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Comparative Evaluation of The Antimicrobial Potency of Four Traditional Nigerian Spices Against Selected Foodborne Pathogens

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Research Article

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Abstract

This study investigated the antimicrobial activities of *Aframomum melegueta* (alligator pepper), *Syzygium aromaticum* (clove), *Tetrapleura tetraptera* (aridan fruit), and Zingiber officinale (ginger) against selected Gram-positive and Gram-negative bacteria. Crude aqueous extracts of the spices were prepared and screened for antimicrobial activity using agar well diffusion assays. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth dilution methods. Test organisms included *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae*, and *Klebsiella spp.* All experiments were performed in triplicate. Alligator pepper and clove exhibited the strongest antibacterial activity, with inhibition zones of up to 14.5 mm against *S. aureus* and 12.5 mm against *P. aeruginosa.* Their MIC values ranged from 12.5–25 mg/mL for Gram-positive bacteria and 25–50 mg/mL for Gram-negative bacteria, with MBC values generally one dilution higher. Ginger showed selective inhibition, strongly affecting *S. aureus* and *S. dysenteriae*, while *E. coli* and *Klebsiella* were less sensitive. Aridan fruit demonstrated weak inhibition across all tested organisms, with MIC ≥ 50 mg/mL and MBC > 100 mg/mL. Overall, Gram-positive organisms were more susceptible than Gram-negative bacteria, consistent with structural differences in cell wall composition.

Paper Description/Highlights

- Traditional Nigerian spices are valued for their medicinal properties, but their comparative efficacy against foodborne pathogens requires systematic validation to combat increasing bacterial resistance.
- Crude aqueous extracts were evaluated using agar well diffusion and broth dilution methods to measure antimicrobial effects against six Gram-positive and Gram-negative bacterial strains.
- Alligator pepper and clove demonstrated the highest potency, particularly against Staphylococcus aureus, while aridan fruit showed negligible activity compared to the other spices.
- This research validates specific spices as effective natural biopreservatives and provides a scientific basis for their potential use in treating gastrointestinal infections.

INTRODUCTION

The global burden of foodborne diseases remains a critical public health challenge, with the World Health Organization (WHO) estimating that unsafe food causes 600 million illnesses and 420,000 deaths annually [1]. Foodborne pathogens, particularly Escherichia coli, Staphylococcus aureus, and Salmonella spp., are the primary etiological agents responsible for widespread outbreaks and systemic infections [2]. This crisis is exacerbated by the escalating threat of antimicrobial resistance (AMR). A landmark 2022 systematic analysis revealed that bacterial AMR was associated with approximately 4.95 million deaths in 2019, with pathogens such as E. coli, K. pneumoniae, and P. aeruginosa listed among the leading causes of mortality [3]. In Sub-Saharan Africa, the prevalence of multidrug-resistant (MDR)

enteric bacteria has reached critical levels, significantly reducing the efficacy of conventional antibiotic therapies [4].

The search for "clean label" food preservation and novel antimicrobial leads has intensified the study of medicinal spices, which serve as reservoirs of structurally diverse bioactive compounds [5]. Spices such as Syzygium aromaticum (Clove) and Aframomum melegueta (Alligator pepper) have been used for millennia in West African traditional medicine and culinary practices. S. aromaticum is renowned for its high concentration of eugenol, a phenolic compound that exerts potent bactericidal activity by disrupting microbial membrane potential and inducing intracellular oxidative stress [6]. A. melegueta contains pungent principles such as paradols and gingerols, which have demonstrated significant inhibitory effects against pro-inflammatory pathways and various microbial strains [7].

Similarly, Zingiber officinale (Ginger) is widely utilized for its therapeutic potential against gastrointestinal disorders, largely due to its complex mixture of terpenes and phenolic ketones [8]. Tetrapleura tetraptera (Aridan fruit) is also incorporated into traditional postpartum diets in Nigeria for its purported antimicrobial benefits, attributed to its saponin and flavonoid content, though its efficacy against specific foodborne isolates requires further systematic validation [9]. Despite the widespread ethnomedicinal use of these spices, comparative evaluations of their crude aqueous extracts the form most commonly consumed remain limited in contemporary literature [10].

