





Comparative Evaluation of the Antibacterial Activity of Culinary Spice Extracts against Selected Bacterial Pathogens

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Abstract	Article History
<p>This study evaluated the antimicrobial activity of ethanolic and aqueous extracts of four commonly used spices clove (<i>Syzygium aromaticum</i>), cinnamon (<i>Cinnamomum verum</i>), ginger (<i>Zingiber officinale</i>), and garlic (<i>Allium sativum</i>) against selected bacterial pathogens: <i>Staphylococcus aureus</i>, <i>Escherichia coli</i>, and <i>Salmonella typhi</i>. Antimicrobial efficacy was assessed using the agar well diffusion method, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the broth dilution technique. The inhibitory effects of the spice extracts were compared with a standard antibiotic (ciprofloxacin), and combination studies were conducted to evaluate possible synergistic interactions. The ethanolic extract of clove exhibited zones of inhibition (ZOI) of 12 mm, 15 mm, and 21 mm against <i>S. typhi</i>, <i>E. coli</i>, and <i>S. aureus</i>, respectively, with corresponding MIC/MBC values ranging from 125–500 mg/ml. Cinnamon extract also demonstrated broad-spectrum activity, producing ZOI of 17 mm, 13 mm, and 16 mm against <i>S. typhi</i>, <i>E. coli</i>, and <i>S. aureus</i>, respectively. In contrast, ginger and garlic extracts showed antimicrobial activity only against <i>S. aureus</i>, with inhibition zones of 21 mm and 15 mm, respectively. Among the aqueous extracts, only clove showed inhibitory activity against <i>S. aureus</i> and <i>E. coli</i>. Overall, ethanolic extracts displayed significantly higher antimicrobial activity than aqueous extracts. <i>Staphylococcus aureus</i> was the most susceptible organism, while <i>E. coli</i> and <i>S. typhi</i> were comparatively less sensitive. Combination studies revealed notable synergistic activity between clove and cinnamon extracts against <i>S. aureus</i>. Although ciprofloxacin produced larger inhibition zones, the spice extracts demonstrated appreciable antimicrobial effects. These findings indicate that selected spices, particularly clove and cinnamon, possess bioactive compounds with potential application as natural antimicrobial agents in the control of foodborne pathogens and microbial infections.</p> <p>Keywords: Antimicrobial activity; Spice extracts; Foodborne pathogens; Minimum inhibitory concentration; Synergistic effects</p>	<p>Received: 22 Nov 2025 Accepted: 30 Dec 2025 Published: 28 Jan 2026</p> <p>Scan QR code to view*</p>  <p>License: CC BY 4.0</p>  <p>Open Access article.</p>
<p>How to cite this paper: Omorordion, N. J., & Chukwujezi, C. G. (2026). Comparative Evaluation of the Antibacterial Activity of Culinary Spice Extracts against Selected Bacterial Pathogens. <i>IPS Interdisciplinary Journal of Biological Sciences</i>, 6(1), 197–210. https://doi.org/10.54117/ijbs.v6i1.119</p>	

Introduction

Spices are plant-derived products widely used to enhance the flavor, aroma, and color of foods and beverages. Beyond their culinary applications, spices have long been recognized for their role in food preservation and medicinal purposes across cultures for thousands of years. Historically, early societies utilized spices not only to improve organoleptic properties but also to extend shelf life and reduce microbial contamination in food products. Plant extracts, in particular, have been employed to enhance the sensory qualities of foods while mitigating the growth of foodborne pathogens, thereby improving safety and storage longevity (Gottardi et al., 2016; Candia et al., 2017; Irshard et al., 2017; Nasaan et al., 2015). Several commonly used spices, including cinnamon, oregano, nutmeg, basil, pepper, thyme, clove, rosemary, ginger, and cumin, are rich in secondary metabolites that exhibit

antimicrobial activity. These compounds are generally recognized as safe (GRAS) and have negligible adverse effects, making them promising candidates for natural antimicrobial agents against both foodborne and human pathogens (Nabavi et al., 2016). The antimicrobial potential of plants is largely attributed to their ability to synthesize diverse phytochemicals through secondary metabolism, which serve as defense mechanisms against bacteria, fungi, and viruses, and simultaneously contribute to food preservation (Makwana et al., 2015; Ayodele et al., 2015). Essential oils derived from plants, such as those from oregano, thyme, garlic, and black cumin, have received considerable attention for their potent antimicrobial properties, which are relevant for controlling microbial contamination in both food and pharmaceutical industries.

The growing prevalence of antibiotic-resistant bacteria has heightened the need for alternative antimicrobial agents. Excessive use of antibiotics has led to the emergence of multidrug-resistant microbes, which are increasingly responsible for serious infections, including cholera, bacterial meningitis, urinary tract infections, and candidiasis (Miladi et al., 2016; Subramani et al., 2017). According to the World Health Organization, infectious diseases were responsible for approximately one-third of the 55 million deaths worldwide in 2011, highlighting the public health impact of microbial infections (Nabavi et al., 2015). Antibiotic-resistant pathogens, including *Staphylococcus aureus* and *Salmonella enteritidis*, frequently contaminate foods such as raw pork, beef, and poultry, further exacerbating foodborne illness risks (Boskovic et al., 2015; Farahani et al., 2018).

Fresh foods, including horticultural produce, seafood, and meat, are particularly susceptible to microbial spoilage, leading to deterioration in sensory quality and increased risk of foodborne diseases (Rawat, 2015; Sevindik & Usual, 2021). Foodborne pathogens remain a major global concern, causing millions of illnesses and deaths annually (Liu et al., 2017). While synthetic preservatives have been used to control microbial growth and extend shelf life, they are often associated with potential carcinogenicity, toxicity, and allergic reactions, prompting a growing interest in natural alternatives such as spices, which are biodegradable, readily available, and associated with minimal health risks.

Spices have been extensively studied for their antimicrobial and antifungal activities, and their incorporation into daily diets is supported by their safety profile and GRAS status (Nabavi et al., 2015). Incorporating spices into food systems not only offers a natural approach to reducing microbial contamination but may also contribute to mitigating the spread of antimicrobial resistance. The antimicrobial properties of spices, such as ginger, garlic, cinnamon, and clove, are enhanced when extracted using different solvents, particularly ethanol, and may exhibit synergistic effects when combined. This study, therefore, aims to investigate and compare the antimicrobial effectiveness of selected culinary spices (*Cinnamomum verum*, *Syzygium aromaticum*, *Zingiber officinale*, and *Allium sativum*) against key foodborne pathogens (*Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*). Specifically, the study evaluates the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of both ethanolic and aqueous spice extracts, assesses the synergistic effects of combined extracts, and compares their efficacy with that of standard antibiotics. The findings are expected to provide scientific evidence supporting the traditional use of spices as natural antimicrobial agents, promote food safety, and offer strategies to mitigate antimicrobial resistance in food systems.

Materials and Methods

Collection of Samples

Fresh ginger (*Zingiber officinale*) and garlic (*Allium sativum*), as well as dried cloves (*Syzygium aromaticum*) and cinnamon sticks (*Cinnamomum verum*), were purchased from a local market. The plant materials were transported to the laboratory

in sterile polyethylene bags and stored at ambient temperature prior to analysis.

Clinical strains of *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli* were obtained from the Medical Microbiology Laboratory of University of Port Harcourt Rivers State, Nigeria. The isolates were confirmed using standard microbiological and biochemical identification procedures and maintained on nutrient agar slants at 4 °C until use.

Preparation of Spices Extracts

Processing of spices

The fresh ginger and garlic were peeled, thoroughly washed and sliced, then sundried for 5 days. All spices were then ground into powder.

Preparation of Aqueous Extracts

Twenty grams (20 g) of each ground spice were boiled with 100 mL of distilled water for 15 minutes. The mixture was allowed to cool to room temperature and subsequently filtered using sterile Whatman filter paper to obtain the aqueous extract.

Preparation of Ethanolic Extracts

Twenty grams (20 g) of each ground spice were soaked in 100 mL of ethanol for 48 hours at room temperature. The resulting mixture was filtered through sterile Whatman filter paper to obtain the ethanolic extract.

