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Antibacterial Activity of Ethanolic and Aqueous Extracts of Zingiber officinale Roscoe on Selected Bacterial Isolates

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Abstract

Background: *Zingiber officinale Roscoe* (Ginger) is a spice commonly known for its nutritional and flavouring value. This study was carried out to assess the antibacterial activities of the ethanolic and aqueous extracts of *Zingiber officinale Roscoe* on selected bacteria isolates.

Materials and Methods: Swab samples were taken to the laboratory for culturing and isolation. The zones of inhibition of the ethanolic and aqueous extracts of *Zingiber officinale Roscoe* on four selected bacteria isolates from the swab samples were tested for antibacterial activity.

Results: The ethanolic extract of *Zingiber officinale Roscoe* exhibited antibacterial activity against all the bacterial isolates at concentrations of 100, 50, and 25 mg/ml. There was no antibacterial activity at 12.5 mg/ml and 6.25 mg/ml. The aqueous extract of *Zingiber officinale Roscoe* exhibited antibacterial activity against all the bacterial isolates at concentrations of 100 mg/ml and 50 mg/ml. There was no antibacterial activity at concentrations of 25, 12.5, and 6.25 mg/ml. Ciprofloxacin, which served as a control, exhibited antibacterial activity at all concentrations on the selected bacterial isolates. The antibacterial activity of ciprofloxacin was significantly higher ($P < 0.05$) than the ethanolic and aqueous extracts of *Zingiber officinale Roscoe*.

Conclusion: The ethanolic and aqueous extracts of *Zingiber officinale Roscoe* showed antibacterial activity at 100 mg/ml and 50 mg/ml concentrations on the selected bacteria isolates. These findings support the potential of *Zingiber officinale Roscoe* as alternative therapeutic agent against bacterial infections.

Keywords: Bacteria, *Zingiber officinale Roscoe*, Ethanolic extract, Aqueous extract, Zone of Inhibition, Ciprofloxacin

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Introduction

Numerous phytochemicals found in plants, fruits, and vegetables have been shown to possess antioxidant and antibacterial properties, often being highlighted for their potential health benefit.[1] In recent years, scientific research has increasingly shifted towards utilizing natural molecules rather than synthetic compounds.[2] This trend is driven by the fact that plant materials contain a diverse array of molecules with significant potential applications across environmental, food, and medical industries.[3] Our environment is teeming with microbes, and our bodies provide a perfect habitat for these microorganisms. While antibiotics are commonly used to manage infections, their overuse has led to the development of resistance in many pathogens, complicating the treatment of infectious diseases.[4] Studies[5,6] have shown that natural products may offer similar antimicrobial benefits as antibiotics, but with fewer side effects.

Zingiber officinale Roscoe (Ginger) is a renowned spice commonly used in various foods due to its nutritional content and flavoring properties.[7] It is a rich source of vitamins, carbohydrates, and minerals.[7] Ginger root is commonly used to alleviate and treat a variety of ailments, including headaches, colds, nausea, and vomiting.[7] Ginger is rich in bioactive constituents, including phenolic and terpene compounds, which contribute to its therapeutic potential. The primary phenolic compounds in ginger are gingerols, shogaols, and paradols.[8] In fresh ginger, gingerols, such as 6-gingerol, 8-gingerol, and 10-gingerol, are the predominant polyphenols. However, upon heat treatment or prolonged storage, gingerols can convert into shogaols, which can subsequently be transformed into paradols through hydrogenation.[8] Other phenolic compounds present in ginger include quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione.[9] Additionally, ginger contains several terpene components, such as β-bisabolene, α-curcumene, zingiberene, α-farnesene, and β-sesquiphellandrene, which are key constituents of ginger essential oils.[8]

Ginger has shown strong antimicrobial properties in inhibiting the growth of various microorganisms, including bacteria, fungi, and viruses, primarily through several mechanisms. It has been found to disrupt biofilm formation in multidrug-resistant strains of *Pseudomonas aeruginosa* by affecting membrane integrity and reducing the level of bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP), a key regulator of biofilm development.[10] Additionally, ginger extracts inhibit biofilm formation, glucan synthesis, and the adherence of *Streptococcus mutans* by down-regulating virulence genes, which have been linked to reduced caries development.[11] Compounds such as gingerenone-A and 6-shogaol in ginger have demonstrated the ability to inhibit *Staphylococcus aureus* by targeting and inhibiting 6-hydroxymethyl-7, 8-dihydropterin pyrophosphokinase.[11] Ginger essential oil, with its lipophilic properties, enhances the permeability of fungal cell walls and membranes, leading to a loss of membrane integrity.[7] Fresh ginger has been effective in inhibiting plaque formation caused by human respiratory syncytial virus (HRSV) in respiratory tract cell lines by blocking viral attachment and internalization.[12] In clinical settings, ginger extract has been shown to lower hepatitis C virus (HCV) loads, reduce α-fetoprotein (AFP) levels, and improve liver function markers such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in Egyptian patients.[13]