This study aims to evaluate the antimicrobial potency of aqueous extracts from Aframomum melegueta, Syzygium aromaticum, Tetrapleura tetraptera, and Zingiber officinale against a panel of clinically relevant Gram-positive and Gram-negative foodborne pathogens. By determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), this research seeks to provide an evidence-based framework for the potential application of these spices as natural biopreservatives and alternative therapeutic agents in the management of resistant bacterial infections [11].

MATERIALS AND METHODS

Plant Material Collection and Preparation of Extracts

Fresh samples of *Aframomum melegueta* (alligator pepper), *Syzygium aromaticum* (clove), *Tetrapleura tetraptera* (aridan fruit), and *Zingiber officinale* (ginger) were procured from local markets in Lagos, Nigeria. Each specimen was authenticated by a taxonomist Dr Adebowale Shonde in the Department of Biological Sciences, Lagos State University of Science and Technology, and voucher specimens were deposited in the departmental herbarium. The plant materials were thoroughly washed, air-dried at room temperature (25 ± 2°C) for two weeks, and ground into fine powder using a sterile electric blender (Philips HR2021, China).

Extraction was carried out using the cold maceration method with 70% ethanol as solvent, following the protocol described by Akinmoladun et al. (2020) [12]. Briefly, 100 g of powdered plant material was soaked in 500 mL of solvent, agitated intermittently, and left for 72 h at room temperature. The extracts

were filtered using Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator (Büchi R-210, Switzerland), and stored at 4°C until use.

Test Microorganisms

Six bacterial strains were employed: *Staphylococcus aureus* (ATCC 25923), Bacillus cereus (ATCC 11778), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Shigella dysenteriae* (clinical isolate), and *Klebsiella pneumoniae* (ATCC 700603). Standard strains were obtained from the Nigerian Institute of Medical Research (NIMR), Lagos. Stock cultures were maintained on nutrient agar slants at 4°C and sub-cultured onto Mueller–Hinton agar (MHA; Oxoid, UK) before experimental use [11].

Antimicrobial Susceptibility Testing (Agar Well Diffusion Assay)

The agar well diffusion method was performed according to CLSI guidelines [11] with modifications from Balouiri et al. (2016). Bacterial suspensions were standardized to 0.5 McFarland standard (~ 1.5×10^8 CFU/mL). Sterile Mueller–Hinton agar plates were inoculated by swabbing the bacterial suspension evenly across the surface. Wells of 6 mm diameter were bored using a sterile cork borer and filled with 100 µL of spice extracts at concentrations of 25%, 50%, 75%, and 100% (diluted with DMSO). Plates were incubated at 37°C for 24 h, and the zones of inhibition (ZOI) were measured in millimeters using a digital Vernier caliper. Ciprofloxacin (10 µg) was used as a positive control, while DMSO served as negative control.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The MIC values were determined using the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) M07-A11 guidelines [13]. Briefly, two-fold serial dilutions of the extracts (ranging from 12.5 to 200 mg/mL) were prepared in 96-well microtiter plates containing Mueller–Hinton broth. Each well was inoculated with 100 µL of a standardized bacterial suspension (1.0 X 10^6 CFU/mL) and incubated at 37°C for 24 h. The MIC was recorded as the lowest concentration of the extract at which no visible growth (turbidity) was observed, a protocol further standardized for plant-derived antimicrobials by Balouiri et al. [14].

DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION (MBC)

For MBC determination, 10 µL aliquots from wells showing no visible growth in the MIC assays were sub-cultured onto fresh Mueller-Hinton Agar (MHA) plates and incubated at 37°C for 24 h. The MBC was defined as the lowest concentration of the extract that produced no bacterial growth, indicating a 99.9%

killing of the initial inoculum, following the standardized bactericidal activity criteria described by CLSI M26-A [15].

DATA ANALYSIS

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation. Statistical differences in antimicrobial activity between various extracts and concentrations were analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post hoc test ($p < 0.05$ considered significant) using IBM SPSS Statistics (Version 25.0) [16]. Pearson correlation analysis was performed to assess the linear relationship between inhibition zone diameters and MIC values [17].

