells, after which the inoculated bacterial and fungal plates were incubated at 37 °C for 24 hours and 35 °C 48 hours, respectively. Upon the

completion of the incubation period, the size of the inhibition zones surrounding each well was recorded. The minimum inhibitory concentration values of each extract were ascertained by preparing a series of five (5) concentrations for each extract (62.5, 31.25, 15.62, and 7.81 mg/mL) using the broth dilution technique. These dilutions were obtained by inoculating the extract and nutrient broth mixture with 1 mL of the standardized microorganisms. Similarly, a negative growth control was prepared by mixing an equal volume of the extract concentrations with similar volumes of nutrient broth containing sterile water. After incubation, the inoculated test tubes were compared to the control test tube to detect any changes or marked turbidity. The lowest concentrations with clear supernatants or no turbidity were confirmed as the MIC of each extract. Likewise, the minimum bactericidal concentration values for each extract were determined by observing the MHA plates with no microbial growth after re-incubating 1 mL of the obtained MICs.

Antibiotic Susceptibility Testing

The antibiotic sensitivity of the bacterial microorganisms was investigated using the disk diffusion method (Shafiei, 2012). Sterile Mueller-Hinton plates were evenly seeded with 0.1 mL of the test organisms using a glass spreader and allowed to stand. The antibiotic discs (Streptomycin, Septrin, Erythromycin, Pefloxacin, Gentamycin, Ampiclox, Augmentin, Amoxicillin, Tarivid, and Ciprofloxacin) were carefully placed onto the agar plates and the impregnated media were incubated at a standard temperature. The obtained diameters of the antibiotic inhibition zones were then measured with a metric ruler and compared to the results of the antimicrobial studies.

Results

The phytochemical tests revealed the presence of varying levels of phytochemicals across the

5228

Myristica fragrans seed extracts. Alkaloids were abundantly found in most of the extracts, while terpenoids, steroids and flavonoids were the least present phytochemical compounds found in the extracts. This means that *Myristica fragrans* seed extracts contained more of alkaloids (Table 1). The antimicrobial susceptibility test results proved that the methanol, and aqueous extracts exhibited different levels of inhibition against the selected test isolates. Out of all the seed extracts, the methanol extract possessed the most significant inhibitory activity with its highest zones of inhibition ranging from 13 to 20 mm for *Escherichia coli*, and its least activity holding a record of 11 to 17 mm for *Staphylococcus aureus*. The aqueous extract also displayed inhibitory activity at a maximum of 13 to 18 mm in *Escherichia coli*, and a minimum of 9 to 15 mm in *Staphylococcus aureus*. Contrastingly, the n-hexane extract showed no inhibitory activity against the selected microbes. This means that

methanol and aqueous extracts had higher concentrations of antimicrobial activities (Table 2). The minimum inhibitory concentration (MIC) tests further confirmed the bacterial and fungal inhibitory activities of the methanol and aqueous seed extracts through the absence of turbidity in the media concentrations of 62.50 and 31.25 mg/mL. This points to the fact that 31.25mg/mL was the MIC that inhibited bacterial and fungal growth (Table 3). The methanolic extract was the sole bactericidal agent, eliminating bacterial growth at a concentration of 62.5 mg/mL which is the minimum bactericidal concentration (Table 4). All selected antibiotics showed varying levels of inhibition against the bacterial strains. The smallest region of inhibition was 12 mm obtained from Ampiclox against *S. aureus*, and Amoxicillin against *P. aeruginosa*. Conversely, the largest recorded zone of inhibition was 23 mm from Streptomycin against *S. aureus* and 25 mm for Tarivid against *E. coli* (Table 5).

Table 1: Phytochemical screening of methanol, n-hexane and aqueous extracts of *Myristica fragrans* seeds

Sample	Flavonoids	Cardiac glycosides	Saponins	Steroids	Terpenoids	Alkaloids	Quinones	Phenols
Nutmeg seed (methanol)	–	+	+++	–	–	+++	++	+
Nutmeg seed (n-hexane)	–	+++	–	–	++	+++	+++	–
Nutmeg seed (Aqueous)	+	–	+	+	–	–	–	+

Key: (–) = Absent, (+) = slightly present, (++) = present, (+++) = highly present

Table 2: Antimicrobial activities (Zones of Inhibitions) of methanol, n-hexane and distilled water extracts of *Myristica fragrans* seeds (mg/mL)