Preparation of Combined Extracts (Synergy Studies)

To evaluate potential synergistic effects, 0.3 mL of each individual spice extract was mixed with 0.3 mL of another spice extract. This process yielded six combinations: Ginger + Garlic, Clove + Cinnamon, Clove + Garlic, Clove + Ginger, Cinnamon + Garlic, Cinnamon + Ginger. These combined extracts were then used to assess the potential enhancement of antimicrobial activity against the selected bacterial pathogens.

McFarland standard

1.175g of Barium chloride (BaCl₂) was dissolved in distilled water to make a 100ml solution and stirred properly mixing the solution. 1ml of concentrated sulfuric acid (H₂SO₄) was diluted in distilled water to make a 100ml solution. 0.5ml of the Barium chloride was then added to 99.5ml of a solution of sulfuric acid and mixed thoroughly.

Preparation and Standardization of Bacterial Inoculum

The clinical strains of microorganisms (*Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*) were precultured in 100ml of nutrient broth and incubated for about 2hrs. The turbidity of the suspension was adjusted using McFarland standard as a reference (1×10⁸ cfu/ml) as described by Tandukar et al. 2017.

Determination of Antimicrobial Activity

Antimicrobial activity is of paramount importance in modern medicine, referring to the ability of a substance to eliminate or impede the growth of microorganisms such as bacteria, viruses, fungi, and protozoa. The antibacterial activity was determined by the diffusion method of Kirby Bauer described.

This method determines the antibacterial activity of the extract.

The agar diffusion method was used for evaluation of the antibacterial activity of ethanol and Aqueous extracts of different spices. Broth cultures of bacterial strains (*Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*) were grown on nutrient agar plates and incubated overnight at 37°C. One plate of each microorganism was taken and the colony was transferred into normal saline under aseptic conditions. The density of each microbial suspension was adjusted to be equal to 0.5 Mcfarland units (approximately 10⁶ CFU/ml for bacteria) to use it as the inoculum for the agar well diffusion assay. Bacterial strains were spread inoculated with a sterile cotton swab on the surface of sterile Mueller Hinton Agar plates so as to achieve a confluent growth. Following inoculation, agar well of 8 mm in diameter, 4 mm in depth and about 2 cm apart were punched in the Mueller Hinton Agar plate with a sterile cork borer. One hundred microliters (100 µl) of the various spice extracts were poured into the labeled wells and kept aside for 3 hours before incubation at 37°C for 18 - 24 hours as described by (Banik et al., 2018). In two other wells, Ciprofloxacin and water was added as positive and negative control respectively.

By using the disc diffusion method, the antibacterial activity of commercial drugs were determined. Subsequent incubation under specified conditions, the results were recorded by evaluating the diameter of the zone of inhibition (ZOI) in mm. The extracts were considered to be active, moderately active and highly active depending on their ability of clear zone parameter, respectively.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Minimum Inhibitory Concentration (MIC) for each test organism was determined by the macrodilution broth method. 4mls of Nutrient broth were added into the test tubes and 0.30ml of the spice extracts were added into the first broth. Serial two-fold dilutions were made with final concentrations ranging from 1000mg/mL to 125mg/mL. Afterwards, 0.1ml of the standardized bacteria suspensions (1 × 10⁶ CFU/mL) was added to each test tubes. The tubes were incubated at 37 °C for 24 h. MIC was defined as the lowest concentration of the antimicrobial agents (spice extract) at which there's no visible growth indicated by no turbidity. This was determined by observing the already incubated tubes for turbidity. The tubes without turbidity are noted and the tube with the lowest concentration is the MIC.

Minimum Bactericidal Concentration (MBC) is the lowest concentration of the antimicrobial agents (spice extract) at which the bacteria is killed. MBC was determined by subculturing the preparations that did not show any bacterial growth in the MIC determination. 1ml from the tubes without turbidity was subcultured and incubated overnight and the lowest concentration of the plate without colony (indicating presence of the organism) was recorded as the MBC.

Results and Discussion

Zone of Inhibition (ZOI) of Standard Antibiotic Disc against Gram-negative pathogens

OFX was sensitive to *Salmonella typhi* and *Escherichia coli* with ZOI of 20mm, NA was only sensitive to *Salmonella typhi* with ZOI 19mm while Nitrofurantoin was sensitive only to *Escherichia coli* with ZOI of 20mm. LBC was sensitive mostly to *Salmonella typhi* with ZOI of 23mm and to *Escherichia coli* with ZOI of 8mm.

Table 1: Zone of Inhibition of Standard Antibiotic Disc against Gram-negative pathogens (*Escherichia coli* and *Salmonella typhi*)

Antibiotic	OFX	IMP	CRO	NA	NF	AUG	GN	LBC
<i>Salmonella typhi</i>	20mm	-	-	19mm	-	-	-	23mm
<i>Escherichia coli</i>	10mm	-	-	-	20mm	-	-	8mm

Key: Ofloxacin (5µg) Imipenem (10µg) Ceftriaxone (30µg) Nalidixic acid (30µg) Nitrofurantoin (30µg) Augmentin (30µg) Gentamicin (10µg) Levofloxacin (5µg)

Zone of Inhibition (ZOI) of standard antibiotic disc against gram positive pathogen (*Staphylococcus aureus*)
GEN and OFX was sensitive to *Staphylococcus aureus* with ZOI of 20mm and 22mm respectively.

Table 2: Zone of Inhibition (ZOI) of standard antibiotic disc against gram positive pathogen (*Staphylococcus aureus*)

	GEN	CTR	ERY	CXC	OFX	AUG	CAZ	CTX
<i>Staphylococcus aureus</i>	20mm	-	-	-	22mm	-	-	-

Key: Gentamicin (10µg) Ceftriaxone (30µg) Erythromycin (15µg) Cloxacillin (5µg) Ofloxacin (5µg), Augmentin (30µg) Cefazidime (30µg) Cefotaxime (30µg).

Antimicrobial activity of Ethanolic Extracts of Spices against Food-borne pathogens.

The results of the assay are summarized in Table 3. Clove and cinnamon extracts displayed varying degrees of growth inhibition across all bacterial species tested, with mean zone diameters of 12-21 mm and 13-17 mm, respectively. Conversely, both ginger and garlic exhibited a narrow

spectrum of activity, showing efficacy solely against *Staphylococcus aureus*, with inhibition zones of 21 mm and 15 mm. The positive control, ciprofloxacin, demonstrated potent, broad-spectrum antimicrobial activity, with inhibition zones significantly larger (22-34 mm) than those produced by any of the tested extracts.

Table 3: Antimicrobial activity of Ethanolic Extracts of Spices against Food-borne pathogens

Spice Extracts	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Cinnamon	17mm	13mm	16mm
Cloves	12mm	15mm	21mm
Ginger	-	-	21mm
Garlic	-	-	15mm
Positive control (Ciprofloxacin)	28mm	22mm	34mm
Negative control (water)	-	-	-

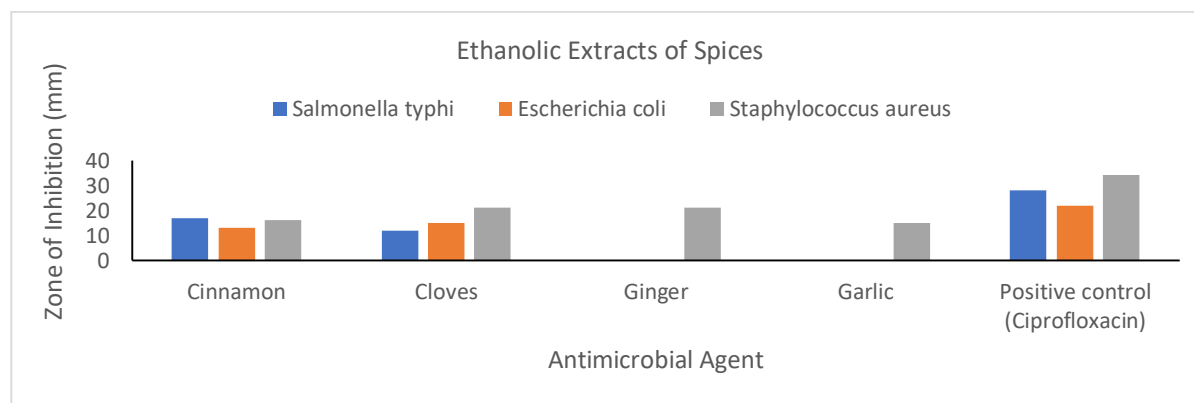


Figure 1: Antimicrobial activity of Ethanolic Extracts of Spices against Food-borne pathogens

Antimicrobial activity of Aqueous Extract of Spices against Food-borne pathogens.

