

Research Article

Phytochemical Screening and Antibacterial Effect of Cardamom and Nutmeg Seed Extracts on Microbes Affecting Oral Health in Humans

Neha Sharma^{1*}, Arunachalam Kalirajan², Ramandeep Saini³, Bishakha Thakur⁴, Divjot Kaur⁵, Munsaka Siankuku⁶

^{1*,3,4,5}Department of Biotechnology, Chandigarh College of Technology, Chandigarh Group of Colleges, Landran (140307), Mohali, Punjab, India.

^{2,6}Department of Chemistry and Biology, School of Natural and Applied Sciences, Mulungushi University, Kabwe (80415), Zambia.

Corresponding Author: Neha Sharma

Department of Biotechnology, Chandigarh College of Technology, Chandigarh Group of Colleges, Landran (140307), Mohali, Punjab, India.

Email: dean.research@cgc.edu.in

Received: 06.05.26, Revised: 04.06.26, Accepted: 01.07.26

ABSTRACT

Background: Microorganisms associated with oral health, including *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus mutans*, can cause local and systemic infections in humans. The increasing prevalence of antibiotic-resistant strains necessitates the search for alternative antimicrobial agents from natural sources. Cardamom and nutmeg are medicinal plants known for their therapeutic properties and rich phytochemical composition.

Methods: Seed extracts of cardamom and nutmeg were subjected to qualitative phytochemical screening to identify major secondary metabolites. Antibacterial activity was evaluated against *E. coli*, *S. aureus*, and *S. mutans* using extract concentrations of 100, 75, 50, and 25 mg. The antimicrobial potential of the extracts was determined by measuring the zone of inhibition, and the minimum inhibitory concentration (MIC) against the test organisms was assessed.

Results: Phytochemical analysis confirmed the presence of flavonoids, alkaloids, tannins, terpenes, saponins, and phenolic compounds in both cardamom and nutmeg seed extracts. The antimicrobial assays demonstrated inhibitory activity of the extracts against all tested microorganisms, with varying degrees of effectiveness depending on the extract concentration and bacterial species. MIC findings further supported the antibacterial efficacy of both seed extracts.

Conclusion: The phytochemical constituents and antibacterial activity exhibited by cardamom and nutmeg seed extracts indicate their potential as natural antimicrobial agents against oral pathogens. These findings suggest that the extracts may serve as promising alternatives to synthetic drugs associated with adverse side effects. Further investigations are required to isolate the active compounds and validate their therapeutic applications.

Keywords: Oral Infections, *Escherichia Coli*, *Staphylococcus Aureus*, *Streptococcus Mutans*, Antimicrobial Activity.

INTRODUCTION

Infection of the oral cavity with microbes may lead to the development of bad breath, dental cavities, and inflammation of gums, and even digestive disorders that could further lead to systemic infections in humans [1]. Experimental reports have demonstrated that *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus mutans* reside in the oral cavity of humans and may also spread in terms of cross-infection in humans [2]. Colonization of bacteria in the human oral cavity and their causative role in inducing disorders of the oral cavity, along with the spread of infection to other viscera, indicates a critical impact of

Staphylococcus aureus on human gut health [3]. *Staphylococcus aureus* can cause acute and chronic systemic infections in humans, ranging from sepsis affecting skin and muscles to cardiac complications due to the damaging effect of bacteria on the endocardium layer [4]. It is known to be among the most ubiquitous and dangerous pathogens, being a causative organism for bone, joint, and urinary tract infections, pneumonia, empyema, inflammation of the stomach, intestine, and meninges in humans [5]. Another oral pathogen, *Streptococcus mutans*, freely resides on the tooth surfaces and initiates tooth caries, thereby affecting oral health in humans. It is

one of the causative organisms responsible for infective endocarditis and is also associated with extraoral pathologies in humans [6]. Besides these bacterial pathogens, *Escherichia coli* is also known as the causative organism of diarrhoea, dysentery, cystitis, pneumonia, bacteremia, and severe abdominal infections in human beings [7]. Considering the serious consequences, researchers have raised the alarm globally related to the spread of drug-resistant microbial pathogens, as one of the most serious threats to public health in the 21st century [8]. On a serious note, microbes can transit from the oral cavity to the gastrointestinal tract through direct translocation, which can adversely affect the human gut and induce microbe-related pathologies in humans [9]. To conquer such microbial wars, investigations related to plant-based secondary metabolites are carried out worldwide for evaluation of their antimicrobial effect against disease-causing pathogens [10]. The search for safe treatment for microbial-induced medical conditions urges the need to develop novel antimicrobial agents that can overcome the challenges related to the growth of antibiotic-resistant microbial strains, thereby preventing systemic infections, along with maintaining oral health. Among the treasures of medicinal and aromatic plants, cardamom and nutmeg are well accepted in the food and pharmaceutical industries due to the presence of therapeutically active secondary metabolites in their cellular organelles [11,12]. Hence, in the present study, phytochemical analysis of cardamom and nutmeg seed extracts was carried out before conducting their antimicrobial assay. Cardamom seeds, scientifically known as *Elettaria cardamomum*, belonging to the Zingiberaceae family, are typically grown in tropical regions all over the globe, such as India, Thailand, Mexico, Zambia, and Tanzania. It is used as a food preservative in several edible dishes and is one of the therapeutically active ancient spices that have been used in native medicines for the prevention and control of several infections [13]. Flavour-rich terpenoids and other bioactives of cardamom seeds make it a well-accepted medicinal plant for treating human disorders [14]. Numerous biological activities of cardamom, including antioxidant, antimutagenic, antibacterial, anti-inflammatory, antidiabetic, cardioprotective, hepatoprotective, and chemoprotective effects [15]. In a similar experiment pace, nutmeg seeds, botanically known as *Myristica fragrans*,