Isolates (mm)	Methanol				n-hexane				Aqueous			
	1000	500	250	125	1000	500	250	125	1000	500	250	125
<i>Pseudomonas aeruginosa</i>	18	17	15	14	–	–	–	–	16	14	12	11
<i>Escherichia coli</i>	20	17	16	13	–	–	–	–	18	16	15	13
<i>Staphylococcus aureus</i>	17	15	14	11	–	–	–	–	15	13	11	9
<i>Candida albicans</i>	18	15	13	–	–	–	–	–	17	15	13	10

Key: (–) = Resistant / no inhibition, S=Susceptible, I=Intermediate, R=Replicate

Table 3: Minimum inhibitory concentrations (MIC) of methanol and aqueous extracts of *Myristica fragrans* seeds (mg/mL)

Methanol					Aqueous			
Isolates	62.50	31.25	15.62	7.81	62.5	31.25	15.62	7.81
<i>Pseudomonas aeruginosa</i>	-	-	+	+	-	-	+	+
<i>Escherichia coli</i>	-	-	+	+	-	-	+	+
<i>Staphylococcus aureus</i>	-	-	+	+	-	-	+	+
<i>Candida albicans</i>	-	-	+	+	-	-	+	+

Key: (-) = No turbidity, (+) =Turbidity

Table 4: Minimum bactericidal concentrations (MBC) of methanol and aqueous extract of *Myristica fragrans* (mg/mL)

Methanol					Aqueous			
Isolates	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
62.50 mg/mL	-	-	-	-	+	+	+	+
31.25 mg/mL	+	+	+	+	+	+	+	+

Key: (-) = No Growth, (+) = Growth

Table 5: Antibiotic sensitivity pattern of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (mm)

Antibiotics (mm)	S	SXT	E	PEF	CN	APX	AU	AM	OFX	CPX
<i>Staphylococcus aureus</i>	23	20	21	17	15	12	18	14	19	21
<i>Pseudomonas aeruginosa</i>	20	15	16	15	19	17	13	12	13	22
<i>Escherichia coli</i>	22	18	20	21	20	14	15	25	21	17

Key: S- Streptomycin, SXT- Septrin, E- Erythromycin, PEF- Pefloxacin, CN- Gentamycin, APX-Ampiclox, AU- Augmentin, AM- Amoxicillin, OFX- Tarivid, CPX- Ciprofloxacin.

Discussion

Antimicrobial resistance is a rapidly growing public health concern, which, if left unaddressed, portends an ill fate for the intervention of antibiotics in infectious disease management. The proliferation of drug resistance is propagated by some widespread multidrug-resistant pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. *Escherichia coli* contributes to the distribution of antimicrobial resistance by exploiting the

horizontal gene transfer mechanism to accumulate and donate multiple resistant genes across homogenous and heterogenous species (Paitan, 2018). This process is further accelerated by the presence of plasmids, which may facilitate the stabilization of the resistant genes in the bacterial chromosome.

Labeled the second leading cause of global antimicrobial resistance (AMR) deaths in 2019, *S.aureus* also presents a major threat to public health worldwide (Gajdács, 2019; Antimicrobial

Resistance Collaborators, 2022). The biofilm forming capacity and high adaptability of *P. aeruginosa* distinguishes it from the typical microbe by not only affording it intrinsic protection from antibiotic susceptibility, but also increasing its ease of transmission (Lin *et al.*, 2021). *Candida albicans* also demonstrates its health-compromising capacity by its characterization as one of the top four critical priority fungal pathogens screened in 2022 (World Health Organisation, 2022). As part of the One-Health approach to mitigate the global burden of antimicrobial resistance, it is imperative that we seek out more potent and less toxic antibiotic alternatives that can hamper or eliminate the growth of resistant microbes.

Thus, the current study screened methanolic, n-hexane and aqueous extracts of *Myristica fragrans* seeds to derive useful insights about their phytochemical and antimicrobial properties against the aforementioned isolates. The phytochemical composition in this study revealed that all extracts of the *M. fragrans* seed contained bioactive phytochemicals. The methanolic and n-hexane extracts commonly expressed cardiac glycosides, alkaloids and quinones, while the aqueous and methanolic extracts commonly exhibited saponins and phenols. The methanolic extract had the highest presence of phytochemicals amongst the seed extracts whereas the aqueous extract had the lowest number of phytochemicals. The detection of observed high phytochemical compounds is an indication of the significant antimicrobial activity of the *M. fragrans* seed recorded in this study. Saponins which were copiously discovered in the methanolic and aqueous extracts. They are known as one of the major secondary metabolites present in plants. Many studies have also explored the antimicrobial and prophylactic activities of saponins against resistant bacteria such as *E. coli*, *Proteus mirabilis* and other clinical isolates (Dong *et al.*, 2020; Tatli Cankaya and Somuncuoglu, 2021; Gitanjali *et al.*, 2023). Similarly, phenolic

