



In-Vitro Antibacterial Activity of Clove Oil Against Food Poisoning and Foodborne Pathogenic Bacteria

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ABSTRACT

As antimicrobial resistance continues to rise, the necessity for discovering natural antimicrobial agents becomes more critical. Clove (*Syzygium aromaticum*) oil is rich in bioactive compounds that exhibit antibacterial properties and can potentially be used as a replacement for conventional antibiotics. Clove oil was obtained by extracting with *n*-hexane in a Soxhlet apparatus and tested for efficacy against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Using disk diffusion methods, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), phytochemical composition was determined using standard methods. Clove oil displayed antibacterial properties that increased with concentration against all the tested bacteria. At 200 mg/mL, the largest diameter of the inhibitory zone was against *P. aeruginosa* (22.00 1.00 mm). For all the bacteria, MIC was 100 mg/mL. But bactericidal efficacy was confirmed only for *P. aeruginosa* at 50 mg/mL. The presence of flavonoids alkaloids phenolic compounds, and terpenoids was identified in the phytochemical analysis. Clove oil demonstrated significant antibacterial activity, with *Pseudomonas aeruginosa* exhibiting the highest susceptibility among the tested organisms. The oil was also found to contain several bioactive phytochemicals with known antimicrobial properties. Further studies are recommended to characterize its chemical constituents using GC-MS analysis, assess its safety profile, and evaluate its antimicrobial efficacy *in vivo*.

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INTRODUCTION

Essential oils (EOs) have received increased interest as natural antibacterial agents and viable replacements or adjuncts to conventional antibiotics in the face of escalating antimicrobial resistance (AMR). Clove oil, obtained from dried flower buds of *Syzygium aromaticum*, is one of the most thoroughly examined essential oils because to its high level of phenylpropanoids, mainly eugenol, as well as β -caryophyllene and eugenyl acetate (Ahamad, 2023; Silva *et al.*, 2024). These ingredients are primarily responsible for the oil's biological action and desirable sensory characteristics.

According to recent research, clove oil and eugenol show broad-spectrum antibacterial

action against both Gram-positive and Gram-negative bacteria, including multidrug-resistant clinical isolates and foodborne pathogens (Salisu *et al.*, 2021; Tariq *et al.*, 2025). The loss of cytoplasmic membrane integrity, intracellular component leakage, oxidative stress induction, and inhibition of biofilm formation and virulence factor expression are among the antimicrobial mechanisms suggested (Nisar *et al.*, 2021; Qian *et al.*, 2020; Armah *et al.*, 2025). Because of these qualities, clove oil is becoming more and more popular as a natural food preservative and as a potential ingredient in antimicrobial formulations.

Clove oil has been demonstrated in the food sector to prevent a variety of food-associated microbes while providing benefits over artificial

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preservatives like potassium sorbate and sodium benzoate, such as increased customer acceptance and perceived safety (Heidelberg, 2024). Several countries, including the US, the EU, and China, have authorized eugenol as a food preservative (Hu *et al.*, 2018).

Despite rising evidence of clove oil's antibacterial activity, more research against clinically significant bacterial infections is needed to evaluate its efficiency and warrant its inclusion in antimicrobial formulations. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* are some of the most common opportunistic pathogens found in healthcare and the population (Lewis, 2021). These organisms have a large number of virulence characteristics and are growing resistant to several antibiotic classes, offering significant therapeutic problems.

Therefore, the present study evaluated the *in vitro* antimicrobial activity of clove oil against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The findings are expected to provide additional scientific evidence supporting the application of clove oil as a natural antimicrobial agent and contribute to ongoing efforts aimed at addressing the global burden of antimicrobial resistance

MATERIALS AND METHODS

Study Area

The study was conducted in Microbiology laboratory, Confluence University of Science and Technology, Kogi State. Osara is located in Adavi Local Government Area of Kogi State

Sample collection and processing

Cloves was purchased from International Market in Lokoja, Kogi state. It was dried under the sun for easy grinding. The dried cloves were grinded into coarse powdered using electronic blender and weighed 480g using a weighing balance.

Preparation of extract using Soxhlet apparatus

The Soxhlet Apparatus was assembled with round bottom flask containing 500ml of N-

Hexane, the extractor was connected to a condenser on the top and the entire set up is fixed securely over a water bath. The N-Hexane in the round bottom flask was heated to reflux. Vapours travelled up into the condenser, where they condensed and dropped into the thimble that contain the clove powder, the siphon arm filled to a certain level, the solvent that contained the extracted was siphoned back in the boiling flask. The cycle was repeated continuously for 2-6 hours until the solvent in the siphon became colourless. After the extraction, the N-Hexane solvent and oil mixture in the flask were subjected to evaporation to remove the N-Hexane. The solvent was evaporated in ceramic container leaving behind the concentrated clove oil.