The results of the aqueous extract assay are summarized in Table 4. Clove extract was the sole preparation to exhibit measurable inhibitory activity, demonstrating a limited

spectrum against *Salmonella typhi* and *Escherichia coli* with zones of 9 mm and 15 mm, respectively. All other aqueous spice extracts, including cinnamon, ginger, and garlic, showed no detectable activity against any of the tested pathogens..

Table 4: Antimicrobial activity of Aqueous Extract of Spices against Food-borne pathogens.

Spice Extracts	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Cinnamon	-	-	-
Clove	9mm	15mm	-
Ginger	-	-	-
Garlic	-	-	-
Positive control (ciprofloxacin)	28mm	22mm	34mm
Negative control (water)	-	-	-

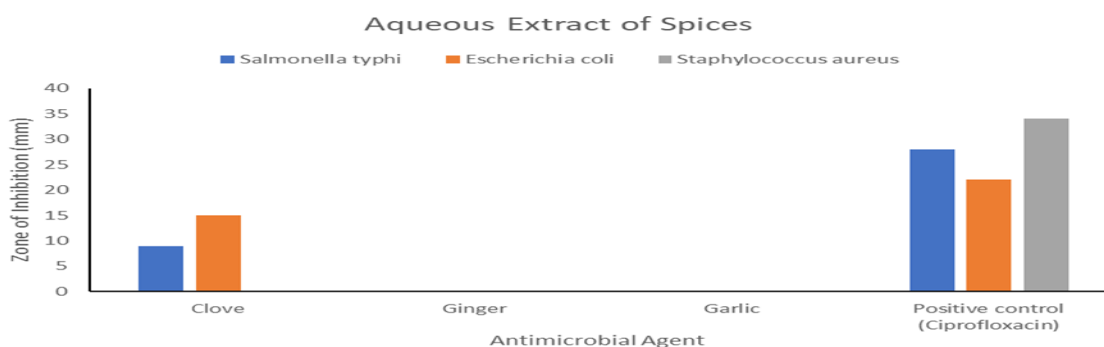


Figure 2: Antimicrobial activity of Aqueous Extract of Spices against Food-borne pathogens.

Synergistic Antimicrobial activity of Ethanolic Extracts of Spices against Food-borne pathogens

The results of the synergy assay for ethanolic extracts are summarized in Table .5. Combinations involving clove consistently produced inhibitory zones against all three pathogens, with diameters ranging from 11-20 mm. The

combination of clove and cinnamon was particularly effective against *Staphylococcus aureus*, yielding a 20 mm zone. In contrast, the ginger and garlic combination showed no synergistic activity. All combinations remained less effective than the ciprofloxacin control.

Table 5: Synergistic Antimicrobial activity of Ethanolic Extracts of Spices against Food-borne pathogens

Combined Spice Extracts	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Clove + Ginger	13mm	18mm	18mm
Clove + Cinnamon	12mm	13mm	20mm
Clove + Garlic	11mm	13mm	16mm
Ginger + Garlic	-	-	-
Cinnamon + Ginger	9mm	12mm	17mm
Cinnamon + Garlic	-	11mm	20mm
Positive control (Ciprofloxacin)	28mm	22mm	34mm
Negative control (water)	-	-	-

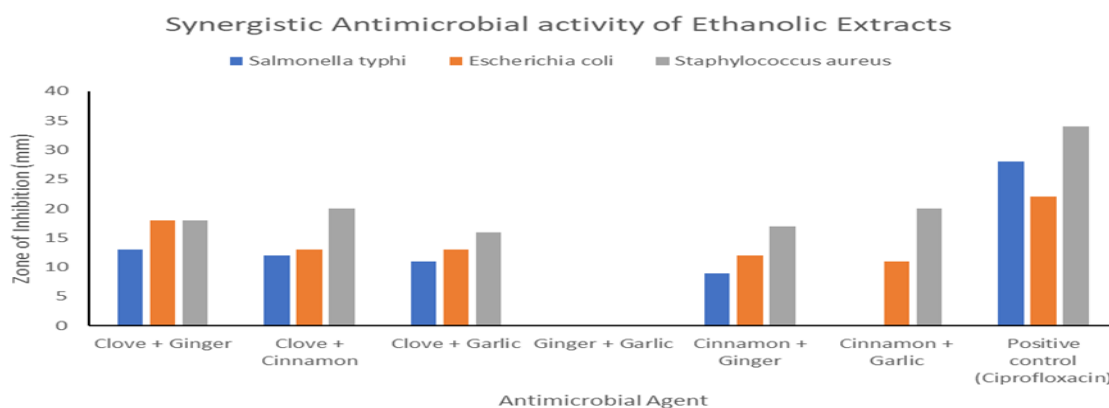


Figure 3: Synergistic Antimicrobial activity of Ethanolic Extracts of Spices against Food-borne pathogens

Synergistic antimicrobial activity of Aqueous Extract of spices against food-borne pathogens.

Only combinations containing clove extract demonstrated weak synergistic activity, with inhibition zones ranging from

8-14 mm. The combination of clove and cinnamon showed the broadest spectrum, inhibiting all three pathogens. All other aqueous combinations failed to produce any detectable zones of inhibition.

Table 6: Synergistic antimicrobial activity of Aqueous Extract of spices against food-borne pathogens.

Combined Spice Extracts	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Clove + Ginger	8mm	12mm	11mm
Clove + Cinnamon	8mm	13mm	10mm
Clove + Garlic	-	14mm	12mm
Ginger + Garlic	-	-	-
Cinnamon + Ginger	-	-	-
Cinnamon + Garlic	-	-	-
Positive control (Ciprofloxacin)	28mm	22mm	34mm
Negative control (water)	-	-	-

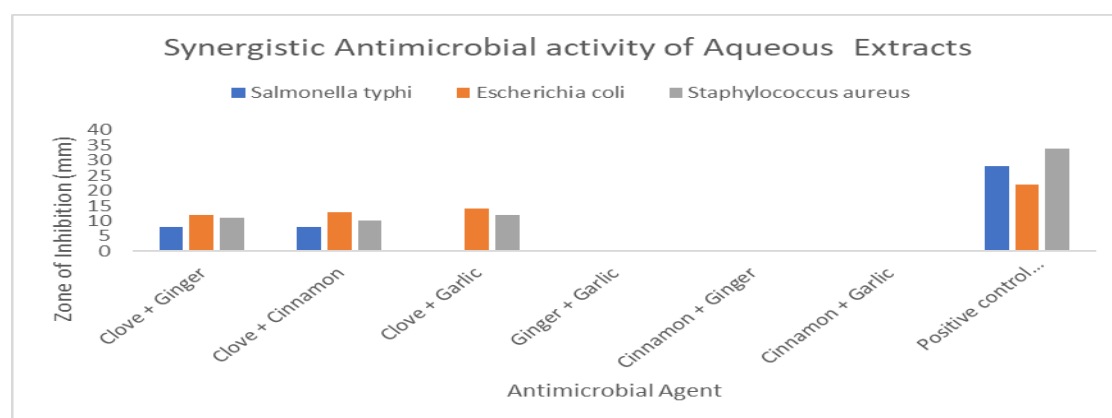


Figure 4: Synergistic antimicrobial activity of Aqueous Extract of spices against food-borne pathogens.

Minimum Inhibitory Concentration (mg/ml) of the Ethanolic Extracts of Spices

The MIC assay for single ethanolic extracts is summarized in Table 7. Cinnamon extract demonstrated the most potent inhibitory activity, with a uniform MIC of 125 mg/ml against all three bacterial pathogens. Clove extract also exhibited a

strong inhibitory effect, with an MIC of 125 mg/ml against *Salmonella typhi* and *Escherichia coli*, though a higher concentration of 250 mg/ml was required for *Staphylococcus aureus*. Ginger and garlic extracts displayed a narrow spectrum of inhibition, with an MIC of 250 mg/ml solely against *Staphylococcus aureus*.