are popularly used as an aromatic in desserts, drinks, and in folk medicine for their antimicrobial, antioxidant, psychostimulant, and antithrombotic effects [16]. Internally, nutmeg seed possesses antidiarrheal, antidysenteric, and antiemetic effects, while on topical application, it gives relief from joint and dental pains in humans [17]. Therapeutic insights of nutmeg seeds highlight its novel drug potential as an aromatic, stimulant, antidysenteric, antimicrobial, and anti-inflammatory agent [18]. Hence, for the development of safe antimicrobial agents in the post-antibiotic era, medicinal plant-based extracts, being a promising source of secondary metabolites and capable of exhibiting antimicrobial effects against antibiotic-resistant microbes, are being well explored scientifically in the current decade. Therefore, in the present study, an investigation related to the bacteriostatic and bactericidal effects of the extracts obtained from the seeds of cardamom and nutmeg was carried out by observing their zone of inhibition, minimum inhibitory concentration, and minimum bactericidal concentration against *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus mutans*.

MATERIALS AND METHODS

Plant Materials

Seeds of cardamom and nutmeg were purchased from a seed store named Akuji Enterprises located in Lusaka, Zambia. Seed samples were authenticated in the department before extraction and antimicrobial study.

Microorganisms

Escherichia coli, *Staphylococcus aureus*, and *Streptococcus mutans* were obtained from the Laboratory of the Department of Microbiology at the University Teaching Hospital. Authentication of the microorganism was carried out by cultural characteristics and microscopic examination. All authenticated bacteria were subcultured on nutrient agar slants and stored at 4°C until used for antimicrobial assay.

Extraction

Foreign organic matter was removed, and the seeds were cleaned carefully. Clean, air-dried cardamom and nutmeg seeds were pulverised separately and crushed into fine powders. Both of the weighed seed powders (100 g each) were subjected to Soxhlet extraction using methanol as solvent separately, and the extract was collected in a sealed glass container after an appropriate siphoning procedure [19,13].

Further, for the preparation of aqueous extract, both of the weighed seed powders (50 g each) were subjected to maceration using double-distilled water as a solvent separately. Maceration of both of the seed powders was collected, filtered, and stored in an airtight glass container at a temperature below 25°C [20,21]. After filtration through Whatman filter paper, the excess solvent was recovered by concentrating the methanolic and aqueous extracts in a rotary vacuum evaporator. Concentrated seed extracts were poured into respective test tubes and labelled for conducting phytochemical studies. Extract solutions were diluted with their appropriate solvents and used further for the preparation of discs to carry out an antimicrobial assay.

Phytochemical Screening

Methanolic and aqueous extracts of cardamom and nutmeg seeds were chemically screened for the detection of the secondary metabolites, viz., alkaloids, flavonoids, saponins, glycosides, tannins, terpenes, phenols, and sterols by using a standard procedure [22,23].

Microbiological Investigations

The experimental study was conducted at the Food and Drug Laboratory at the University Teaching Hospital in Lusaka, Zambia.

Preparation of Mueller-Hinton Agar Plates

Mueller-Hinton agar (MHA) plates were prepared according to the instructions of the manufacturer for susceptibility testing studies. Firstly, 39 grams of the powder were weighed on an electric balance and then transferred into a conical flask containing 1 L of distilled water. The contents were then mixed by gently swirling the conical flask over the heat for a minute. Afterwards, it was sterilised in an autoclave at 121°C for 15 minutes and then left to cool at room temperature. The solution was then carefully poured into Petri dishes for the solidification process and stored at 8°C in the refrigerator.