Qualitative phytochemical screening

Clove oil was subjected to qualitative phytochemical screening utilizing standard preliminary phytochemical tests to evaluate the presence of several bioactive constituents, including alkaloids, glycosides, flavonoids, tannins, saponins, and phenolic compounds (Tasneem and Narsegowda, 2018). These assays were used to determine the major secondary metabolites in the oil. All tests included appropriate positive and negative controls to confirm the results' reliability and correctness.

1. **Alkaloids** were detected using Mayer's, Dragendorff's, and Wagner's tests. The formation of creamy white, orange-brown, and reddish-brown precipitates, respectively, indicated a positive reaction.
2. **Flavonoids** were identified using the alkaline reagent test, lead acetate test, and Shinoda (magnesium-HCl reduction) test. The appearance of an intense yellow coloration reversible by acid addition, yellow precipitate formation, and pink to red coloration, respectively, confirmed the presence of flavonoids.
3. **Tannins** were assessed using ferric chloride and gelatin tests. The development of blue-black or green-black coloration following ferric chloride addition and white precipitate formation with gelatin

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solution indicated hydrolysable and condensed tannins.

4. **Saponins** were detected using the foam test, where persistent frothing (≥ 1 cm) lasting at least 15 min signified a positive result.
5. **Glycosides** were evaluated using the Keller–Kiliani test for cardiac glycosides and Fehling's test following acid hydrolysis. The formation of a reddish-brown interfacial ring and a brick-red precipitate, respectively, indicated glycoside presence.
6. **Phenolic compounds** were confirmed by a blue coloration upon addition of ferric chloride solution, while terpenoids were detected using the Salkowski test, indicated by a reddish-brown ring at the interface

Preparation of Clove Oil Concentrations

Dimethyl sulfoxide (DMSO) was used to reconstitute the clove oil concentrations for antimicrobial assays. A stock solution (200 mg/mL) was prepared by dissolving 1 g of clove oil in 5 mL of DMSO. Serial dilutions were performed to obtain 100 mg/mL and 50 mg/mL concentrations using equal-volume dilution with DMSO.

Test Bacterial

The test bacterial used in this study were *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. These pure isolates were obtained from the Department of Microbiology, Confluence University of Science and Technology, Osara, Kogi State.

The isolates were maintained on agar slants at 4°C and subsequently sub-cultured for 24 hours

Preparation of Culture Media

The required quantities of dehydrated Nutrient Agar were weighed and prepared using distilled water according to the manufacturer's instructions.

Antibacterial Sensitivity Test

The antibacterial activity of clove oil was evaluated using the disc diffusion method on Mueller–Hinton agar. Sterile filter paper discs were impregnated with clove oil at concentrations of 200, 100, and 50 mg/mL and placed on agar plates inoculated with standardized bacterial suspensions. Plates were incubated at 37 °C for 24 h, after which zones of inhibition were measured in millimeters. All tests were performed in duplicate, and results were compared with standard antibiotic controls.

The surfaces of the agar plates were inoculated with standardized inocula equivalent to 0.5 McFarland turbidity standards of the test bacterial and fungal isolates (Abalaka *et al.*, 2016). A sterile 6 mm cork borer was then used to create wells in the inoculated agar plates. Using a micropipette, different concentrations of the extract were dispensed into the wells (Salisu *et al.*, 2017).

The plates were allowed to stand at room temperature for 30 minutes to facilitate diffusion of the extracts into the agar medium before incubation. The culture plates were incubated at 37°C for 24 hours. The diameters of the zones of inhibition were measured using a transparent metre rule, and the mean values were recorded to the nearest whole number (Irowa *et al.*, 2020). DMSO served as the negative control, while chloramphenicol was used as positive controls.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of clove oil were determined using a modified broth dilution method. Concentration of 100, 50, 25 and 12.5 mg/mL of clove oil were prepared in sterile nutrient broth and inoculated with bacterial suspensions standardized to 0.5 McFarland turbidity. Following incubation at 37 °C for 18–24 h, tubes were examined for turbidity. Aliquots from tubes showing no visible growth were sub-cultured onto nutrient agar to determine bactericidal activity. The lowest concentration



inhibiting visible growth was recorded as the MIC, while the lowest concentration showing no colony growth on agar was recorded as the MBC. All experiments were performed in duplicate in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI, 2020)