Table 7: Minimum Inhibitory Concentration (mg/ml) of the Ethanolic Extracts of Spices

Spice Extracts	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Cinnamon	125mg/ml	125mg/ml	125mg/ml
Clove	125mg/ml	125mg/ml	250mg/ml
Ginger	-	-	250mg/ml
Garlic	-	-	250mg/ml

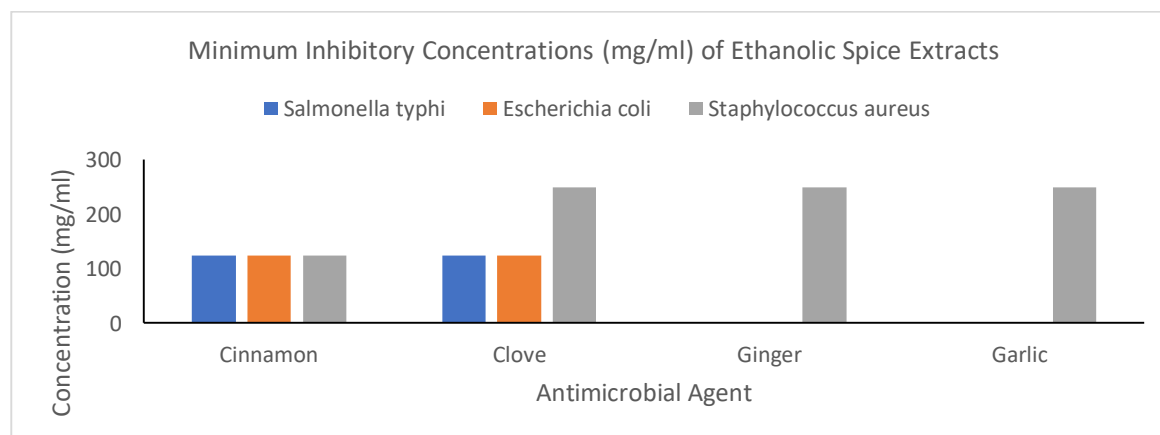


Figure 5: Minimum Inhibitory Concentration (mg/ml) of the Ethanolic Extracts of Spices

Minimum Bactericidal Concentration (mg/ml) of the Ethanolic Extracts of Spices

The MBC assay for single ethanolic extracts is summarized in Table 8. Cinnamon extract was the most effective bactericidal agent, requiring 250 mg/ml to kill *Salmonella typhi* and

Staphylococcus aureus, and 500 mg/ml for *Escherichia coli*. Clove, ginger, and garlic extracts each required a higher concentration of 500 mg/ml to exert a bactericidal effect on *Staphylococcus aureus*, and showed no lethal activity against the other two pathogens at the tested concentrations.

Table 8: Minimum Bactericidal Concentration (mg/ml) of the Ethanolic Extracts of Spices

Spice Extracts	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Cinnamon	250mg/ml	500mg/ml	250mg/ml
Clove	500mg/ml	500mg/ml	500mg/ml
Ginger	-	-	500mg/ml
Garlic	-	-	500mg/ml

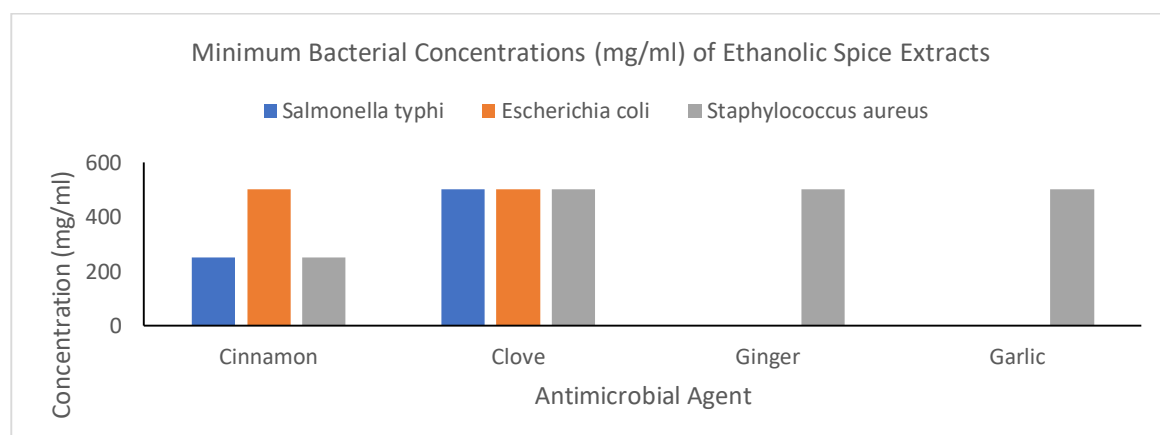


Figure 6: Minimum Bactericidal Concentration (mg/ml) of the Ethanolic Extracts of Spices

Minimum Inhibitory Concentration (mg/ml) of Aqueous Extract of spices

The MIC results for single aqueous extracts are summarized in Table 9. Clove extract was the only preparation to exhibit any

inhibitory activity, with an MIC of 125 mg/ml against *Escherichia coli* and 500 mg/ml against *Salmonella typhi*. No inhibitory activity was detected for the aqueous extracts of cinnamon, ginger, or garlic against any of the tested pathogens.

Table 9: Minimum Inhibitory Concentration (mg/ml) of Aqueous Extract of spices

Spice Extracts	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Cinnamon	-	-	-
Cloves	500mg/ml	125mg/ml	-
Ginger	-	-	-
Garlic	-	-	-

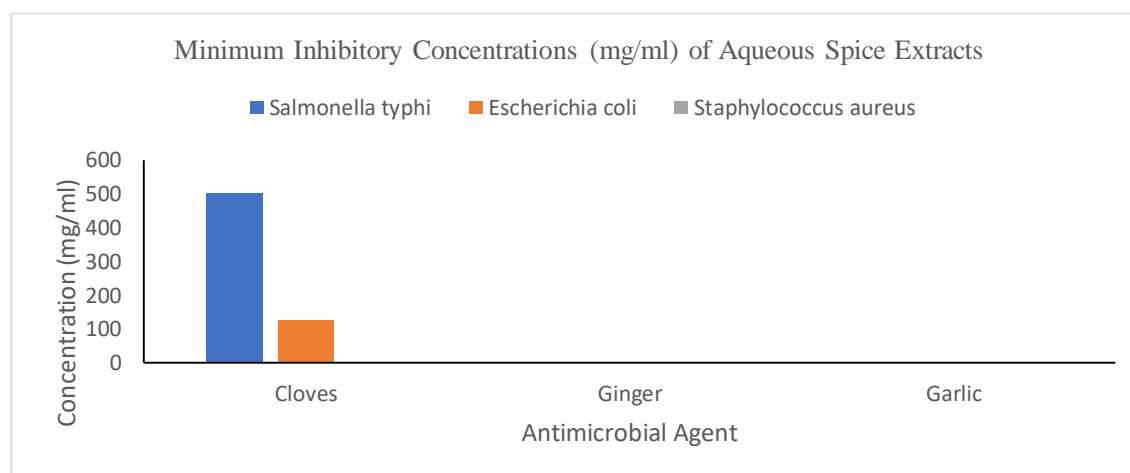


Figure 7: Minimum Inhibitory Concentration (mg/ml) of Aqueous Extract of spices

Minimum Bactericidal Concentration (mg/ml) of Aqueous Extract of spices

The MBC results for single aqueous extracts are summarized in Table 10. Clove extract was the only preparation with

measurable bactericidal activity, requiring 500 mg/ml to kill *Escherichia coli* and a high concentration of 1000 mg/ml to kill *Salmonella typhi*. No bactericidal activity was observed for the aqueous extracts of cinnamon, ginger, or garlic.