Disc Diffusion Method

Antimicrobial susceptibility studies were done using the disk diffusion technique as described by Kirby-Bauer. Whatman filter paper was used to prepare circular discs using a hole punch, and discs were placed in various test tubes of the different concentrations (100%, 75, 50%, and 25%) of the cardamom and nutmeg seed extracts. The discs were allowed to soak in seed extracts for 24 hours, after which antimicrobial susceptibility tests were performed [24,25]. Test tubes in discs soaked in distilled water and ampicillin (30 µg) served as a standard and a control, respectively.

Preparation of Inoculum

The inoculum preparation was performed according to the American Society of Microbiology [26] (2016). Individual samples of the four colonies of microbes of interest, *S. aureus*, *E. coli*, and *S. mutans*, were touched with the help of sterile individual inoculating loops. The organisms were subsequently put in test tubes, respectively, using sterile saline (2-3ml). The test tubes were swirled gently to have a stable and homogeneous suspension. The turbidity of each suspension was equated to a 0.5 McFarland standard before inoculating the MHA plates [25].

Inoculation of Mueller-Hinton Agar Plate

The general procedure reported by the American Society for Microbiology was used to inoculate the Mueller-Hinton Agar plate [26]. A sterilised swab was used to swab the inoculum tube and whip against the inner part of the test tube to stick microorganisms onto the swab. In addition, to conduct inoculation of the MHA plate, the swab was streaked thrice on the entire surface of the agar. This was done by turning the plate 60 ° precisely every time to ensure that the inoculum was distributed evenly. The excess liquid was picked up with the swab to ensure that the MHA plate was kept in a dry state. This was repeated until all three bacteria had been inoculated on their respective MHA plates, and the swab was then discarded in a special container to avoid contamination. The agar plates were allowed to dry partially (lids) and allowed to be left undisturbed for 3 minutes at room temperature until the discs containing seed extracts were placed on them [25,27].

Placement of the Susceptibility Discs

The discs of susceptibility had been inoculated as per the American Society of Microbiology [26]. Clean forceps were sterilized by heating them over a flame and left to cool. The respective test tubes were then emptied carefully using the forceps, and one disc was removed. The discs were put down carefully on the surface of the agar with the air flow chamber partially covered after the lid of the petri dish was removed. The agar was pressed at the tip with the forceps such that the discs were entirely stuck to the surface. To prevent any contamination, the lid of each petri plate was held at the top of the surface of agar, and this procedure was repeated until all discs were placed on the different MHA plates in contact with different bacteria. The MHA plates were all set and incubated at 35 °C, and then the evaluation was performed after an additional 24 hours of incubation [28]. The zone of inhibition

was measured (mm) after the experiments were repeated three times with a caliper scale.

Estimation of MIC and MBC Values

The minimum concentration of the methanol and aqueous extract of the cardamom and nutmeg seeds was established by establishing the minimum level of seed extract that inhibited the growth of the tested micro-organisms. Further, the minimum bactericidal concentration was taken to be the highest concentration of seed extracts applied on the disc that did not result in bacterial growth as observed on the Mueller-Hinton plates after incubation at temperatures lower than 30 °C. The estimation of MBC is made very simply by the lack of bacterial colonies formed on the surface of the medium in the presence of a particular concentration of seed extracts [29].

Statistical Analysis

Observations related to the minimum inhibitory concentration of methanolic and aqueous extracts obtained from cardamom and nutmeg seeds against the tested bacterial strains were analyzed by one-way ANOVA using SPSS software program. Antibacterial data were recorded as mean $\pm$ standard error, and the comparison among the different concentrations of seed extract was made, followed by least significant differences with a p-value $\leq$ 0.05 considered significant.

RESULTS

Extraction

Solvent extract was concentrated to a dried powder and stored in an amber-colored glass container at a temperature below 25°C. Extractive values and color of cardamom and nutmeg seeds (Table 1).

Table 1. Extractive Values of Cardamom and Nutmeg Seeds

Seeds	Solvent	Extractive Value	Color
Cardamom	Methanol	8.5%	Yellowish Green
	Aqueous	2.5%	Light Greenish
Nutmeg	Methanol	9.5%	Brownish Black
	Aqueous	3.25%	Light Brownish

Phytochemical Screening

Phytochemical screening reveals that alkaloids, glycosides, terpenes, tannins, and saponins are present in the methanolic extract of cardamom seeds, whereas tannins and saponins were found present in the aqueous extract. Besides this, phytochemical analysis of nutmeg seed extracts reveals that secondary metabolites such as alkaloids, flavonoids, tannins, and terpenes were identified in the methanolic extract; however, alkaloids, tannins, and saponins were detected in the aqueous extract

of nutmeg seeds. The results indicate that the alkaloids were present in higher amounts in the aqueous extract of nutmeg seeds in comparison to extracts of cardamom seeds. This could be in line with the intense color that was observed in the nutmeg extract after the phytochemical analysis of alkaloids. It was also observed that phenolics and tannins were present in both of the seeds, pointing towards their antioxidant and astringent properties. Results of phytochemical screening (Tables 2 & 3).

