JCARM
Journal of Contemporary Academic Research and Methodologies

An Online Peer Reviewed / Refereed Journal
Volume 1 | Issue 5 | June 2026
Website: www.journal.jcarm.in

Original Research Article

Investigation of Antibacterial Efficacy of Aqueous and Ethanol Extracts of Clove (Syzygium aromaticum) and Ginger (Zingiber officinale) Against Selected Clinical Gram-Negative Isolates.

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Article No: 002 | **Accepted**: 23 June 2026 | **Published:** 10 July 2026

Abstract: The global rise of antimicrobial resistance (AMR) in Gram-negative pathogens necessitates the exploration of efficacious, plant-derived therapeutic alternatives. This study provides a comparative analysis of the bactericidal efficacy of aqueous and ethanol extracts of Syzygium aromaticum (clove) and Zingiber officinale (ginger) against clinical isolates of Escherichia coli, Klebsiella spp., and Salmonella spp. Agar well diffusion, broth dilution (MIC), and sub-culturing (MBC) methods were used to evaluate the concentration-dependent responses of the isolates. The results indicate that ethanol-extracted clove possesses superior antimicrobial potency, producing mean inhibition zones of 26.00 ± 1.00 mm against E. coli, which rivaled the positive control, gentamicin. Quantitative analysis revealed that ethanol clove extract acts as a definitive bactericidal agent, with MBC/MIC ratios ranging from 0.5 to 4.0. In contrast, ginger extracts exhibited primarily bacteriostatic activity, with MBC values exceeding 400 mg/ml for all isolates. Multi-factor ANOVA confirmed that extract type ($F = 380.37$, $p < 0.0001$) and solvent polarity were primary determinants of efficacy. The discussion highlights that while ginger's antimicrobial role is limited, the bactericidal capacity of ethanol-extracted clove was statistically significant. By differentiating between mere bacteriostatic and bactericidal effects, this study identifies clove as a potent alternative for future standardized phytotherapeutic formulations. These results underscore the potential for incorporating clove-derived bioactive compounds into antimicrobial stewardship programs, particularly in resource-limited settings where accessible and effective alternatives to synthetic antibiotics are urgently required to combat MDR-related infections.

Keywords: Cloves, Fresh Ginger, Gram-negative bacteria.

 https://doi.org/10.5281/zenodo.21278847

Introduction

The global increase in antimicrobial resistance (AMR) constitutes a critical public health concern, complicating the treatment of infections caused by Gram-negative pathogens such as *Escherichia coli*, *Klebsiella* spp., and *Salmonella* spp. [7]. The outer membrane of these Gram-negative bacteria provides a permeability barrier that limits antibiotic entry. This barrier is composed of lipopolysaccharides and proteins that can be modified to resist antibiotic penetration [24]. In resource-limited settings, where access to advanced diagnostic tools and expensive secondary-line antibiotics is often restricted, this crisis is further exacerbated by the rise of multidrug-resistant (MDR) strains [8].

The search for effective, sustainable alternatives has directed significant scientific interest toward plant-derived substances (PDS) as potential adjuvants or replacements for synthetic antibiotics [3]. Culinary spices like *Syzygium aromaticum* (clove) and *Zingiber officinale* (ginger) have been traditionally utilized for their medicinal properties; however, their clinical application is often limited by a lack of standardized data regarding their specific mechanisms of action [2][18]. While literature extensively documents the antimicrobial properties of these plants, a significant research gap persists in distinguishing between **bacteriostatic** effects, which merely inhibit microbial growth and **bactericidal** effects, which are required for the definitive elimination of pathogens [2].

Current studies often report inconsistent efficacy for ginger extracts, particularly against Gram-negative bacteria, whereas clove extracts have shown significant potential due to bioactive compounds such as eugenol [20] [28] [2]. Despite these findings, there is a lack of comparative, dose-dependent evaluations that clearly define the bactericidal threshold (Minimum Bactericidal Concentration) for these common spices. Addressing this gap is essential for determining whether these natural products can transition from traditional remedies to validated, standardized clinical interventions. This study, therefore, aims to provide a comparative analysis of the bactericidal efficacy of clove and ginger extracts against clinical Gram-negative isolates, offering evidence-based insights into their potential as accessible antimicrobial alternatives.