Table 10: Minimum Bactericidal Concentration (mg/ml) of Aqueous Extract of spices

Spice Extracts	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Cinnamon	-	-	-
Cloves	1000mg/ml	500mg/ml	-
Ginger	-	-	-
Garlic	-	-	-

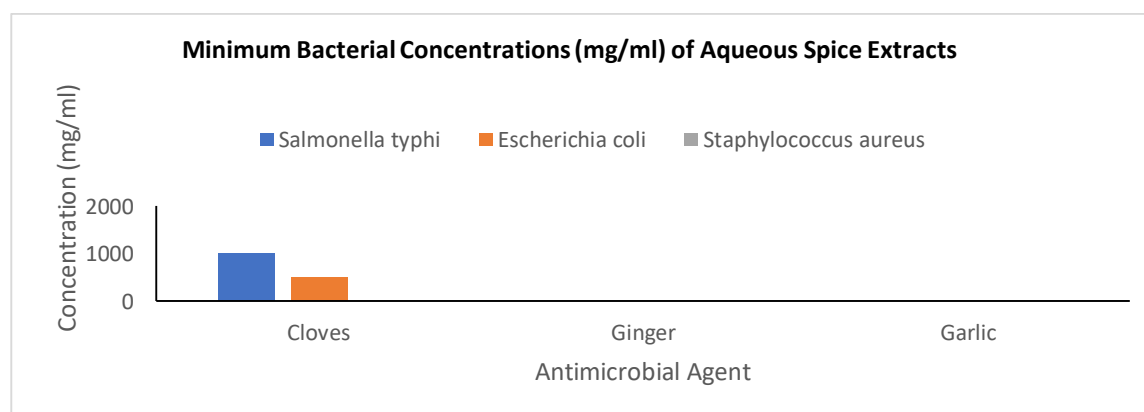


Figure 8: Minimum Bactericidal Concentration (mg/ml) of Aqueous Extract of spices

Minimum Inhibitory Concentration of Combined (Synergy) Spice Ethanolic Extracts

The MIC assay for synergistic ethanolic combinations is summarized in Table 11. The combination of clove and ginger was the most potent, demonstrating the lowest MIC values of

125 mg/ml against both *Escherichia coli* and *Staphylococcus aureus*. The majority of other clove-based combinations, such as clove with cinnamon or garlic, showed consistent MICs of 250 mg/ml across the pathogens. The combination of ginger and garlic showed no detectable inhibitory activity.

Table 11: Minimum Inhibitory Concentration of Combined (Synergy) Spice Ethanolic Extracts

Combined Spice Extracts	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Clove + Ginger	250mg/ml	125mg/ml	125mg/ml
Clove + Cinnamon	250mg/ml	250mg/ml	250mg/ml
Clove + Garlic	250mg/ml	250mg/ml	250mg/ml
Ginger + Garlic	-	-	-
Cinnamon + Ginger	250mg/ml	250mg/ml	125mg/ml
Cinnamon + Garlic	-	250mg/ml	250mg/ml

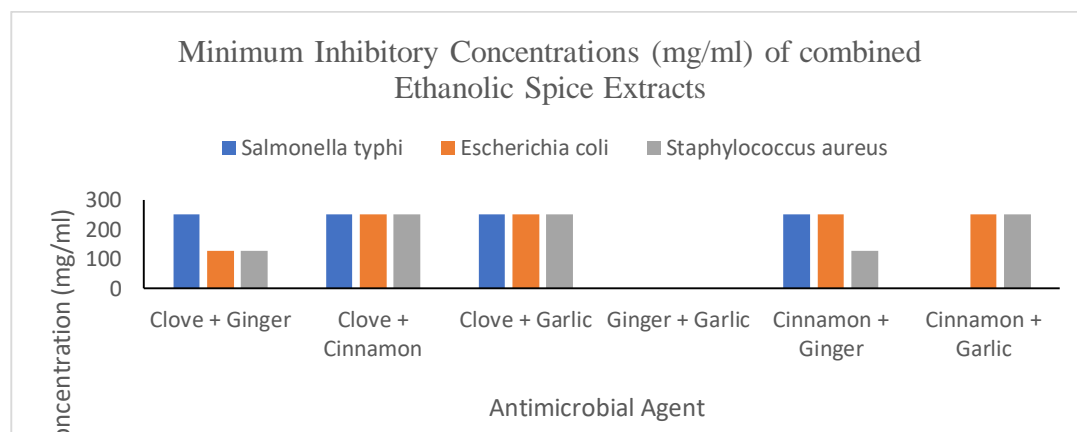


Figure 9: Minimum Inhibitory Concentration of Combined (Synergy) Spice Ethanolic Extracts

Minimum Bactericidal Concentration (mg/ml) of Combined (Synergy) Ethanolic Spice Extracts

The MBC assay for synergistic ethanolic combinations is summarized in Table 12. The clove and ginger combination were the most effective, with an MBC of 250 mg/ml against

Escherichia coli and *Staphylococcus aureus*. Other combinations, such as clove with cinnamon and cinnamon with garlic, required significantly higher concentrations, up to 1000 mg/ml, to achieve a bactericidal effect against *Staphylococcus aureus*.

Table 12: Minimum Bactericidal Concentration (mg/ml) of Combined (Synergy) Ethanolic Spice Extracts

Combined Spice Extracts	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Clove + Ginger	500mg/ml	250mg/ml	250mg/ml
Clove + Cinnamon	500mg/ml	500mg/ml	1000mg/ml
Clove + Garlic	500mg/ml	500mg/ml	500mg/ml
Ginger + Garlic	-	-	-
Cinnamon + Ginger	500mg/ml	500mg/ml	250mg/ml
Cinnamon + Garlic	-	500mg/ml	1000mg/ml

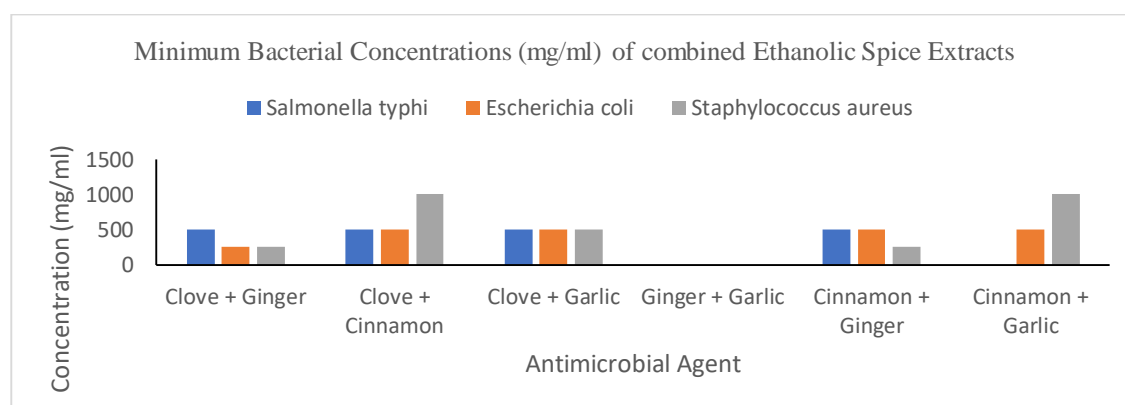


Figure 10: Minimum Bactericidal Concentration (mg/ml) of Combined (Synergy) Ethanolic Spice Extracts

Minimum Inhibitory Concentration (mg/ml) of Combined (Synergy) Aqueous Spice Extracts

The MIC results for synergistic aqueous combinations are summarized in Table 13. Activity was confined to combinations containing clove. The most effective pairs, clove

with ginger and clove with cinnamon, demonstrated MICs of 250 mg/ml against *Escherichia coli* and *Staphylococcus aureus*. All other combinations failed to inhibit bacterial growth at the tested concentrations.