Table 2. Phytochemical Screening of Cardamom Seed Extract

Extract	Alkaloids	Flavonoids	Cardiac Glycosides	Terpenes	Tannins	Saponins	Phenolics
Methanol	+	-	+	+	+	+	+
Aqueous	-	-	-	-	+	+	+

(+ Present, - Absent)

Table 3. Phytochemical Screening of Nutmeg Seed Extract

Extract	Alkaloids	Flavonoids	Cardiac Glycosides	Terpenes	Tannins	Saponins	Phenolics
Methanol	+	+	-	+	+	-	+
Aqueous	+	-	-	-	+	+	+

(+ Present, - Absent)

Microbiological Investigations

The zone of inhibition of microorganisms and minimum inhibitory concentration were

observed during antimicrobial investigation of extracts obtained from the seeds of cardamom and nutmeg against all three pathogens.

Estimation of Zone of Inhibition

Observation indicates that 100mg/ml extracts were most effective against *E. coli*, *S. aureus*, and *S. mutans*. However, aqueous extracts exhibited a weak inhibitory effect on the growth of bacteria except at 100mg/ml. That was found effective. Figure 1A, 1B, and 1C represent confirmatory images for the antimicrobial activity of methanolic and aqueous extracts of

cardamom against *E. coli*, *S. aureus*, and *S. mutans*. The clear zones are an indication that no bacterial colonies grew around the discs, hence representing antibacterial activity, and the discs with partial clear zones around them are an indication that a few colonies were formed around the discs, indicating low inhibition of the test sample (Table 4).

Table 4. Zone Of Inhibition of Microorganisms (Mm) With Cardamom Seeds

Extract(Mg/MI)	Solvent	E. Coli	S. Aureus	S. Mutans
100	Methanol	28.6±1.05	27.4±0.70	32.6±0.85
	Water	10.6±0.66	9.6±0.25	10.8±0.40
75	Methanol	23.8±0.36	16.4±0.75	27.9±1.34
	Water	7.6±1.10	7.7±0.26	7.6±0.50
50	Methanol	10.5±0.25	10.6±1.20	13.9±0.58
	Water	5.3±0.75	4.9±0.20	5.4±0.26
25	Methanol	5.4±0.23	6.8±0.20	7.4±0.20
	Water	2.7±0.40	3.1±0.35	3.5±0.20
Ampicillin	-	29.67±0.25	28.43±0.80	26.63±0.92
Control	DMSO	-	-	-

- indicates no inhibition

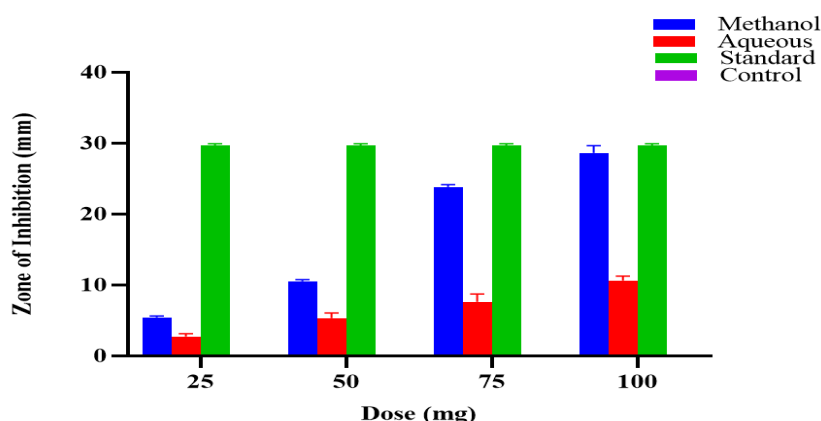


Figure 1A. Graph Representing the Antibacterial Effect of Cardamom Seed Extracts on *E. coli*.

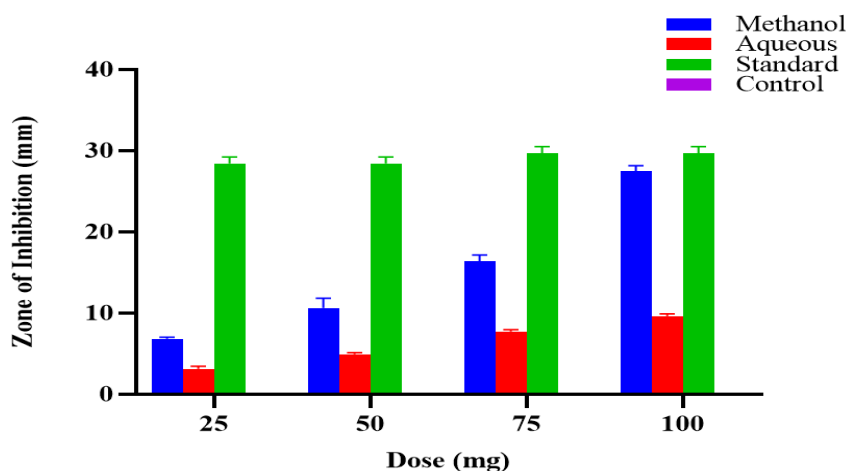


Figure 1B. Graph Representing the Antibacterial Effect of Cardamom Seed Extracts on *S. Aureus*