RESULTS

The Result of Antibacterial Activity of Clove Oil

Antibacterial activity of clove oil against all tested organisms exhibited concentration-dependent as shown in Table 1. At 200 mg/mL, the highest zone of inhibition was recorded

against *Pseudomonas aeruginosa* (22.00 ± 1.00 mm), followed by *Klebsiella pneumoniae* (10.00 ± 1.00 mm) and *Staphylococcus aureus* (10.00 ± 1.00 mm). At 100 mg/mL, the inhibition zones decreased to 17.00 ± 0.00 mm, 4.00 ± 1.00 mm, and 6.00 ± 0.00 mm, respectively, while at 50 mg/mL, values further declined to 6.00 ± 1.00 mm, 2.50 ± 0.50 mm, and 3.00 ± 1.00 mm. The positive control produced inhibition zones of 6.00 ± 1.00 mm against *P. aeruginosa*, 15.00 ± 0.00 mm against *K. pneumoniae*, and 6.00 ± 1.00 mm against *S. aureus*, whereas the negative control showed no inhibitory activity (0.00 mm) against all organisms.

Table 1: Antibacterial Activity of Clove Oil

Concentration /Organisms	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>
200	22.00±1.00 ^d	10.00±1.00 ^c	10.00±1.00 ^d
100	17.00±0.00 ^c	4.00±1.00 ^b	6.00±0.00 ^c
50	6.00±1.00 ^b	2.50±0.50 ^b	3.00±1.00 ^b
+ve control	6.00±1.00 ^b	15.00±00 ^d	6.00±1.00 ^c
-ve control	0.00±00 ^a	0.00±00 ^a	0.00±00 ^a

Results Represent Mean ± Standard Error Mean of duplicate determinations. Values with the same subscript on the same column and not significantly different at (P < 0.05). +ve control (25mg/mL) = chloramphenicol, -ve control = DMSO

Minimum Inhibitory and Bactericidal Concentrations

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of clove oil against the test organisms are presented in Table 2. The MIC for all tested bacterial species was determined to be 100 mg/mL, indicating that this concentration was sufficient to inhibit visible bacterial growth. Bactericidal activity was observed only against *P. aeruginosa* at an MBC of 50 mg/mL, while no bactericidal effect was detected against *S. aureus* and *K. pneumoniae* within the tested concentration range. This suggests that clove oil exhibits predominantly bacteriostatic effects against these organisms under the experimental conditions.

Table 2: Minimum Inhibitory and Minimum Bactericidal Concentrations

Bacterial Species	MIC (mg)	MBC (mg)
<i>Staphylococcus aureus</i>	100	0
<i>Pseudomonas aeruginosa</i>	100	50
<i>Klebsiella Pneumoniae</i>	100	0

Phytochemical Composition of Clove Oil

Qualitative phytochemical analysis of clove oil revealed the presence of flavonoids, alkaloids, phenolic compounds, and terpenoids, while tannins, cardiac glycosides, saponins, and steroids were absent as shown in Table 3. The detection of phenols and terpenoids is consistent with the known chemical composition of clove oil, particularly its high eugenol content. These phytochemicals are widely associated with antimicrobial activity and may contribute synergistically to the inhibitory effects observed against the tested bacterial species.

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Table 3: Qualitative phytochemical analysis of Clove Oil

Test	Observation	Inference
Flavonoids	Yellow coloured is observed	+
Tanin	No formation of blue-black coloration	-
Glycosides	No brown correlation formation	-
Saponins	No formation of frothing or foaming	-
Alkaloids	Formation of yellow precipitate	+
Phenol	Formation of greenish coloration if formed	+

DISCUSSION OF FINDINGS

Clove (*Syzygium aromaticum*) essential oil is well-known for its robust antibacterial capabilities, making it an effective natural preservative in food products, especially meat and meat derivatives, where it extends shelf life and improves food safety (Valarezo *et al.*, 2025). In the current investigation, clove oil shown strong antibacterial activity against all tested bacterial pathogens, with inhibitory effects rising with concentration. *P. aeruginosa* had the highest susceptibility, with a zone of inhibition of 22.00 ± 1.00 mm at 200 mg/mL, while *K. pneumoniae* and *S. aureus* were less susceptible.