Table 13: Minimum Inhibitory Concentration (mg/ml) of Combined (Synergy) Aqueous Spice Extracts

Combined Spice Extracts	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Clove + Ginger	500mg/ml	250mg/ml	250mg/ml
Clove + Cinnamon	500mg/ml	250mg/ml	250mg/ml
Clove + Garlic	-	250mg/ml	250mg/ml
Ginger + Garlic	-	-	-
Cinnamon + Ginger	-	-	-
Cinnamon + Garlic	-	-	-

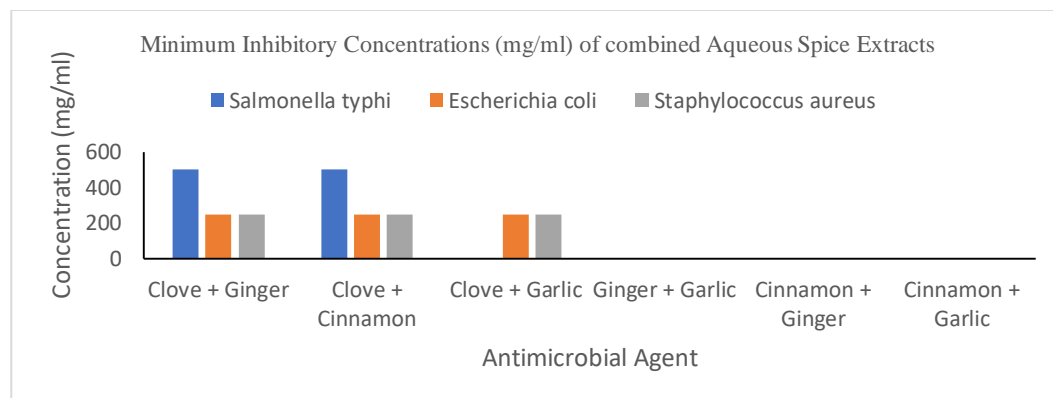


Figure 11: Minimum Inhibitory Concentration (mg/ml) of Combined (Synergy) Aqueous Spice Extracts

Minimum Bactericidal Concentration (mg/ml) of Combined (Synergy) Aqueous Spice Extracts

The MBC results for synergistic aqueous combinations are summarized in Table 14. Bactericidal activity was only observed in clove-containing combinations. The MBC for

these active pairs was 500 mg/ml against *Escherichia coli* and *Staphylococcus aureus*, and 1000 mg/ml against *Salmonella typhi*. All other aqueous combinations exhibited no bactericidal activity.

Table 14: Minimum Bactericidal Concentration (mg/ml) of Combined (Synergy) Aqueous Spice Extract

Combined Spice Extracts	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Clove + Ginger	1000mg/ml	500mg/ml	500mg/ml
Clove + Cinnamon	1000mg/ml	500mg/ml	500mg/ml
Clove + Garlic	-	500mg/ml	500mg/ml
Ginger + Garlic	-	-	-
Cinnamon + Ginger	-	-	-
Cinnamon + Garlic	-	-	-

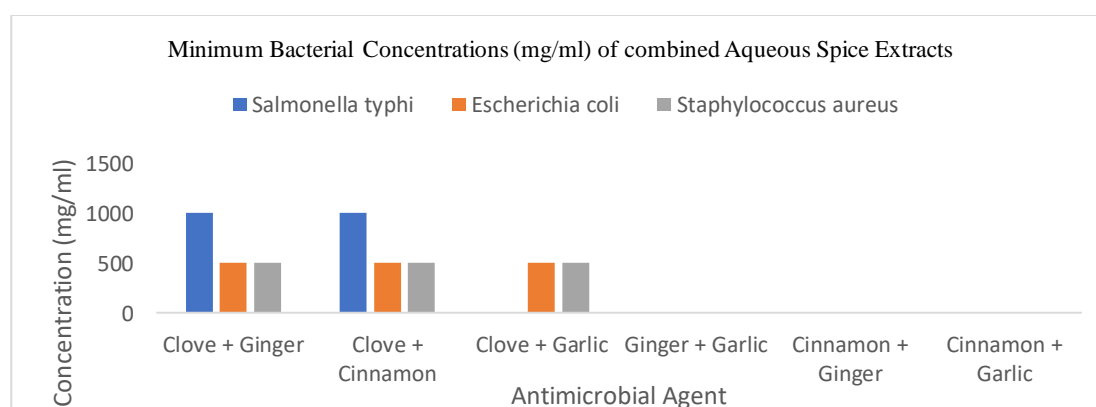


Figure 12: Minimum Bactericidal Concentration (mg/ml) of Combined (Synergy) Aqueous Spice Extracts

Percentage Effectiveness Compared to Standard Antibiotic (Ciprofloxacin)

The comparison is based on the zone of inhibition from Tables 3 and 5 as it provides a direct, visual measure of antimicrobial

strength. Ciprofloxacin's zones (28mm for *Salmonella typhi*, 22mm for *Escherichia coli*, and 34 mm for *Staphylococcus aureus*) are used as the 100% benchmark.

Table 15: Percentage Effectiveness Compared To Standard Antibiotic (Ciprofloxacin 85% - 100%)

Spice Extracts	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Single Ethanolic Extracts			
Cinnamon	61%	59%	47%
Cloves	43%	68%	62%
Ginger	0%	0%	62%
Garlic	0%	0%	44%
Best Synergistic Ethanolic Extracts			
Clove + Ginger	46%	82%	55%
Clove + Cinnamon	43%	59%	59%
Cinnamon + Garlic	0%	58%	59%
Single Aqueous Extract			
Clove	32%	68%	0%
Best Synergistic Aqueous Extract			
Clove + Cinnamon	29%	59%	29%
Clove + Garlic	0%	64%	35%

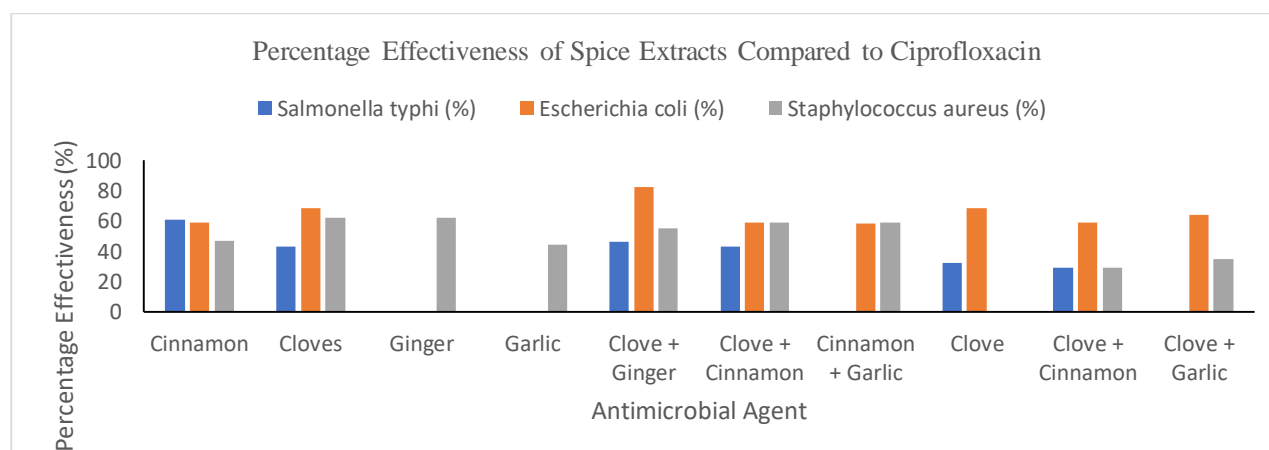


Figure 13: Percentage Effectiveness Compared to Standard Antibiotic (Ciprofloxacin)

Discussion

The evaluation of ethanolic extracts from common spices reveals significant antimicrobial potential against food-borne pathogens, with efficacy varying considerably between the different spices and bacterial species. According to (Tijjani, et al., 2017), an antimicrobial agent which the diameter of the zone of inhibition is above 3 mm, the organism is said to be sensitive but if it is 2 mm or less than that, the organism is said to have resistance over the particular agent.

Therefore, considering the statement of Tijjani et al., and comparing the result obtained in this study, shows that the Clove and cinnamon extracts displayed varying degrees of growth inhibition across all bacterial species tested, with Zones of Inhibition (ZOI) of 12-21 mm and 13-17 mm, respectively. Conversely, both ginger and garlic exhibited a narrow spectrum of activity, showing efficacy solely against *Staphylococcus aureus*, with inhibition zones of 21 mm and 15 mm. The positive control, ciprofloxacin, demonstrated potent, broad-spectrum antimicrobial activity, with inhibition zones significantly larger (22-34 mm) than those produced by any of the tested extracts.