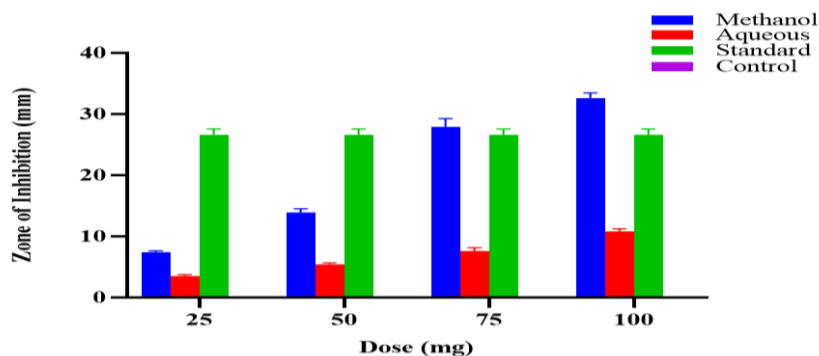


Figure 1C. Graph Representing the Antibacterial Effect of Cardamom Seed Extracts on S. Mutans

Estimation of Zone of Inhibition

Observation indicates that 100 mg/ml methanolic extracts and aqueous extract of nutmeg seeds exhibited a high zone of inhibition against E. coli, S. aureus, and S. mutans. During the antimicrobial assay, it was observed that 25-75 mg/ml methanolic extracts were less effective in comparison to 100mg/ml. However, aqueous extracts exhibited less inhibitory effect on the growth of any bacteria as compared to methanolic extracts and the standard. Figures 2A,2B and 2C represent confirmatory images for the

antimicrobial activity of the methanolic extract of nutmeg against E. coli, S. aureus, and S. mutans. The zone of inhibition clearly marks the area with the inhibitory effect of nutmeg seeds, with an indication that bacterial colonies fail to grow around the discs, hence representing antibacterial activity. Colonies formed around the discs, indicating partial or weak inhibition of the seed extract, pointing towards inefficacy of the seed extract against bacteria under investigation. The degree of sensitivity with reference to the zones that were observed against all three bacteria (Table 5).

Table 5. Zone Of Inhibition of Microorganisms (Mm) With Nutmeg Seeds

Extract(Mg/MI)	Solvent	E. Coli	S. Aureus	S. Mutans
100	Methanol	30.8±0.60	30.6±0.86	33.53±1.80
	Water	9.3±0.10	11.4±1.30	12.1±0.30
75	Methanol	27.3±1.84	18.4±1.00	29.53±0.96
	Water	7.3±0.26	8.6±0.49	9.5±0.41
50	Methanol	12.5±0.90	13.6±0.64	15.86±0.56
	Water	4.8±0.35	6.8±0.30	7.5±0.56
25	Methanol	6.7±0.41	8.7±0.60	9.1±0.36
	Water	2.5±0.32	4.5±0.51	5.6±0.36
Ampicillin	-	31.46±0.66	31.03±0.68	34.23±0.90
Control	DMSO	-	-	-

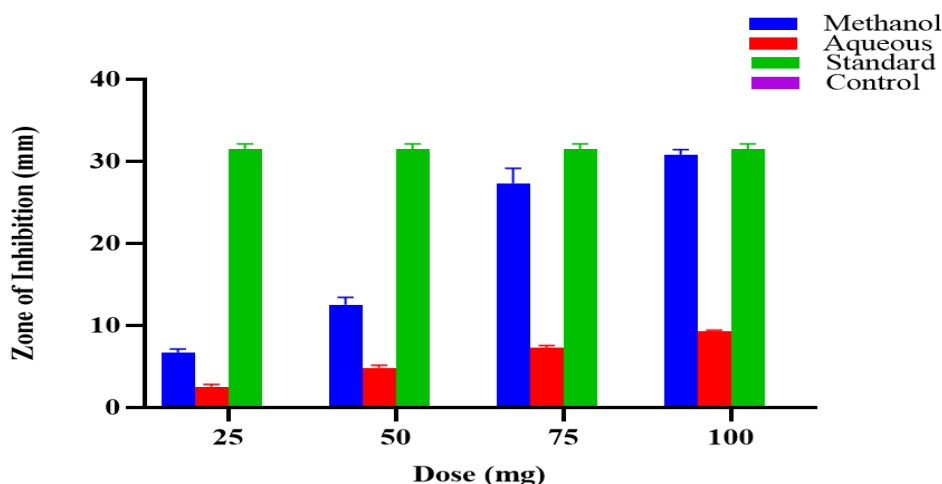


Figure 2A. Graph Representing the Antibacterial Effect of Nutmeg Seed Extracts on E. coli