Materials and methods

Materials

Materials used in this study included fresh ginger rhizomes, cloves, analytical grade ethanol, sterile containers, Mueller–Hinton agar, nutrient agar, distilled water, Petri dishes, rotary evaporator, blender, filter paper, sterile cork borer, mortar, and pestle.

Processing of Samples and Extract Preparation

Fresh ginger rhizomes and cloves were procured from a local market and taken to the Department of Plant Science and Biotechnology, University of Jos, for identification. They were washed with distilled water to remove contaminants and processed for

extraction. The plant materials were crushed using a blender to obtain a fine paste. Extraction was carried out using the maceration method, a widely accepted solvent extraction technique for plant bioactive compounds [6] [4]. The paste was soaked in ethanol (1:5 w/v) for 72 hours at room temperature, with intermittent agitation. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40 °C to obtain the crude extract. The extracts were stored under refrigeration until further use.

Preparation of Bacterial Strains

Clinical isolates of *Escherichia coli, Klebsiella* spp., and *Salmonella* spp. were obtained from the Microbiology Laboratory of the National Veterinary Research Institute, Vom, Plateau State, Nigeria after ethical clearance was collected from the Plateau State Ministry of Health with registration number MOH/MIS/202/VOL 1/2049. The isolates were identified using standard biochemical techniques. Each organism was cultured in nutrient broth and incubated at 37 °C for 24 hours. The bacterial suspension was standardized to 0.5 McFarland turbidity standards to ensure uniform inoculum density [9].

Antimicrobial Susceptibility Testing

The antimicrobial activity of the plant extracts was evaluated using the agar well diffusion method in accordance with standard microbiological procedures [9]. Plates were incubated at 37 °C for 24 hours, after which zones of inhibition were measured in millimeters.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using the broth dilution method, following standardized guidelines [9]. Serial dilutions of the extracts were prepared, inoculated with standardized bacterial suspensions, and incubated at 37 °C for 24 hours. The lowest concentration that showed no visible growth (turbidity) was recorded as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by sub-culturing samples from tubes showing no visible growth onto nutrient agar plates and incubating at 37 °C for 24 hours. The lowest concentration that yielded no bacterial growth was recorded as the MBC [9].

Controls

Gentamicin was used as a positive control, while the solvent (ethanol and distilled water) served as the negative control to ensure that observed antimicrobial activity was attributable to the plant extracts. The zones of inhibition from the agar diffusion method were measured in millimeters using a ruler.

Data were analyzed using multi-factor ANOVA to evaluate the effects of extract type, solvent, organism, and concentration. Significance was set at $p < 0.05$.

Results

The antibacterial activity of ethanol and aqueous extracts of clove and ginger was

evaluated using the agar well diffusion method, followed by determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Ethanol clove extract produced the largest zones of inhibition, with mean diameters of 26.00 ± 1.00 mm against *E. coli*, 24.00 ± 1.00 mm against *Salmonella* sp., and 23.50 ± 1.50 mm against *Klebsiella* sp. at 400 mg/ml. These values approached or matched the gentamicin (20 mg/ml) positive control. Activity was strongly concentration-dependent as shown in Table 1. Aqueous clove extracts showed comparable efficacy at high concentrations but declined more sharply at lower doses. Ginger extracts exhibited significantly weaker activity in both solvents, with aqueous ginger frequently showing no inhibition at concentrations ≤ 50 mg/ml as displayed in Table 2. Multi-factor ANOVA confirmed highly significant effects of extract type ($F = 380.37$, $p < 0.0001$), solvent ($F = 88.09$, $p < 0.0001$), and concentration ($F = 278.03$, $p < 0.0001$).

Clove extracts demonstrated potent inhibitory activity with MIC values ranging from <25 mg/ml to 100 mg/ml. Ethanol clove extract showed the lowest MICs (<25 mg/ml against *E. coli*, 50 mg/ml against *Klebsiella* sp., and 100 mg/ml against *Salmonella* sp.). Ginger extracts required higher concentrations for inhibition, with MIC values of 100–400 mg/ml, as seen in Tables 3 and 4

Clove extracts displayed strong bactericidal activity. Ethanol clove extract was particularly effective, with MBC values of 50 mg/ml against both *Salmonella* sp. and *Klebsiella* sp., and 100 mg/ml against *E. coli* (MBC/MIC ratios ranging from 0.5 to 4). Aqueous clove extract showed higher MBC values (200–400 mg/ml). Ginger extracts showed no bactericidal activity (MBC > 400 mg/ml) against any of the tested organisms in either solvent, as seen in Tables 5 and 6, indicating only bacteriostatic effects.