The antimicrobial effectiveness of clove demonstrated a broad spectrum of activity, with inhibition zones exceeding 10 mm and MIC values ranging from 250 - 125mg/mL against a range of microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*. Clove showed higher Inhibitory effect on *Staphylococcus aureus* (21 ± 1.0mm) and moderate activity against the *Escherichia coli* (15 ± 1.0mm) and *Salmonella typhi* (12 ± 1.0mm). Badei et al. 2002 tested the antimicrobial activities of cardamom, cinnamon and clove essential oils (EOs) against nine Gram-positive bacterial strains, four Gram-negative bacterial strains, seven molds, and two yeasts, compared with phenol, using the disc diffusion method. Clove EO showed the highest antimicrobial activity, and the antimicrobial spectra (diameter of inhibition zones) of 10% clove EO was 1.48 times as that of 10% phenol. Angienda et al. 2010 investigated the antimicrobial activities of EOs of four spices against *Salmonella typhimurium* (*S. typhimurium*), *E. coli*, *B. cereus*, and *Listeria innocua* (*L. innocua*) by agar diffusion test. Clove EO showed the most effective inhibition against both Gram-positive bacteria and Gram-negative bacteria

compared with three other EOs, with the MICs ranging from 1.25% v/v (*B. cereus*) to 2.50% v/v (*S. typhimurium* and *E. coli*). Liang et al. 2003 observed the antimicrobial activities of seven spices, and different concentrations of extracts and EOs in each spice were used to test the effects on the growth of spoilage microorganisms in apple cider by total plate counts. Clove products showed the strongest antimicrobial activities compared with other spices tested. In addition, previous studies indicate that clove exhibits antioxidant capabilities comparable to those of butylated hydroxytoluene (BHT), a prevalent synthetic antioxidant utilized in food preservation. This suggests the potential for clove to serve as a natural preservative, enhancing oxidative stability, antioxidant attributes, flavor, shelf life, and food coloration (Idowu et al., 2021). The antimicrobial and antioxidant activities of clove were mainly attributed to the presence of secondary metabolites. A study conducted by Suleiman et al. revealed that the ethanolic extract of clove flower bud appeared to be rich in flavonoids (26.8%), phenolic acid (20.8%), and tannins (4.9%) (Suleiman et al., 2019), whose antioxidant effects were already well-known, similar to another phytochemical screening of clove made by Upadhyaya et al. 2018. In addition, the extract of clove flower bud with stronger antimicrobial capacity was also found to exhibit higher phenolic content (El-Maati et al., 2016), indicating that phenolic compounds that contributed to the antioxidant activity also displayed antibacterial capacity. Moreover, some components mainly existing in volatile oil also participated in the contribution of antibacterial activity, such as eugenol, isoeugenol, eugenyl acetate, caryophyllene, and humulene. Eugenol was even classified as a substance generally regarded as safe by Food and Drug Administration (FDA). Cinnamon displayed a broad spectrum of antimicrobial activity against *Staphylococcus aureus* ($16 \pm 1.0\text{mm}$), *Escherichia coli* ($13 \pm 1.0\text{mm}$) and *Salmonella typhi* ($12 \pm 1.0\text{mm}$) and it showed the ability to inhibit microbial growth as well as kill the microorganisms. The antimicrobial activities of cinnamon were evaluated in some studies. Gupta et al. 2008 compared the antimicrobial activities of cinnamon extract (50% ethanol) and EO against 10 bacteria and 7 fungi by the agar well diffusion method. Cinnamon EO was more effective than cinnamon extract against tested microorganisms. Cinnamon extract showed the highest activities. Ceylan et al. 2004 tested the antibacterial effects of cinnamon, sodium benzoate, potassium sorbate, and their combinations on *E. coli* at 8 and 25 °C in apple juice. The results showed that 0.3% w/v cinnamon provided 1.6 log CFU/mL reduction on *E. coli* at 8 °C and 2.0 log CFU/mL reduction at 25 °C. This discovery is mostly connected to cinnamaldehyde, a chemical component found in Cinnamon. Cinnamaldehyde can be used to prevent bacterial growth by inhibiting cell wall formation, cell membrane function, protein synthesis, or nucleic acid synthesis. The antimicrobial activity of Ginger and Garlic on the other hand only showed particularly against Gram-positive bacteria (*Staphylococcus aureus*) with Ginger having a ZOI of $21 \pm 1.0\text{mm}$ and Garlic with ZOI of $15 \pm 1.0\text{mm}$. (Abdallah 2017). The phenolic compounds in ginger work as denaturing agents, preventing microbial growth by modifying cell permeability and causing bacterial cells to shatter. Most phenolic compounds are metal chelators that bind to active

sites of metabolic enzymes, limiting enzyme activity as well as bacterial metabolism and reproduction. Ginger has been shown to inhibit the growth of colon bacteria as well as other pathogens, such as *Salmonella*, *E. coli*, *Staphylococci*, *Proteus sp.*, and *Streptococci*. Joe et al. 2009 reported the antimicrobial effects of garlic, ginger, and pepper ethanol extracts on *K. pneumoniae*, *S. aureus*, *M. morgani*, *C. albicans*, *E. coli*, and *P. vulgaris* using the filter paper assay. Garlic extract exerted superior antibacterial activities at all concentrations. Indu et al. 2006 tested the antibacterial activities of 5 spice extracts against 20 serogroups of *E. coli*, 8 serotypes of *Salmonella*, *L. monocytogenes*, and *A. hydrophila* by the agar well method and filter paper method. The results indicated that ginger extract possessed inhibitory effects on two serogroups of *E. coli*.

The antimicrobial activity of spice extracts is mainly attributed to their phytochemicals. Phenolic compounds, such as phenolic acids, flavonoids, and tannins are among the most abundant and widely distributed groups of secondary metabolites in edible plants. The antibacterial activity of these compounds present in the spices involves many modes of action, such as destroying cell membrane morphology, altering membrane fatty acids, depleting proton motive force, causing reactive oxygen damage, impairing enzymatic mechanisms for energy production and metabolism, disrupting normal functionality of proteins, and inhibiting nucleic acid synthesis (Elisha et al., 2017, Marchese et al., 2017).

The antibacterial activity seemed to be bacteria-dependent, and Gram-positive bacteria (*Staphylococcus aureus*) were more susceptible to the tested spice extracts than Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*), which was in accordance with many previous studies (Benmeziane et al., 2018, Nagy et al., 2015). Different from Gram-positive bacteria, Gram-negative bacteria have an outer membrane rich in lipopolysaccharides, as well as a unique periplasmic space. In general, Gram-negative bacteria were more resistant to antibiotics than Gram positive bacteria. The resistance is due to the differences in their cell wall composition. In Gram negative bacteria the outer membrane acts as a great barrier to many environmental substances including antibiotics. Presence of thick murein layer in the cell wall prevents the entry of the inhibitor. The complex composition and spatial structure of lipopolysaccharides form a barrier for penetration of antimicrobial agents, besides, the presence of enzymes in periplasmic space may break down intrusive molecules, preventing the antibacterial drugs entering intracellular environment.