compounds have demonstrated strong antimicrobial activity against pathogens due to their hydroxyl groups (Álvarez-Martínez *et al.*, 2020). According to recent studies, p-coumaric acid, a phenolic compound, has shown notable reduction in the growth of *Bacillus subtilis*, *Shigella dysenteriae*, *Streptococcus pneumoniae*, and others (Liu *et al.*, 2020; Kaur and Kaur, 2022). The bactericidal and fungicidal potential possessed by these phytochemicals can be ascribed to their ability to hinder the proliferation of microbes through the eradication of molecular targets, improvement of microbial cell wall permeability and suppression of cell protein synthesis (Hochma *et al.*, 2021; Kebede, Gadisa and Tufa, 2021, Mohammed *et al.*, 2021).

The results of the antimicrobial screening revealed that the methanolic and aqueous extracts of the *Myristica fragrans* seed possess potent inhibitory activities against the tested isolates. Both extracts impeded the survival of the test microorganisms, with the methanolic extract yielding the highest recorded zone (20 mm) against *E. coli*. These findings are supported by previous studies (Dzotam and Kuete 2023; Nikouie and Kariminik, 2023). Dzotam and Kuete (2023), reported that the methanolic extract of *Myristica fragrans* seed yielded significant antimicrobial activities against *E. coli*, *Klebsiella pneumoniae*, and *P. aeruginosa*. Nikouie and Kariminik (2023) also reported similar antimicrobial activities of methanolic extracts of *Myristica fragrans* seeds on ESBL-producing *Klebsiella* and *Acinetobacter* bacterial isolates. The significant inhibitory activities exhibited by the methanolic extract can be attributed to its polarity which encourages the solubility of most antimicrobial compounds of plant origin (Mudzengi *et al.*, 2017). Earlier studies have affirmed the efficiency of methanol as an extraction solvent by observing its versatility in the extraction of varied potent phytochemical compounds with low-water solubility such as flavonoids, terpenoids and alkaloids (Nakum and

Bhoya, 2022; Kumar *et al.*, 2023). Conversely, the comparatively smaller zones of inhibition demonstrated by the aqueous extract may be on account of the extract's tendency to easily decompose, leading to the loss of relevant phytochemicals (Adesina *et al.*, 2013; Mercy *et al.*, 2017). Unlike the other extracts, the n-hexane extract failed to display any notable inhibition against the test microorganisms. This result differs from the findings of Panggabean *et al.* (2019), which demonstrated the significant activity of the hexane extract of *Myristica fragrans* against pathogenic microbes isolated from tempeh sausage. According to Nwinyi *et al.* (2009), this disparity can be attributed to plant inactivity, which may stem from the plant or sample's age, the solvent(s) of extraction, extraction techniques, and the collection timeframe of the plant samples.

In accordance with the results of the antimicrobial studies, the MIC and MBC findings showed inhibition of microbial growth at low concentrations of 31.25 and 62.5 mg/mL respectively. The low MICs observed in the methanolic and aqueous extracts indicate the efficacy of *Myristica fragrans* and its potential in the management of diseases caused by these microbes. Additionally, the presence of growth in the minimum bactericidal concentration test conducted on the aqueous extract confirms its non-bacteriostatic nature. Ashish *et al.* (2013) corroborated this result in their study, wherein the MIC results demonstrated a similar range of concentrations for the selected nutmeg extracts. The MIC value of 31.25mg/mL reported in this study indicated that it was the minimum concentration of the extracts that prevented the isolates growth while the 62.5 mg/mL reported for MBC means the concentration that killed the isolates.