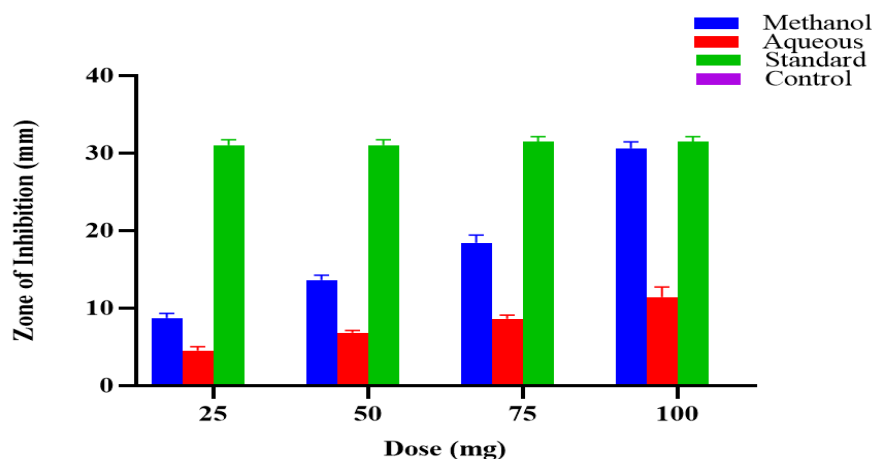


Figure 2B. Graph Representing the Antibacterial Effect of Nutmeg Seed Extracts on *S. Aureus*

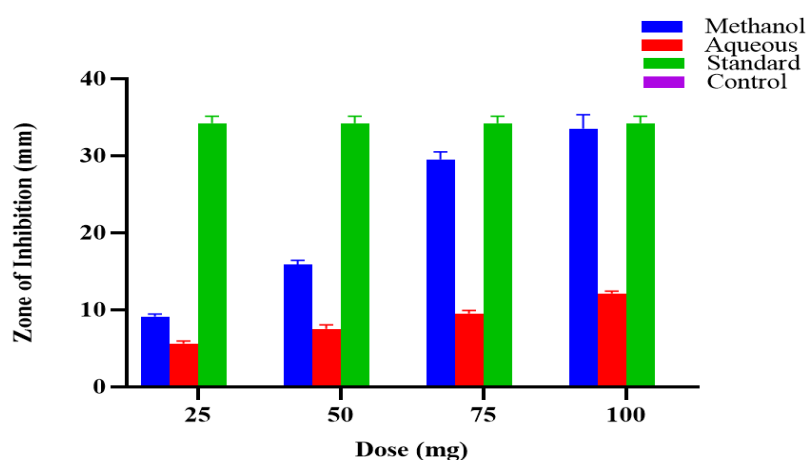


Figure 2C. Graph Representing the Antibacterial Effect of Nutmeg Seed Extracts on *S. Mutans*

Determination of Minimum Inhibitory Concentration

The lowest concentration of cardamom and nutmeg seeds extract that inhibits the growth

of the test microorganisms, and with a complete zone of inhibition was observed for consideration as minimum inhibitory concentration (Table 6).

Table 6. MIC Determination with Cardamom and Nutmeg Seeds

Seeds	Extract	E. Coli	S. Aureus	S. Mutans
Cardamom	Methanol	50	75	50
	Aqueous	-	-	-
Nutmeg	Methanol	25	50	50
	Aqueous	100	100	100

Statistical Analysis

As per statistical analysis with Graph Pad Prism, the comparison graphs representing bacteriostatic action of the aqueous and methanolic extracts of cardamom and nutmeg seeds against the test organisms were analyzed. Graphs suggest that the methanolic extracts exhibited greater inhibitory effect against all three test microorganisms as compared to the aqueous extracts. Comparatively, the antimicrobial effect of the

methanolic extracts of the cardamom and nutmeg seeds was found to be two to threefold higher than that of the aqueous extracts.

DISCUSSION

In the present study, phytochemical screening indicates the presence and absence of phytoconstituents in the seed extract of cardamom and nutmeg on the basis of chemical identification tests. With reference to the zone of inhibition and MIC, it was observed that E.