RESULTS

Antimicrobial Activity of Medicinal Spices

Alligator Pepper Extract

The antibacterial activity of Aframomum melegueta extract is presented in Table 1. The extract produced inhibition zones ranging from 3.0 ± 0.2 mm to 14.5 ± 0.3 mm across concentrations. The lowest MIC and MBC values were observed against Staphylococcus aureus (12.5 mg/mL and 25 mg/mL, respectively), while the highest values were recorded for Escherichia coli.

Table 1
Zone of inhibition (mm), MIC, and MBC of Aframomum melegueta extract (mean of triplicates).

Organism	25%	50%	75%	100%	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus	3.0 ± 0.2	7.0 ± 0.1	8.0 ± 0.2	14.5 ± 0.3	12.5	25
Bacillus cereus	6.2 ± 0.2	7.0 ± 0.2	7.4 ± 0.3	8.5 ± 0.2	25	50
Escherichia coli	6.0 ± 0.1	6.8 ± 0.2	6.0 ± 0.1	7.0 ± 0.2	50	100
Pseudomonas aeruginosa	5.0 ± 0.2	7.5 ± 0.3	10.8 ± 0.4	12.5 ± 0.2	25	50

CLOVE EXTRACT

Table 2 summarizes the activity of Syzygium aromaticum. Inhibition zones ranged from 3.0 ± 0.2 mm to 14.5 ± 0.2 mm. The MIC and MBC values followed the same trend as those observed for alligator pepper, with S. aureus showing the highest sensitivity.

Table 2
Zone of inhibition (mm), MIC, and MBC of Syzygium aromaticum extract

Organism	25%	50%	75%	100%	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus	3.0 ± 0.2	7.0 ± 0.2	8.0 ± 0.1	14.5 ± 0.2	12.5	25
Bacillus cereus	6.2 ± 0.3	7.0 ± 0.2	7.4 ± 0.3	8.5 ± 0.3	25	50
Escherichia coli	6.0 ± 0.2	6.8 ± 0.2	6.0 ± 0.2	7.0 ± 0.3	50	100
Pseudomonas aeruginosa	5.0 ± 0.2	7.5 ± 0.3	10.8 ± 0.3	12.5 ± 0.2	25	50

ARIDAN FRUIT EXTRACT

The antimicrobial response to Tetrapleura tetraptera extract is shown in Table 3. Inhibition zones were comparatively lower, ranging from 1.0 ± 0.1 mm to 6.0 ± 0.2 mm. MIC values were consistent at 50 mg/mL for all organisms, while MBC values exceeded 100 mg/mL for most isolates.

Table 3
Zone of inhibition (mm), MIC, and MBC of Tetrapleura tetraptera extract.

Organism	25%	50%	75%	100%	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus	1.0 ± 0.1	2.5 ± 0.1	3.0 ± 0.1	3.5 ± 0.2	50	> 100
Bacillus cereus	1.0 ± 0.2	1.5 ± 0.2	2.0 ± 0.1	2.5 ± 0.2	50	> 100
Escherichia coli	2.0 ± 0.2	1.5 ± 0.1	2.0 ± 0.2	2.5 ± 0.2	50	> 100
Pseudomonas aeruginosa	2.0 ± 0.1	3.8 ± 0.2	5.0 ± 0.3	6.0 ± 0.2	50	100

GINGER EXTRACT

The activity of Zingiber officinale extract is shown in Table 4. Inhibition zones ranged from 2.0 ± 0.2 mm to 9.0 ± 0.3 mm. MIC values varied from 25 mg/mL to 50 mg/mL depending on the organism.

Table 4
Zone of inhibition (mm), MIC, and MBC of Zingiber officinale extract.

Pathogen	50 mg/mL	100 mg/mL	MIC (mg/mL)	MBC (mg/mL)
Escherichia coli	8.0 ± 0.2	2.0 ± 0.2	50	> 100
Shigella dysenteriae	4.0 ± 0.2	9.0 ± 0.3	25	50
Klebsiella	2.0 ± 0.1	3.0 ± 0.2	50	> 100
Staphylococcus aureus	8.0 ± 0.3	9.0 ± 0.2	25	50

COMPARATIVE ANTIBACTERIAL ACTIVITY AT 100% CONCENTRATION

A comparative summary of inhibition zones at full concentration is presented in Fig. 1. Alligator pepper and clove produced the highest inhibition zones against S. aureus (14.5 mm), while Aridan fruit showed the lowest activity across pathogens.