In the antibiotic susceptibility test, some antibiotics such as Streptomycin, Tarvid, Gentamycin and others showed higher inhibition zones against the test isolates compared to the

extracts of *Myristica fragrans* seed. However, according to the CLSI standards, some of the isolated microorganisms were resistant to some antibiotics such as Ampiclox, Erythromycin and Pefloxacin (CLSI, 2020). The discrepancy between the effectiveness of the antibiotics and the seed extracts may be related to the purified nature of standard antibiotics, and the comparatively crude composition of plant extracts. Previous studies; Ekozin *et al.*, (2025), Ovuru *et al.*, (2024) have affirmed that plant extracts are likely to contain impure chemicals that are inactive and as such incapable of antimicrobial action. The results of the antimicrobial activity reported in this study could be because of the impact of extraction method phytochemicals yield and the concentration of available bioactive compounds. On the contrary, synthetic antibiotics tend to contain their active ingredients in their purest form, thereby ensuring consistent potency levels in their natural condition (Williams, 2021).

Conclusion

The spice, *Myristica fragrans*, is locally considered a curative agent for numerous diseases. The outcomes of this study demonstrated that the methanolic and aqueous extracts of *Myristica fragrans* seeds contain effective bioactive substances and significant antimicrobial properties. Based on the current findings, *M. fragrans* serves as a promising contender for natural antimicrobial agents that can act against diseases caused by *S. aureus*, *P. aeruginosa*, *C. albicans*, and *E. coli*. Furthermore, there is need for more studies on the mechanism of actions exhibited by the bioactive compounds present in the *M. fragrans* seeds extract against the microbial isolates to foster a holistic understanding of their medicinal capabilities. Also, worthy of further exploration is the standardization of extraction techniques for higher yield, purification and isolation of the observed phytochemicals and the extract toxicity so as to determine its safety for use in the treatment of

health challenges associated with the studied isolates. This may aid the discovery of novel compounds, and consequently, the production and formulation of new drugs.

References

- Abdulsattar, J., Orabi, M. and Nasi, Z. (2021). Phytochemical profile, antimicrobial, antioxidant activity and Cyclooxygenase 2 inhibitory properties of nutmeg (*Myristica fragrans*) seeds extract. *Egyptian Journal of Chemistry*, 65(1), 317-326. <https://doi.org/10.21608/ejchem.2021.78192.3831>
- Antimicrobial Resistance Collaborators. (2022). Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis. *The Lancet*, 399(10325), 629–655. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0)
- Ashish, D. G., Vipin, K. B., Vikash, B. and Nishi, M. (2013). Chemistry, antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans* houtt). *Journal of Genetic Engineering and Biotechnology*, 11(1), 25-31. doi.org/10.1016/j.jgeb.2012.12.001
- Adesina, I. A., Adebote, V. T., Elehinafe, T. R. and Enerijiofi, K. E. (2013). Growth inhibition of *Aspergillus niger* and *Penicillium italicum* by seed kernel oil from Mango (*Mangifera indica* L.). *Journal of Natural Sciences Research*, 3(9), 61-64.
- Akortha, E. E., Aluyi, H. S. A. and Enerijiofi, K. E. (2010). Plasmid encoded amoxicillin resistance in common bacterial pathogens from patients in University of Benin Hospital, Benin City, South Western Nigeria. *Journal of Medicine and Medical Sciences*, 1(10), 478-484.
- Allel, K., Day, L., Hamilton, A., Lin, L., Furuya-Kanamori, L., Moore, C.E., Van Boeckel, T., Laxminarayan, R. and Yakob, L. (2023). Global antimicrobial-resistance drivers: an ecological country-level study at the human–animal interface. *The Lancet Planetary Health*, 7(4), e291-e303. [https://doi.org/10.1016/S2542-5196\(23\)00026-8](https://doi.org/10.1016/S2542-5196(23)00026-8)

Conflict of Interest Statement

There is no conflict of interest in this study.