The observed dose-dependent response shows that increasing clove oil concentrations increases the availability and diffusion of bioactive chemicals capable of inhibiting bacterial growth. Essential oils have been shown to exhibit similar concentration-dependent antimicrobial properties, which are typically attributed to the cumulative action of their bioactive ingredients on microbial cell structures and metabolic pathways (Burt, 2004; Nazzaro *et al.*, 2013).

The potent effectiveness of clove oil against *P. aeruginosa* is especially interesting given the pathogen's inherent resistance to numerous conventional antibiotics (Yang *et al.*, 2025). This opportunistic pathogen has multiple resistance mechanisms, including efflux pumps, reduced outer membrane permeability, and

biofilm-forming ability, all of which contribute to its persistence and resistance to antimicrobial treatment (Pang *et al.*, 2019). Despite these defence mechanisms, the significant inhibition shown in this study implies that clove oil's active ingredients may transcend some of these barriers. This conclusion is congruent with the findings of Devi *et al.* (2010), who discovered that clove oil and its main constituent, eugenol, significantly broke bacterial membranes and reduced the growth of numerous harmful microbes.

The minimum inhibitory concentration (MIC) results indicate clove oil's antibacterial activity, since a concentration of 100 mg/mL inhibited observable growth of all tested bacterial species. The consistent MIC values show a broad-spectrum inhibitory activity against both Gram-positive and Gram-negative bacteria. Interestingly, bactericidal action was discovered only against *P. aeruginosa*, with an MBC value of 50 mg/mL, but no bactericidal impact was recorded against *S. aureus* or *K. pneumoniae* in the dose range examined.

These results imply that, in the experimental settings used, clove oil primarily functions as a bacteriostatic agent against the latter organisms. Because bacteriostatic drugs inhibit bacterial growth, allowing host immune responses to eradicate infections, while bactericidal treatments directly destroy bacterial cells, the contrast between bacteriostatic and bactericidal activity is therapeutically significant (Pankey and Sabath, 2004).

The antibacterial activity shown in this study could be ascribed to the phytochemical elements found in clove oil. A qualitative phytochemical screening revealed the presence of flavonoids, alkaloids, phenolic chemicals, and terpenoids, all of which are known to have antibacterial activities. Among these chemicals, phenolics, particularly eugenol, are regarded as the most important bioactive components of clove oil. Eugenol has been widely reported to have antimicrobial actions by disrupting cell membranes, increasing membrane permeability, leaking intracellular components, and inhibiting critical enzymatic activity required for bacteria life (Marchese *et al.*, 2017). Furthermore, the

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hydrophobic property of eugenol allows it to penetrate bacterial lipid membranes, causing structural instability and loss of cellular integrity (Hyldgaard *et al.*, 2012).

Flavonoids may have contributed to the reported antibacterial activity. Flavonoids have been shown to impair nucleic acid production, interfere with bacterial energy metabolism, and affect cytoplasmic membrane function (Cushnie and Lamb, 2011). Similarly, alkaloids have antibacterial activities through mechanisms that hinder DNA replication and protein synthesis, whereas terpenoids can destabilize microbial membranes and interfere with cellular respiration processes (Cowan, 1999). The presence of these phytochemicals in clove oil may cause synergistic interactions that increase its overall antibacterial potency beyond the activity of individual constituents.

The absence of tannins, saponins, cardiac glycosides, and steroids suggests that the antibacterial activity of the clove oil investigated in this study is primarily associated with its phenolic and terpenoid-rich composition rather than these secondary metabolite classes. This finding is consistent with previous studies that identified eugenol-rich clove oil as one of the most powerful antibacterial essential oils due to its high phenolic content (Chaieb *et al.*, 2007). The high correlation between the identified phytochemicals and antimicrobial activity shown in this investigation supports the concept that the antibacterial effects of clove oil come from the combined and potentially synergistic actions of several bioactive substances.

CONCLUSION

The results of this investigation support earlier findings that clove oil has broad-spectrum antibacterial activity and is an effective natural antimicrobial agent. Its potential as a source of alternative or adjunct antimicrobial drugs in the face of growing antimicrobial resistance is highlighted by its capacity to inhibit clinically relevant bacteria, especially *P. aeruginosa*. To fully comprehend its therapeutic potential, more research incorporating chemical characterisation by gas chromatography–mass spectrometry,

assessment of synergistic interactions with standard antibiotics, and in vivo efficacy tests is necessary.

Conflict of interest

The authors declared no conflict of interest.

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