MIC AND MBC COMPARISON

Figure 2 presents the MIC and MBC values across the four extracts. Alligator pepper and clove consistently demonstrated the lowest MIC/MBC values, whereas Aridan fruit showed the highest.

ANTIMICROBIAL INDEX

The antimicrobial index values for each extract are shown in Fig. 3. Alligator pepper and clove exhibited the highest index values across pathogens tested.

STATISTICAL SUMMARY

A summary of the overall antimicrobial performance of the extracts is presented in Fig. 4. Alligator pepper and clove showed the highest mean zone of inhibition, while Aridan fruit had the lowest.

Figure 4. **Statistical summary of antimicrobial activity (Mean ± SD).**

DISCUSSION

The present study evaluated the antimicrobial activities of aqueous extracts from four medicinal spices *Aframomum melegueta* (alligator pepper), *Syzygium aromaticum* (clove), *Tetrapleura tetraptera* (aridan fruit), and *Zingiber officinale* (ginger) against a panel of Gram-positive and Gram-negative bacteria. The findings demonstrate varying levels of antimicrobial potency, with alligator pepper and clove showing the strongest inhibitory profiles, ginger exerting moderate selective effects, and aridan fruit exhibiting the weakest activity.

The broad-spectrum activity of *Aframomum melegueta* observed in this study corroborates previous reports highlighting its rich phytochemical profile, particularly phenolic compounds and flavonoids, which contribute to antibacterial activity [12]. The inhibition of Staphylococcus aureus and Pseudomonas aeruginosa at low MIC values (12.5–25 mg/mL) suggests that the extract possesses both bacteriostatic and bactericidal potential. Similar dose-dependent responses of *A. melegueta* against Gram-positive organisms have been attributed to its high content of gingerol-like compounds and terpenoids, which disrupt bacterial membranes and interfere with protein synthesis [18].

The results obtained for Syzygium aromaticum align with extensive literature documenting the strong antimicrobial efficacy of clove oil and extracts, largely attributed to eugenol as the principal bioactive compound [19]. The present study confirms that clove exhibited potent activity against both Gram-positive and Gram-negative bacteria, with MIC and MBC values comparable to those of alligator pepper. This reinforces the therapeutic potential of clove as a natural antimicrobial, particularly given its established clinical applications in dentistry and wound management [20].

In contrast, *Tetrapleura tetraptera* showed poor inhibition across test organisms, requiring higher MIC and MBC values (≥ 50 mg/mL). This suggests limited antimicrobial efficacy, consistent with previous studies that describe *T. tetraptera* more as a functional spice with nutritional and antioxidant benefits than as a potent antimicrobial agent [21]. The weak bactericidal activity observed in this study may be due to a lower concentration of phenolic constituents compared to alligator pepper and clove. Nonetheless, its inhibitory effect against *Pseudomonas aeruginosa*, albeit modest, indicates that it may still contribute synergistically in polyherbal formulations [22].

Zingiber officinale demonstrated moderate antimicrobial activity, with notable inhibition against *Staphylococcus aureus* and *Shigella dysenteriae.* The unexpected observation that *E. coli* inhibition was stronger at lower concentrations (8.0 mm at 50 mg/mL versus 2.0 mm at 100 mg/mL) may reflect concentration-dependent antagonism or the presence of inhibitory compounds that precipitate at higher concentrations, reducing bioavailability [23]. Previous studies have emphasized the role of gingerols and shogaols as major antibacterial constituents, which act by disrupting microbial membranes and inhibiting quorum sensing [25]. These findings position ginger as a promising candidate for adjunct therapies targeting enteric pathogens.