E. coli, *S. aureus*, and *S. mutans* were found sensitive to the methanol extracts (25, 50, 75, 100 mg/ml) of cardamom and nutmeg seeds. The effectiveness and observation of the zone of inhibition with the methanolic extract can be attributed to the presence of an appropriate amount of alkaloids, terpenes, tannins, phenolics, or saponins in the cardamom seeds and the presence of alkaloids, flavonoids, terpenes, phenolics, and tannins in nutmeg seeds. All three bacteria, *E. coli*, *S. aureus*, and *S. mutans*, also exhibited less sensitivity to the aqueous extract of cardamom and nutmeg seeds; this can be tied to the presence of tannins or phenolics in the cardamom and nutmeg seeds extract. Experiment findings suggest that the highest zone of inhibition was produced with 100 mg/ml methanolic extract of cardamom and nutmeg seeds against *E. coli* and *S. mutans*; however, *S. aureus* was found to be less sensitive to both of the extracts. It was also observed that the extracts of lower concentration, viz, 25, 50, 75 mg of both cardamom and nutmeg extracts, showed minimal inhibition zones, and this variation in the degree of sensitivity of the bacterial strains towards the extracts may be due to different antimicrobial potency of phytochemicals biosynthesized in the cardamom and nutmeg seeds. The zone of inhibition produced by the methanolic and aqueous extracts of the cardamom and nutmeg seeds highlights their potential for antibacterial use. Furthermore, the zone of inhibition produced by the methanolic extracts of cardamom and nutmeg seeds was almost closer to the zone of inhibition produced by the standard drug. The results of the present study were found in accordance with numerous experiments related to the antibacterial effect of aromatic plants that were conducted by the researchers in the past[30,31]. On the whole, both cardamom and nutmeg seed extracts exhibited antimicrobial activity against *E. coli*, *S. aureus*, and *S. mutans*.

CONCLUSION

Experimental findings reveal that phytochemicals screened in the methanolic and aqueous extracts of cardamom and nutmeg possess antibacterial activity against *E. coli*, *S. aureus*, and *S. mutans*, with the lowest sensitivity being observed by *S. aureus* with both extracts. Further synergistic studies could be carried out for the bioassay of phytochemicals.

Author Contributions:

Conceptualization: Neha Sharma and Arunachalam Kalirajan; Methodology: Neha Sharma, Arunachalam Kalirajan, and Munsaka Siankuku; Investigation: Neha Sharma, Bishakha Thakur, Divjot Kaur, and Munsaka Siankuku; Data Curation: Neha Sharma and Ramandeep Saini; Formal Analysis: Ramandeep Saini and Arunachalam Kalirajan; Visualization: Bishakha Thakur and Divjot Kaur; Writing—Original Draft Preparation: Neha Sharma and Ramandeep Saini; Writing—Review and Editing: Arunachalam Kalirajan, Ramandeep Saini, and Munsaka Siankuku; Supervision: Arunachalam Kalirajan- All authors have read and approved the final version of the manuscript.

Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement:

The data supporting the findings of this study are available within the article. Additional data related to this study are available from the corresponding author upon reasonable request.

Funding:

The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical Approval:

This study did not involve human participants, human tissues, or animal experimentation. Therefore, ethical approval was not required.

REFERENCES

1. Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. *Clinical Microbiology Reviews*. 2000;13(4):547-568.
2. Gendron R, Grenier D, Maheu-Robert LF. The oral cavity is a reservoir of bacterial pathogens for focal infections. *Microbes and Infection*. 2000;2(8):897-906.
3. McCormack MG, Smith AJ, Akram AN, Jackson M, Robertson D, Edwards G. *Staphylococcus aureus* and the oral cavity: An overlooked source of carriage and infection? *American Journal of Infection Control*. 2015;43(1):35-37.
4. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*. 2015;28(3):603-661.