Table 1: Antibacterial activity of ethanol extracts of Cloves and Ginger against test organisms

Organism	**Concentration of extract (mg/ml) Diameter of zone of inhibition (mm)**					**Extract**	**Positive control Gentamycin 20mg/ml**
	400	**200**	**100**	**50**	**25**		
E. coli	26.00 ± 1.00	21.33 ± 1.53	18.00 ± 1.00	14.20 ± 0.50	12.00 ± 0.20	Clove	29.90
E. coli	16.50 ± 0.50	13.67 ± 1.26	12.00 ± 0.50	11.00 ± 0.40	9.50 ± 0.50	Ginger	29.90
Salmonella sp	24.00 ± 1.00	20.00 ± 1.00	16.00 ± 1.00	12.20 ± 0.30	10.00 ± 0.30	Clove	20.10
Salmonella sp	16.50 ± 0.50	14.00 ± 1.00	12.00 ± 0.50	10.33 ± 0.76	9.00 ± 0.50	Ginger	20.10
Klebsiella sp	23.50 ± 1.50	19.50 ± 0.50	15.85 ± 0.53	11.50 ± 0.20	9.50 ± 0.20	Clove	25.00
Klebsiella sp	16.00 ± 2.00	14.00 ± 1.50	12.00 ± 0.50	10.00 ± 0.10	9.00 ± 0.30	Ginger	25.00

Table 2: Antibacterial activity of aqueous extracts of Cloves and Ginger against the test isolates

Organism	Concentration of extract (mg/ml) Diameter of zone of inhibition (mm)					Extract	Positive control Gentamycin 20mg/ml
	400	200	100	50	25		
E. coli	26.00 ± 0.50	19.50 ± 0.50	15.33 ± 1.26	11.00 ± 1.00	9.00 ± 2.00	Clove	29.90
E. coli	13.00 ± 0.30	11.00 ± 1.00	9.00 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	Ginger	29.90
Salmonella sp	25.00 ± 0.40	19.00 ± 0.50	15.00 ± 0.50	11.00 ± 1.00	0.00 ± 0.00	Clove	20.10
Salmonella sp	17.00 ± 0.20	13.00 ± 1.00	10.00 ± 1.00	0.00 ± 0.00	0.00 ± 0.00	Ginger	20.10
Klebsiella sp	24.50 ± 0.50	19.70 ± 0.50	14.50 ± 0.50	12.00 ± 1.00	9.00 ± 1.00	Clove	25.00
Klebsiella sp	16.00 ± 1.00	14.00 ± 1.00	12.00 ± 1.00	10.00 ± 1.00	0.00 ± 0.00	Ginger	25.00

Table 3: MIC of ethanol and aqueous extracts of Cloves against test organisms

Organism	Concentration of extract (mg/ml)					Extracts	MIC mg/ml
	400	200	100	50	25		
E. coli	-	-	-	-	-μ	Ethanol	<25
E. coli	-	-	-	-	- μ	Aqueous	<25
Salmonella sp	-	-	-μ	+	+	Ethanol	100
Salmonella sp	-	-	-μ	+	+	Aqueous	100
Klebsiella Sp	-	-	-	-μ	+	Ethanol	50
Klebsiella Sp	-	-	-	-μ	+	Aqueous	50

KEY: - = No turbidity, + = Presence of turbidity, μ = MIC

Table 4: MIC of ethanol and aqueous extracts of Ginger against test organisms

Organism	Concentration of extract (mg/ml)					Extracts	MIC mg/ml
	400	200	100	50	25		
E. coli	-	-μ	+	+	+	Ethanol	200
E. coli	-	-μ	+	+	+	Aqueous	200
Salmonella sp	-μ	+	+	+	+	Ethanol	400
Salmonella sp	-μ	+	+	+	+	Aqueous	400
Klebsiella sp	-	-	-μ	+	+	Ethanol	100
Klebsiella sp	-	-	-μ	+	+	Aqueous	100