The comparative analysis of inhibition zones, MIC, and MBC values revealed that alligator pepper and clove extracts were most effective against Gram-positive organisms, particularly *S. aureus*, while Gram-negative bacteria such as *E. coli* and *Klebsiella spp.* were more resistant. This observation is in line with the established understanding that Gram-negative bacteria possess an outer membrane barrier that reduces permeability to hydrophobic plant-derived compounds [25]. Nevertheless, the activity observed against Pseudomonas aeruginosa is particularly significant, as this pathogen is notoriously resistant to conventional antibiotics.

Overall, the results underscore the biomedical relevance of these spices as potential sources of antimicrobial agents. The observed bactericidal activity of alligator pepper and clove suggests their

applicability in therapeutic formulations for treating infections caused by multidrug-resistant Gram-positive pathogens. Moreover, the moderate effects of ginger highlight its value as an adjunctive treatment, while the weak activity of aridan fruit points toward a primarily supportive or synergistic role rather than standalone antimicrobial efficacy. These findings support the growing consensus on the role of medicinal plants as reservoirs of novel antimicrobial compounds in the fight against antimicrobial resistance [26, 27].

CONCLUSIONS

The present study shows that the four Nigerian spices differed markedly in antimicrobial strength against the tested foodborne pathogens. Aframomum melegueta and Syzygium aromaticum were the most active, showing the most consistent inhibitory and bactericidal effects, especially against Staphylococcus aureus and other susceptible Gram-positive organisms. Zingiber officinale displayed moderate, pathogen-dependent activity, while Tetrapleura tetraptera exhibited the least antimicrobial effect under the conditions tested. Overall, the findings support the traditional use of alligator pepper and clove as promising sources of natural antibacterial agents and suggest that these spices may be useful as leads for the development of alternative or adjunctive antimicrobial preparations. Further work should focus on isolation of active compounds, toxicity evaluation, and assessment of synergistic effects with conventional

Abbreviations

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Declarations

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Conflict of interest: The authors declare no conflict of interest.