The evaluation of aqueous extracts from cinnamon, clove, ginger, and garlic reveals significantly reduced antimicrobial potential compared to their ethanolic counterparts, highlighting the crucial role of extraction solvent in determining bioactive compound solubility and subsequent efficacy. The results demonstrate a stark contrast in activity profiles, with most aqueous preparations showing limited to no inhibitory effects against the tested food-borne pathogens. The aqueous extract of clove stood as the sole exception, demonstrating measurable inhibitory activity with zones of $9 \pm 1.0\text{ mm}$ against *Salmonella typhi* and $15 \pm 1.0\text{mm}$ against

Escherichia coli. This retention of activity, though substantially weaker than its ethanolic extract, suggests that certain antimicrobial components of clove possess sufficient hydrophilicity for partial extraction into water. The active compound eugenol, while predominantly hydrophobic, may form soluble complexes or undergo partial hydrolysis in aqueous solutions, yielding antimicrobial derivatives. Furthermore, the Minimum Inhibitory Concentration (MIC) values for aqueous clove extract—500 mg/ml for *Salmonella typhi* and 125 mg/ml for *Escherichia coli*—indicate that its efficacy is both pathogen-dependent and requires significantly higher concentrations than the ethanolic extract. The relatively better performance against *Escherichia coli* proves that certain water-soluble phenolics from spices can still disrupt membrane function in Gram-negative bacteria, albeit less efficiently than their lipid-soluble counterparts. Conversely, the aqueous extracts of cinnamon, ginger, and garlic showed a complete absence of inhibitory activity against all tested pathogens. This universal lack of efficacy underscores a fundamental phytochemical principle: the primary antimicrobial compounds in these spices are largely non-polar and possess poor solubility in aqueous solvents. The inactivity of aqueous cinnamon extract directly contrasts with the potent activity of its ethanolic form and can be attributed to the insolubility of cinnamaldehyde, its key bioactive component. Shan et al. emphasized that cinnamaldehyde's mechanism relies on its ability to integrate into lipid membranes, a property contingent on its hydrophobicity and lost in aqueous extraction. Similarly, the absence of activity in aqueous garlic extract is particularly instructive. While garlic is renowned for its potent antimicrobial compound allicin, this molecule is not only hydrophobic but also highly unstable in water, rapidly degrading into less active compounds. Therefore, the preparation and storage time of the aqueous extract likely resulted in the degradation of any allicin that may have initially dissolved, leading to the observed null result. This contrasts with traditional uses of crushed fresh garlic, where allicin is released immediately before application. The narrow spectrum of ginger's ethanolic extract, which targeted only *Staphylococcus aureus*, was entirely lost in its aqueous form, indicating that its gingerols and shogaols are also effectively insoluble in water, a finding consistent with the physicochemical profiles. The profound performance chasm between aqueous and ethanolic extracts is a central finding that aligns with the broader scientific consensus on phytochemical extraction.

The synergistic effect of combination of every two spice was performed by agar well diffusion method to detect their zone of inhibition along with their MIC and MBC for all of the tested organisms. In a combined extract of clove and cinnamon, a broader activity (20 ± 1.0 mm ZOI) against *Staphylococcus aureus* was observed, whereas they individually showed 21mm and 16mm, respectively. Against *Escherichia coli*, ZOI of 13mm was observed in contrast to their individual effects of 15mm and 13mm respectively. This combination also showed ZOI of 12mm in contrast to the individual effects of 12mm and 17mm respectively. Moreover, cinnamon extract combined with ginger, garlic extract showed better effect against *Staphylococcus aureus* than individual effect with ZOI of 17mm and 20mm respectively while their

individual ZOI is 16mm, 21mm and 15mm respectively. Cinnamon combined with Ginger showed ZOI of 9mm and 12mm for *Salmonella typhi* and *Escherichia coli* respectively and Cinnamon combined with Garlic showed ZOI of 11mm against *E. coli* in contrast to the zero activity of garlic on *Escherichia coli*. Furthermore, individually, garlic, and ginger exhibited no antimicrobial activity on *Escherichia coli* and *Salmonella typhi* however, in combination with clove extract, they showed an extensive zone of inhibition (18mm and 13mm) for *Escherichia coli* respectively and (13mm and 11m respectively) for the *Salmonella typhi*. The effect of combination of spices extracts have proven to be feature of antimicrobial and antioxidant treatment due to number of important considerations viz (i) they increase activity through use of compounds with synergistic or additive activity, (ii) they thwart drug resistance (ii) they decrease required doses, reducing both cost and adverse/toxic side effects and (iv) they increase the spectrum of activity (Baljeet et al. 2015). The efficiency of the combination of extracts can be more promising against the microbial growth where individual action is less likely to be effective. Combination of various compounds may have contributed to the observed synergistic and additive effects. The multiple mode of action may include degradation of cell wall, disruption of cytoplasmic membrane, leakage of cellular components, alteration of fatty acid and phospholipids constituents, changes in synthesis of DNA and RNA and destruction of protein translocation (Baljeet et al. 2015). Hence it is possible that combining spice extracts could lead to synergistic or additive inhibitory potential against both food spoilage and pathogenic microorganisms. Most studies attributed additive and synergistic effects to phenolic and alcoholic compounds. Synergism often results from components of one spice supporting the other while improving the total efficiency. Previously few reports were present on the synergistic/antagonistic effects of spice extracts especially on food-spoilage microorganisms (Baljeet et al., 2015, Banik et al., 2018). A specific policy has been adopted by the World Health Organization (WHO) that primary health care sectors in developing countries throughout the world require more effective and efficient traditional medical practice. The antimicrobial activities of combined extract were more effective since antimicrobial properties of herbs and spices not only depend on their chemical compositions but also on their lipophilic properties, water solubility and various compounds that may have contributed to the observed additive effects (Baljeet et al., 2015). Therefore, combinations of spices and other natural antimicrobial agents may increase food shelf life by destroying food spoilage organisms and can be used as alternative drugs to treat mild sickness instead of using commercial drugs as well. Mostly, the natural bioactive compound has shown to affect the structure and integrity, permeability or functionality of cytoplasmic membrane, and the efflux system of target bacteria. The antimicrobial activity of alternative plant bioactive components has shown to activate immune cells and enhance the growth of beneficial gut flora. In fact, the efficacy of bioactive compound is regulated by the target site and structure of bacterial cells as well as environmental factors like redox potential, moisture content, hydrophilicity, temperature, pH, acidity and availability of nutrients of target bacteria.

For the ethanolic extracts of spices compared with Ciprofloxacin (antibiotic for positive control), Clove displayed the highest microbial percentage efficiency against *Escherichia coli* and *Staphylococcus aureus* with 68% and 62% respectively, whereas cinnamon had the highest efficiency of 61% against *Salmonella typhi*. Hence, they are almost as potent as the c\ Ciprofloxacin. When combined together, Clove + ginger had percentage efficiency of 82% against *Escherichia coli*. This combination proves to be more effective when combined (synergy) than in individual states. In Aqueous state, clove still proved highly potent with efficiency of 68% and when combined with garlic has the most efficiency of 64% both against *Escherichia coli*. Hence these spices extracts may serve as a complementary or alternative antimicrobial agents used in place of antibiotics for the treatment of infections caused by these pathogens. The use of spices is prehistoric. In vitro and in vivo studies have examined their role in food safety and preservation, in addition to their primary use as flavoring and coloring compounds. Spices can prevent and treat cancer, aging, and metabolic, neurological, cardiovascular, and inflammatory problems. Spices contain antibacterial and antifungal chemicals that can kill spoilage microorganisms and fight human infections.

Conclusion

This study examined the antimicrobial activities of ethanolic and aqueous extracts of clove, cinnamon, ginger, and garlic, both individually and in combination, against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*, and compared their effects with a standard antibiotic. The findings showed that all the extracts possessed varying degrees of antimicrobial activity, with ethanolic extracts generally more effective than aqueous extracts. Among the spices, clove and cinnamon exhibited the strongest inhibitory effects, while the combined extracts showed broader activity, suggesting possible synergistic interactions. *Staphylococcus aureus* was more sensitive than *Escherichia coli* and *Salmonella typhi*, likely due to differences in cell wall structure. Although the extracts did not surpass the standard antibiotic, their significant inhibitory effects indicate that these spices contain active compounds capable of suppressing microbial growth. This supports their potential use as natural antimicrobial agents or food preservatives, providing a safer alternative to synthetic chemicals.

Recommendations

- i) Food industries, health professionals, and researchers should be encouraged to explore and adopt natural antimicrobial agents such as spices and herbs as safer and eco-friendly alternatives to synthetic preservatives and antibiotics.
- ii) It is recommended that in vivo studies and food model experiments be carried out to assess the extracts' real-life preservative and therapeutic effectiveness, stability, and safety.
- iii) Toxicological studies should be performed to determine the safety of using these plant extracts in foods or medicinal formulations, especially at higher concentrations or in combination.
- iv) Future investigations should include more microorganisms, including other Gram-positive and Gram-negative bacteria, as

well as fungi, to provide a more comprehensive evaluation of antimicrobial potential.

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