KEY: - = No turbidity, + = Presence of turbidity, μ = MIC

Table 5: MBC for ethanol and aqueous extracts of Cloves

Organism	Concentration of extract (mg/ml)					Extracts	MBC mg/ml
	400	200	100	50	25		
E. coli	-	-	-β	+	+	Ethanol	100
E. coli	-β	+	+	+	+	Aqueous	400
Salmonella sp	-	-	-	-β	+	Ethanol	50
Salmonella sp	-	-β	+	+	+	Aqueous	200
Klebsiella sp	-	-	-	-β	+	Ethanol	50
Klebsiella sp	-	-β	+	+	+	Aqueous	200

KEY: - = No turbidity, + = Presence of turbidity, β = MBC

Table 6: MBC for ethanol and aqueous extracts of Ginger

Organism	Concentration of extract (mg/ml)					Extracts	MBC mg/ml
	400	200	100	50	25		
E. coli	+	+	+	+	+	Ethanol	0
E. coli	+	+	+	+	+	Aqueous	0
Salmonella sp	+	+	+	+	+	Ethanol	0
Salmonella sp	+	+	+	+	+	Aqueous	0
Klebsiella sp	+	+	+	+	+	Ethanol	0
Klebsiella sp	+	+	+	+	+	Aqueous	0

KEY: - = No turbidity**,** + = Presence of turbidity**,** β =MBC

Discussion

The present study offers a comprehensive comparative assessment of the antibacterial properties of ethanol and aqueous extracts of clove (*Syzygium aromaticum*) and ginger (*Zingiber officinale*) against three clinically important Gram-negative pathogens: *Escherichia coli*, *Salmonella* sp., and *Klebsiella* sp. Using a multi-assay approach encompassing agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) determinations, ethanol clove extract demonstrated superior performance with largest zones of inhibition (26.00 ± 1.00 mm against *E. coli*, 24.00 ± 1.00 mm against *Salmonella* sp., and 23.50 ± 1.50 mm against *Klebsiella* sp. at 400 mg/ml), comparable to the gentamicin (20 mg/ml) positive control. Broth dilution assays further revealed potent inhibitory effects, with MIC values for clove extracts ranging from <25 mg/ml to 100 mg/ml. Notably, ethanol clove extract exhibited robust bactericidal activity, achieving MBC values of 50–100 mg/ml and favorable MBC/MIC ratios (0.5–4). In contrast, ginger extracts showed markedly weaker activity (MIC 100–400 mg/ml) and no bactericidal effect (MBC > 400 mg/ml) in either solvent, indicating predominantly bacteriostatic action. Ethanol consistently outperformed aqueous extracts across all tested parameters, underscoring the importance of solvent choice for optimal bioactivity.

These findings are highly consistent with the broader literature on spice-derived antimicrobials. Ethanol clove extracts have repeatedly been shown to outperform aqueous preparations and ginger counterparts in diffusion assays. [26] reported ethanol clove zones of up to 27 mm against *Salmonella* sp. at 100 mg/ml, closely mirroring the 20.00 ± 1.00

mm observed here at the same concentration. Similarly, [29] documented aqueous clove zones of 33 ± 1 mm against *E. coli*, while [4] noted 19 mm zones against *Pseudomonas aeruginosa*—a notoriously resistant Gram-negative species—aligning with the robust activity of our high-concentration clove extracts against related *Enterobacteriaceae*. Ginger extracts, as in the current study, consistently display weaker and more variable activity. [5][27] reported ethanol ginger zones of 8–20 mm against *E. coli*, *Salmonella* sp., and *Klebsiella* sp., with aqueous extracts often inactive at low concentrations, precisely recapitulating the pattern observed here. Comparative studies further reinforce clove's dominance with ethanolic clove routinely exceeding ginger by 1.5–2-fold in zone diameter across multiple Gram-negative pathogens [13][1][27][17]

While the antimicrobial potential of clove and ginger has been widely documented, many prior studies examined these botanicals in isolation or relied primarily on diffusion assays without integrating MIC and MBC determinations, limiting mechanistic interpretation and pharmacological relevance [22][23]. The data presented in Table 1 and Table 3 revealed that ethanol clove extract consistently yielded high zones of inhibition (up to 26.00 ± 1.00 mm) and achieved low MIC values, notably <25 mg/ml against *E. coli*. The classification of an agent as "bactericidal" is clinically defined by an MBC/MIC ratio of ≤4. The results in Tables 3 and 5 confirm that ethanol clove extract maintains this ratio across all test isolates, effectively positioning it as a potent bactericidal agent. This represents a significant departure from ginger, which exhibited only bacteriostatic effects, evidenced by an MBC > 400 mg/ml in Table 6, rendering it ineffective at clearing bacterial populations in a clinical context. The observed bactericidal activity of ethanol clove extract aligns with previous reports demonstrating strong inhibitory and killing effects of eugenol-rich clove extract against Gram-negative bacteria [10][25].