Data availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

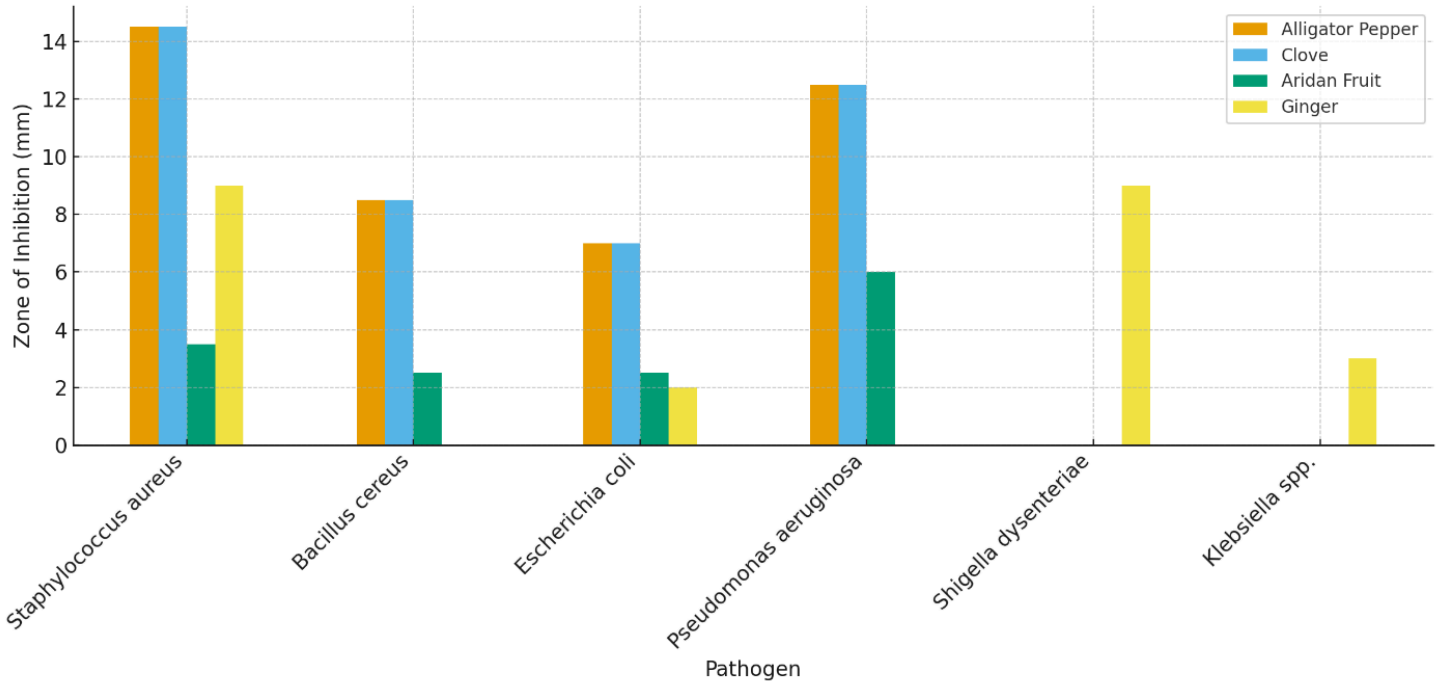


Figure 1

Comparative summary of inhibition zones at 100% concentration