5. DeLeo FR, Diep BA, Otto M. Host defense and pathogenesis in *Staphylococcus aureus* infections. *Infectious Disease Clinics of North America*. 2009;23(1):17-34.
6. Lemos JA, Palmer SR, Zeng L, et al. The biology of *Streptococcus mutans*. *Gram-Positive Pathogens*. 2019;435-448.
7. Braz VS, Melchior K, Moreira CG. *Escherichia coli* is a multifaceted pathogenic and versatile bacterium. *Frontiers in Cellular and Infection Microbiology*. 2020;10.
8. Salam MA, Al-Amin MY, Salam MT, et al. Antimicrobial resistance: A growing serious threat to global public health. *Healthcare*. 2023;11(13):1946.
9. Mueller M, Tainter CR. *Escherichia coli* infection. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2025.
10. Keita K, Darkoh C, Okafor F. Secondary plant metabolites as potent drug candidates against antimicrobial-resistant pathogens. *SN Applied Sciences*. 2022;4(8).
11. Pandian A. *Ethnopharmacology and phytochemistry of cardamom*. 2021.
12. Al-Rawi SS, Ibrahim AH, Ahmed HJ, Khudhur ZO. Therapeutic and pharmacological prospects of nutmeg seed: A comprehensive review for novel drug potential insights. *Saudi Pharmaceutical Journal*. 2024;32(6):102067.
13. Abdullah A, Ahmad N, Tian W, et al. Recent advances in the extraction, chemical composition, therapeutic potential, and delivery of cardamom phytochemicals. *Frontiers in Nutrition*. 2022;9.
14. Ghazi AM, Na'ma AJ, Kshash QH, Jasim NS. Antibacterial activity of *Elettaria cardamomum* extracts on the growth of some gingivitis-inducing bacteria in culture media. *International Journal of Drug Delivery Technology*. 2020;10(1):38-45.
15. Yahyazadeh R, Ghasemzadeh Rahbardoar M, Razavi BM, Karimi G, Hosseinzadeh H. Effect of *Elettaria cardamomum* (cardamom) on metabolic syndrome: Narrative review. *Iranian Journal of Basic Medical Sciences*. 2021;24(11):1462-1469.
16. Panggabean KA, Rusmarilin H, Suryanto D. Utilization of nutmeg seed (*Myristica fragrans* Houtt.) extract as an antimicrobial on tempeh sausage. *IOP Conference Series: Earth and Environmental Science*. 2019;260(1):012087.
17. Ibrahim MA, Cantrell CL, Jeliaskova EA, Astatkie T, Zheljaskov VD. Utilization of nutmeg (*Myristica fragrans* Houtt.) seed hydrodistillation time to produce essential oil fractions with varied compositions and pharmacological effects. *Molecules*. 2020;25(3):565.
18. Verma NK, Singh AK, Maurya A. *Myristica fragrans* (nutmeg): A brief review. *EAS Journal of Pharmacy and Pharmacology*. 2021;3(5):133-137.
19. Redfern J, Kinninmonth M, Burdass D, Verran J. Using Soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. *Journal of Microbiology and Biology Education*. 2014;15(1):45-46.
20. Morsy NFS. A comparative study of nutmeg (*Myristica fragrans* Houtt.) oleoresins obtained by conventional and green extraction techniques. *Journal of Food Science and Technology*. 2016;53(10):3770-3777.
21. Latifasari N, Faizah F, Kurniawati AD. Effect of time extraction cardamom seeds in aqueous solvent on physical, total phenol, and flavonoid properties. *Journal of Applied Food Technology*. 2023;11(1):1-7.
22. Shailesh. Preliminary phytochemical and antimicrobial screening of *Syzygium aromaticum*, *Elettaria cardamomum* and *Piper nigrum* extracts. *Journal of Pharmacognosy and Phytochemistry*. 2015;4:85-89.
23. Mendhekar SY, Balsaraf CD, Bangar MS, Jadhav SL, Gaikwad DD. Pharmacognostic, phytochemical, physicochemical and TLC profile study of mace (aril) of *Myristica malabarica* Lamk. (*Myristicaceae*). *Journal of Phytopharmacology*. 2017;6(6):329-334.
24. Biemer JJ. Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. *Annals of Clinical and Laboratory Science*. 1973;3(2):135-140.
25. Schumacher A, Vranken T, Malhotra A, Arts JJC, Habibovic P. In vitro antimicrobial susceptibility testing methods: Agar dilution to 3D tissue-engineered models. *European Journal of Clinical Microbiology and Infectious Diseases*. 2017;37(2):187-208.

26. Wicks JH, Nelson RL, Krejcarek GE. Rapid inoculum standardization system: A novel device for standardization of inocula in antimicrobial susceptibility testing. *Journal of Clinical Microbiology*. 1983;17(6):1114-1119.
27. Patel K, Bunachita S, Agarwal AA, Bhamidipati A, Patel UK. Comprehensive overview of antibiotic selection and its influencing factors. *Cureus*. 2021.
28. Famuyide IM, Aro AO, Fasina FO, Eloff JN, McGaw LJ. Antibacterial and antibiofilm activity of acetone leaf extracts of nine under-investigated South African *Eugenia* and *Syzygium* species (Myrtaceae) and their selectivity indices. *BMC Complementary and Alternative Medicine*. 2019;19(1).
29. Phuyal N, Jha PK, Raturi PP, Rajbhandary S. In vitro antibacterial activities of methanolic extracts of fruits, seeds, and bark of *Zanthoxylum armatum* DC. *Journal of Tropical Medicine*. 2020;2020:1-7.
30. Akrayi HFS. Antibacterial effect of seed extracts of cardamom (*Elettaria cardamomum*) against *Staphylococcus aureus* and *Proteus mirabilis*. *Tikrit Journal of Pure Science*. 2012;17(2).
31. Gupta AD, Bansal VK, Babu V, Maithil N. Chemistry, antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans* Houtt). *Journal of Genetic Engineering and Biotechnology*. 2013;11(1):25-31.