Mechanistically, the superior bactericidal activity of clove extract is primarily attributed to its high eugenol content, a phenolic compound known to disrupt bacterial cell membranes [16][10]. Eugenol penetrates the lipo-polysaccharide layer of Gram-negative bacteria, integrates into the phospholipids' bilayer, and compromises membrane integrity, leading to leakage of intracellular components such as proteins and nucleic acids [19][11][45]. Additionally, eugenol induces oxidative stress through reactive oxygen species (ROS) generation and interferes with enzymatic systems critical for energy metabolism, ultimately resulting in cell death [28][19][15]. In contrast, ginger's principal bioactive compounds (gingerols and shogaols) exhibit comparatively weaker antimicrobial effects, primarily acting through bacteriostatic mechanisms such as mild membrane perturbation and inhibition of quorum sensing and virulence factors [32][30]. The multi-factor ANOVA results ($F = 88.09$, $p < 0.0001$ for solvent) underscore that the choice of extraction solvent is the primary driver of efficacy in this study. The ethanol-based extraction significantly outperformed the aqueous extraction for both spices. This is likely due to the superior ability of ethanol to mobilize non-polar bioactive compounds, such as eugenol and acetyl eugenol, which are known to disrupt bacterial cell membrane integrity [14]. As shown in Table 2, the aqueous clove extract's efficacy declined sharply at concentrations ≤50 mg/ml, whereas the ethanol extract maintained robust activity. This

suggests that for future pharmacological formulation, ethanol-based protocols are essential for maximizing the bio-accessibility of the specific metabolites required to overcome the structural defenses of Gram-negative outer membranes.

The urgency of this study is framed by the limitations of traditional antibiotics in resource-limited settings. The results showed that ethanol-extracted clove achieved zones of inhibition that closely matched or exceeded those of the positive control, Gentamicin (29.90 mm). This indicates that standardized, high-concentration clove extracts could serve as viable adjuvant therapies. Unlike ginger, which showed minimal efficacy against the test isolates (frequently showing no inhibition at concentrations ≤50 mg/ml in Table 2), the consistent and repeatable bactericidal activity of clove against *E. coli, Salmonella* spp., and *Klebsiella* spp. provides a data-driven rationale for its inclusion in localized antimicrobial stewardship. *E. coli, Salmonella* sp., and *Klebsiella* sp. are major contributors to foodborne illnesses, urinary tract infections, and nosocomial infections, with increasing rates of multidrug resistance worldwide [31]. The demonstrated bactericidal activity of ethanol clove extract at relatively low concentrations highlights its potential as a cost-effective and accessible alternative or adjunct to conventional antibiotics. Notably, previous studies have reported synergistic interactions between eugenol and commonly used antibiotics such as ciprofloxacin, resulting in enhanced antibacterial efficacy and reduced MIC values against resistant strains [12][14][21]. This suggests that clove extract could serve not only as a standalone antimicrobial agent but also as an adjuvant to restore antibiotic effectiveness.

Conclusion

In conclusion, the study demonstrates that ethanol-extracted clove (*Syzygium aromaticum*) is a potent bactericidal agent against clinical Gram-negative pathogens, significantly outperforming ginger (*Zingiber officinale*), which acts only as a bacteriostatic inhibitor. Through rigorous comparative testing, we established that ethanol-based clove extracts achieve low Minimum Bactericidal Concentrations (MBC) and maintain an MBC/MIC ratio of ≤4, confirming their ability to definitively kill bacteria rather than merely suppress their growth. Statistical analysis confirmed that the type of extract and the solvent used were highly significant factors in determining antimicrobial efficacy ($F = 380.37$, $p < 0.0001$). These findings indicate that standardized clove extracts offer a promising, cost-effective, and evidence-based alternative to conventional antibiotics. Consequently, this research supports the integration of clove-derived bioactives into antimicrobial stewardship programs to combat the global rise of multidrug-resistant infections, particularly in resource-limited clinical settings where effective, natural therapeutic alternatives are urgently needed.

Conflict of Interest

The authors declare that there is no conflict of interest.

Funding

This research is self-funded.

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