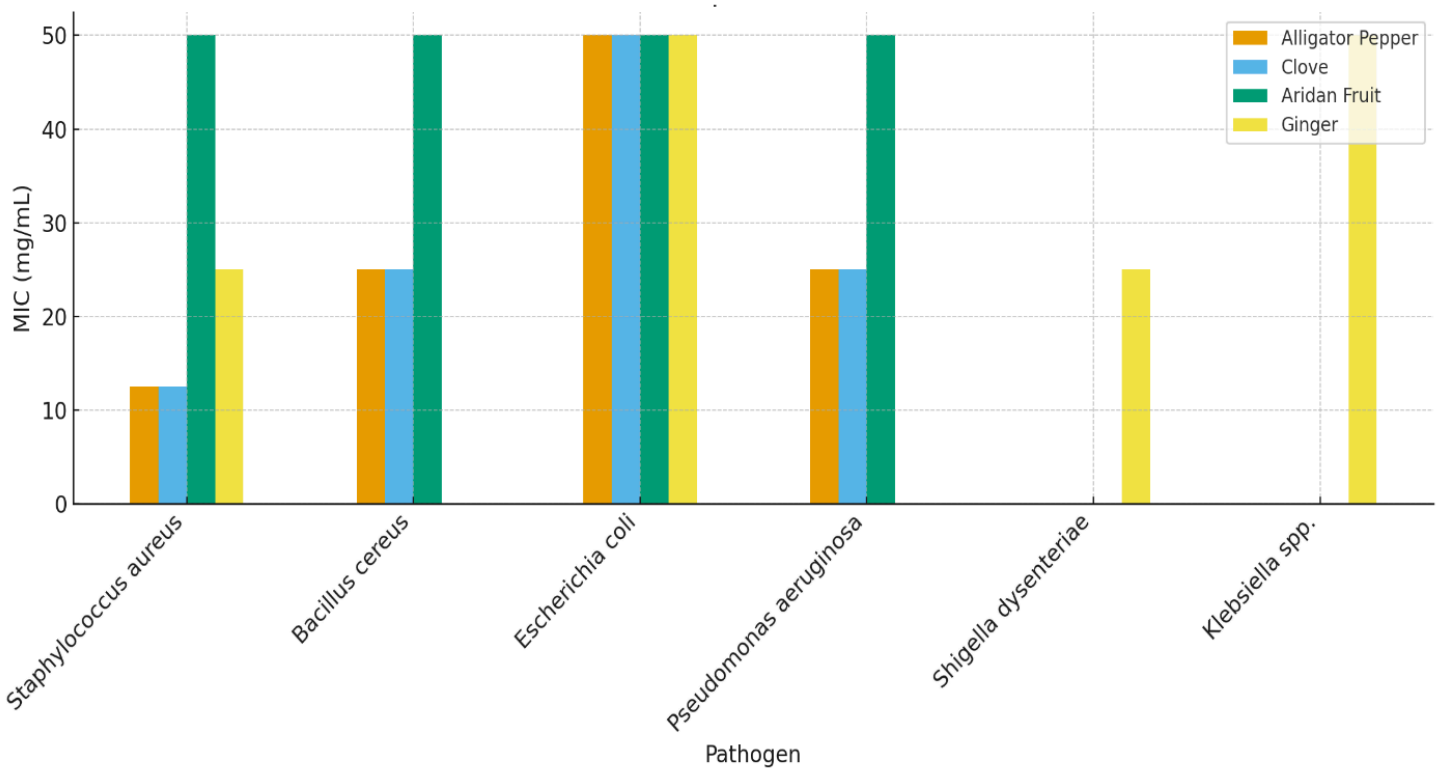


Figure 2

MIC and MBC comparison of extracts

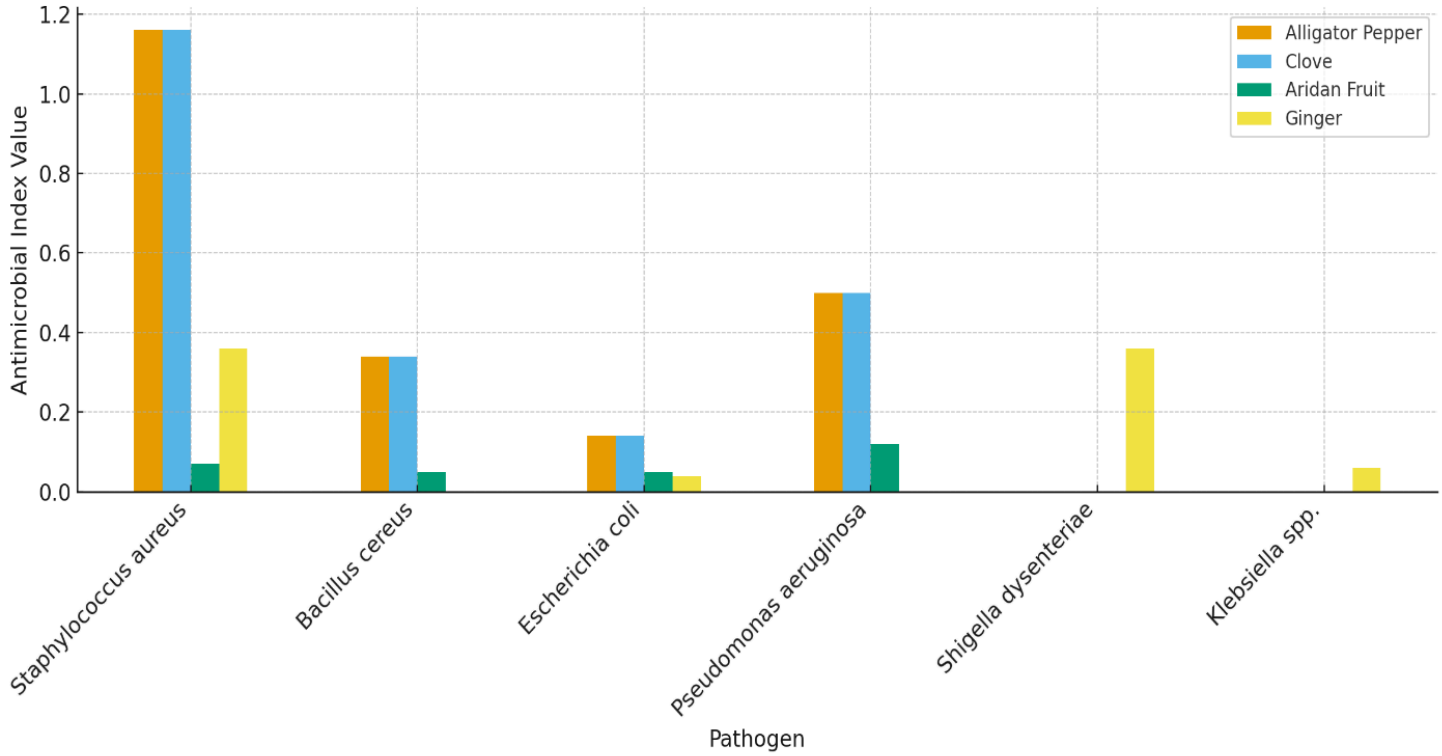


Figure 3

Antimicrobial index of extracts.