- Álvarez-Martínez, F. J., Barrajón-Catalán, E., Encinar, J. A., Rodríguez-Díaz, J. C. and Micol, V. (2020). Antimicrobial Capacity of Plant Polyphenols against Gram-positive Bacteria: A Comprehensive Review. *Current Medicinal Chemistry*, 27(15), 2576–2606. <https://doi.org/10.2174/0929867325666181008115650>
- Anand, U., Jacobo-Herrera, N., Altemimi, A. and Lakhssassi, N. (2019). A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. *Metabolites*, 9(11), 258. <https://doi.org/10.3390/metabo9110258>
- Aware, C. B., Patil, D. N., Suryawanshi, S. S., Mali, P.R., Rane, M.R., Gurav, R.G. and Jadhav, J.P. (2022). Natural bioactive products as promising therapeutics: A review of natural product-based drug development. *South African Journal of Botany*, 151, 512–528. <https://doi.org/10.1016/j.sajb.2022.05.028>
- Ben Lagha, O. M., Zakaria, M. P., Ismail, I. S., Nor-Khaizura, M. A. R. and Rukayadi, Y. (2020). Antibacterial activity of nutmeg (*Myristica fragrans* Houtt.) extract against foodborne pathogens on raw beef during chilled and frozen storage. *Food Research*, 4(2), 380 – 388. [doi.org/10.26656/fr.2017.4\(2\).280](https://doi.org/10.26656/fr.2017.4(2).280)
- Chandra, P., Mk, U., Ke, V., Mukhopadhyay, C., U. D. A. and V, R. (2021). Antimicrobial resistance and the post antibiotic era: better late than never effort. *Expert opinion on drug safety*, 20(11), 1375-1390. <https://doi.org/10.1080/14740338.2021.1928633>
- CLSI. (2020). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute

- Din, M. U., Ali, A., Yasir, M., Jilani, M. I., Shoaib, S., Latif, M., Ahmad, A., Naz, S., Aslam, F., Iqbal, M., and Nazir, A. (2021). Chemical Composition and in vitro Evaluation of Cytotoxicity, Antioxidant and Antimicrobial Activities of Essential Oil Extracted from *Myristica Fragrans* Houtt. *Polish Journal of Environmental Studies*, 30(2), 1585-1590. <https://doi.org/10.15244/pjoes/124738>
- Dong, S., Yang, X., Zhao, L., Zhang, F., Hou, Z. and Xue, P. (2020). Antibacterial activity and mechanism of action saponins from *Chenopodium quinoa* Willd. husks against foodborne pathogenic bacteria. *Industrial Crops and Products*, 149, 112350. <https://doi.org/10.1016/j.indcrop.2020.112350>
- Dzotam, J.K. and Kuete, V. (2023). *Myristica fragrans* as a potential source of antibacterial agents. *Advances in botanical research*, 107, 213–237. <https://doi.org/10.1016/bs.abr.2022.08.017>
- Egharevba-Ojo, P., Igue, E. E., Enerijiofi, K. E. and Amengialue, O. O. (2024). Phytochemical screening and anti-*Helicobacter pylori* activity of *Bryophyllum pinnatum*, *Ocimum gratissimum* and *Vernonia amygdalina* *Journal of Bioscience and Biotechnology*, 13 (2), 163 – 171. doi.org/10.69085/jbb20242163
- Ekozin, A .A., Isola, O. B., Onyigen, O. H., Omojoyegbe, R. T., Enerijiofi, K. E. and Olaitan E. O. (2025). Mechanistic insights into *Ageratum conyzoides* L. in spontaneous abortion: A computational and pharmacological study. *Pharmaceutical Research - Natural Products- Elsevier*, 6, 100143. doi.org/10.1016/j.prenap.2025.100143
- Enabulele, S. A., Oboh, F. O. J. and Uwadiae, E. O. (2014). Antimicrobial, nutritional and phytochemical properties of *Monodora myristica* seeds. *IOSR Journal of Pharmacy and Biological sciences*, 9(4),1 - 6. doi.org/10.9790/3008-09430106
- Enerijiofi, K. E. and Isola, O. B. (2019). Preliminary Phytochemical screening and *invitro* antibacterial activities of aqueous and ethanol extracts of *Ageratum conyzoides* L. Leaf, Stem, Flower and Root on some Bacterial isolates associated with Diarrhoea. *Nigerian Journal of Pure and Applied Sciences*, 32 (2), 3480 - 3489 <http://dx.doi.org/10.19240/njpas.2019.B09>
- Enerijiofi, K. E., Akapo, F. H. and Erhabor, J. O. (2021). GC-MS Analysis and antibacterial activities of *Moringa oleifera* leaf extracts on selected clinical bacterial isolates. *Bulletin of the National Research Centre*, 45 (179), 1 -10. doi.org/10.1186/s42269-021-00640-9
- Erhabor, J. O., Oshomoh, E. O, Enerijiofi, K. E., Erhabor, C. R., Ogbonnaya, C. S. and Idu, M. (2017). Antibacterial activity and Phytochemical Screening of the Acetone extracts of the Root of Aloe vera (L.) Burm. F. against microorganisms associated with male infertility. *Samuel Adegboyega University Science and Technology Journal*, 2(1), 7 - 16.
- Feyisayo, A.K. and Oluokun, O.O. (2013). Evaluation of antioxidant potentials of *Monodora myristica* (Gaertn) dunel seeds. *African Journal of Food Science* 7(9):317-324. doi.org/10.5897/AJFS2013.1020
- Gajdacs, M. (2019). The continuing threat of methicillin-resistant *Staphylococcus aureus*. *Antibiotics*, 8(2), 52. <https://doi.org/10.3390/antibiotics8020052>
- Gitanjali, J., Ram, D.S.D., Kavitha, R., Amalan, V., Alahmadi, T.A., Alharbi, S.A., Kandasamy, S., Shanmuganthan, R. and Vijayakumar, N. (2023). Antimicrobial, antioxidant, anticancer, and antithrombotic, competency of saponins from the root of *Decalepis hamiltonii*. *Environmental Research*, 231, 116096. <https://doi.org/10.1016/j.envres.2023.116096>
- Hochma, E., Yarmolinsky, L., Khalfin, B., Nisnevitch, M., Ben-Shabat, S. and Nakonechny, F. (2021). Antimicrobial Effect of Phytochemicals from Edible Plants. *Processes*, 9(11), 2089. <https://doi.org/10.3390/pr9112089>

- Ibrahim, K.M., Naem, R.K. and Abd-Sahib, A.S. (2013) Antibacterial activity of nutmeg (*Myristica fragrans*) seed extract against some pathogenic bacteria. *Al-Nahrain Journal of Science*, 16(2),188-192. doi.org/10.22401/JNUS.16.2.29
- Ikokuwu, G. M., Oseghale, I. D., Omoregie, I., Ralph-Okhiria, O. H. and Ighile, E. F. (2023). Emerging Trends in Antimicrobial Resistance and Novel Therapeutic Strategies. *International Journal of Pathogen Research*, 12(3), 10 – 26. doi.org/10.9734/ijpr/2023/v12i3226
- Kaur, J. and Kaur, R. (2022). *p*-Coumaric Acid: A Naturally Occurring Chemical with Potential Therapeutic Applications. *Current Organic Chemistry*, 26(14), 1333 – 1349. doi.org/10.2174/1385272826666221012145959
- Kebede, T., Gadisa, E. and Tufa, A. (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PLOS ONE*, 16(3), e0249253. https://doi.org/10.1371/journal.pone.0249253
- Kumar, A., P, N., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., K, S. and Oz, F. (2023). Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. *Molecules*, 28(2), 887. https://doi.org/10.3390/molecules28020887
- Lin, Q., Pilewski, J.M. and Di, Y.P. (2021). Acidic microenvironment determines antibiotic susceptibility and biofilm formation of *Pseudomonas aeruginosa*. *Frontiers in Microbiology*, 12:747834. https://doi.org/10.3389/fmicb.2021.747834
- Liu, X., Ji, D., Cui, X., Zhang, Z., Li, B., Xu, Y., Chen, T. and Tian, S. (2020). *p*-Coumaric acid induces antioxidant capacity and defense responses of sweet cherry fruit to fungal pathogens. *Postharvest Biology and Technology*, 169, 111297. doi.org/10.1016/j.postharvbio.2020.111297
- Mercy, G. A., Light, F. W. and Gospel, A. (2017). Qualitative and Quantitative Phytochemical screening of Some Plants Used in Ethnomedicine in the Niger Delta Region of Nigeria. *Journal of Food and Nutrition Sciences*, 5(5), 198-205. doi.org/10.11648/j.jfns.20170505.16
- Mohammed, M.J., Anand, U., Altemimi, A.B., Tripathi, V., Guo, Y. and Pratap-Singh, A. (2021). Phenolic Composition, Antioxidant Capacity and Antibacterial Activity of White Wormwood (*Artemisia herba-alba*). *Plants*, 10(1), 164. https://doi.org/10.3390/plants10010164
- Mudzengi, C.P., Murwira, A., Tivapasi, M., Murungweni, C., Burumu, J.V. and Halimani, T. (2017). Antibacterial activity of aqueous and methanol extracts of selected species used in livestock health management. *Pharmaceutical Biology*, 55(1):1054–1060. https://doi.org/10.1080/13880209.2017.1287744
- Nagja, T., Vimal, K., and Sanjeev, A. (2016). *Myristica fragrans*; a comprehensive review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 8(2), 27 - 30. https://journals.innovareacademics.in/index.php/ijpps/article/view/8552.
- Nakum, S. and Bhoya, U. (2022). Comparative Study on In-Vitro Antioxidant activities by ABTS, DPPH, FRAP Assessment Methods and Phytochemical Analysis in Different Solvents Extracted from *Tridax procumbens*. *World Scientific News*, 170, 16 - 31.
- Namra, N., Rafia, R., Ayesha, M. and Jihene, B.G. (2016). Nutmeg: A review on uses and biological properties. *International Journal of Chemical and Biochemical Sciences*, 9:107-110
- Nikouie, E. and Kariminik, A. (2023). Antibacterial Effects of Methanolic Extract of *Myristica fragrans* against *Klebsiella pneumoniae* and *Acinetobacter baumannii* Producing broad-spectrum beta-lactamase. *Biotechnological Journal of Environmental Microbiology*, 1(4), 193 - 200