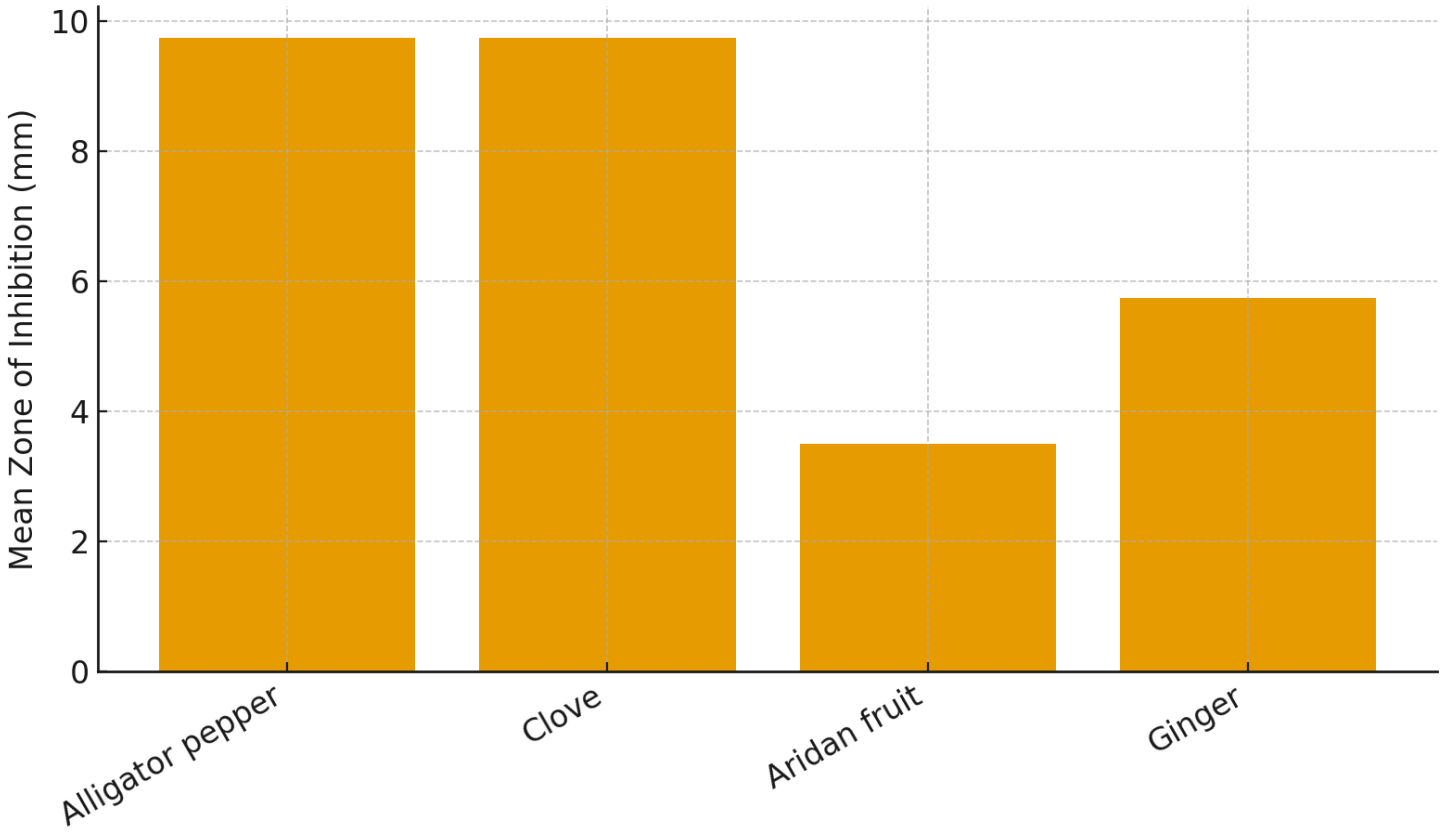


Figure 4

Statistical summary of antimicrobial activity (Mean ± SD).