- Nwinyi, O.C., Nwodo, C.S., Olayinka, A.O., Ikpo, C.O. and Ogunniran, K. O. (2009). Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus*. *African Journal of food Science*, 3(3), 77 - 81
- Okiki, P.A., Nwobi, C.P., Akpor, O.B., Adewole, E. and Agbana, R.D. (2023). Assessment of nutritional and medicinal properties of nutmeg. *Scientific African*, 19: e01548. doi.org/10.1016/j.sciaf.2023.e01548
- Ovuru, K. F., Izah S. C., Yasmin, H., Enerijiofi, K. E., Das, M. and Ogwu, M. C. (2024). Microbial Contaminants of Herbal Remedies: Health Risks and Sustainable Quality Control Strategies. In *Herbal Medicine Phytochemistry. Reference Series in Phytochemistry*. Izah, S. C., Ogwu, M. C., Akram, M. (eds). Springer Nature, Cham. 1571-1600. doi.org/10.1007/978-3-031-43199-9_9
- Paitan, Y. (2018). Current trends in antimicrobial resistance of *Escherichia coli*. *Escherichia coli, a versatile pathogen*, 181-211. https://doi.org/10.1007/82_2018_110
- Panggabean, K.A., Rusmrlin, H. and Suryanto, D. (2019). The utilization of nutmeg seed (*Myristica fragrans* Houtt) extract as an antimicrobial on tempeh sausage. *Earth and Environmental Science*, 260, 1 - 7. doi.org/10.1088/1755-1315/260/1/012087
- Pulingam, T., Parumasivam, T., Gazzali, A. M., Sulaiman, A. M., Chee, J. Y., Lakshmanan, M., Chin, C. F. and Sudesh, K. (2022). Antimicrobial resistance: Prevalence, economic burden, mechanisms of resistance and strategies to overcome. *European Journal of Pharmaceutical Sciences*, 170, 106103. https://doi.org/10.1016/j.ejps.2021.106103
- Rahu, M. I., Naqvi, S. H. A., Memon, N. H., Idrees, M., Kandhro, F., Pathan, N. L., Sarker, M. N. I. and Aqeel Bhutto, M. (2021). Determination of antimicrobial and phytochemical compounds of *Jatropha curcas* plant. *Saudi journal of biological sciences*, 28(5), 2867 – 2876. https://doi.org/10.1016/j.sjbs.2021.02.019
- Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A. and Alqumber, M. A. A. (2023). Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. *Healthcare*, 11(13), 1946. https://doi.org/10.3390/healthcare11131946
- Shafiei, Z., Shuhairi, N.N., Md Fazly Shah Yap, N., Harry Sibungkil, C.A. and Latip. (2012). Antibacterial activity of *Myristica fragrans* against oral pathogens. *Evidence-Based Complementary and Alternative Medicine*, 2012(1): 825362.
- Tatli Cankaya, I. I. and Somuncuoglu, E. I. (2021). Potential and Prophylactic Use of Plants Containing Saponin-Type Compounds as Antibiofilm Agents against Respiratory Tract Infections. *Evidence-Based Complementary and Alternative Medicine*, 2021, 1 – 14. https://doi.org/10.1155/2021/6814215
- Williams, N. (2021). *Herbal versus synthetic medicines*. [online] News Medical. Available at: https://www.news-medical.net/health/Herbal-versus-Synthetic-Medicines.aspx.
- World Health Organization. (2022). *WHO fungal priority pathogens list to guide research, development and public health action*. World Health Organization.