Bacterial conjunctivitis presents with signs and symptoms of redness, discharge, swelling, irritation and discomfort, tearing, light sensitivity and itching.[14] *Staphylococcus aureus* is one of the most frequently isolated bacteria in cases of bacterial conjunctivitis, particularly in adults and those with pre-existing ocular conditions.[14] *Streptococcus pneumoniae* bacterium is seen in many bacterial conjunctivitis cases, particularly in children.[10] Bacteria typically enter the conjunctival sac through direct contact with infected secretions, contaminated surfaces, or adjacent mucosal tissues (e.g., nasal or sinus mucosa). The management of this condition typically involves both pharmacological and non-pharmacological approaches aimed at alleviating symptoms, eradicating the infection, and preventing transmission. Topical antibiotics are the cornerstone of treatment for bacterial conjunctivitis, particularly when symptoms are moderate to severe or if there is a risk of complications.[14]

The use of plant extracts in the treatment of infections is widely advocated due to their antibacterial properties. With the rising concerns of antibiotic resistance, there is a need to explore natural remedies as potential alternatives to conventional antibiotics. This is also applicable to the treatment of ocular infections such as

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bacterial conjunctivitis. Overall, ginger's antimicrobial effects are attributed to its ability to inhibit bacterial biofilm formation, disrupt ergosterol biosynthesis in fungi, and block viral attachment and internalization.[15] This study was carried out to assess the antibacterial activities of the ethanolic and aqueous extracts of *Zingiber officinale Roscoe* on selected bacteria isolates.

Materials and Methods

Collection of Plant Material

The fresh ginger rhizomes were obtained from the local market at Owerri. The rhizomes were identified and authenticated by Prof. Mrs. C.P. Anyanwu, a botanist at the Department of Crop Science and Technology, Federal University of Technology, Owerri. A voucher number (FUTO/001/574/2024) was assigned to it. The rhizomes were then taken to the Department of Microbiology Laboratory, Federal University of Technology Owerri, for processing and extraction. The rhizomes were washed thoroughly with distilled water and oven dried at 50°C. The rhizomes were ground into fine powder under laboratory conditions using a sterile electric blender, weighed and stored in an airtight container and then stored in dry plastic container until time of extraction.[15]

Ethanolic Extraction of *Zingiber officinale Roscoe*

The ethanolic extraction of *Zingiber officinale Roscoe* rhizome was done using the soxhlet extraction process as described by Fairbrother.[15] Three hundred milliliters (300 ml) of 95% ethanol was poured into a Soxhlet flask, and 100 grams of the ginger was placed into the extractor, and the reflux arm plugged with cotton wool to avoid flow of particles. The Soxhlet apparatus was mounted on a heating mantle set at the boiling point of the solvent (78°C). When the solvent was boiling, the vapor was evaporated through the extractor arm into the extraction chamber, while the condenser at the top condensed the vapor. The liquid condensate dripped into the center which contained the *Zingiber officinale Roscoe* rhizome sample to be extracted. The extract filled the siphon tube, where it flowed back down into the soxhlet flask. This was allowed to continue to circulate until the extraction was completed. It was then removed, and then the ethanol was evaporated to remain only the *Zingiber officinale Roscoe* extract in the soxhlet flask.

Aqueous Extraction of *Zingiber officinale Roscoe*

The aqueous extraction of *Zingiber officinale Roscoe* was done using the cold maceration process as described by Hidayat and Wulandari.[16] Thirty grams of *Zingiber officinale Roscoe* sample was weighed into a sterile 250 ml conical flask. One hundred milliliters of distilled water was poured into the *Zingiber officinale Roscoe* powder, corked, and allowed to soak at a ratio of 3:25 and left to macerate for 24 hours at a temperature of 4°C. The mixture was then filtered after 24 hours using sterile cheese filter cloth and the *Zingiber officinale Roscoe* extract was then evaporated using hot air oven or rotary vacuum evaporator at 60°C to remain only the extract.

Collection and Transportation of Bacteria Swabs

Bacteria swabs were collected from four patients who presented with signs and symptoms of bacterial conjunctivitis at the Department of Optometry Eye Clinic, Federal University of Technology, Owerri, Nigeria. Upon examination of the external ocular tissues using a pen light, samples were gently obtained from the inferior conjunctival sac with a sterile swab stick from patients who presented with red eyes and purulent conjunctival discharges. Ethical approval for the study was obtained from the Ethics Committee, School of Health Technology, Federal University of Technology, Owerri, Nigeria, with reference number FUT/SOHT/C1/062 dated May 3, 2024. All the participants provided informed consent to be part of the study and participation in the study was voluntary. The Amies transport medium was used to transport the swab samples to the Microbiology Department Laboratory at Federal University of Technology, Owerri for culturing and identification of microorganisms.[17]

Identification of Bacteria Isolates

The media preparation was done according to the manufacturer's specification[15] prior to collection of swabs. The swab samples underwent inoculation on Nutrient agar, Blood agar, Mac-Conkey agar, and Chocolate agar plates to isolate and grow individual bacterial colonies from the mixed microbial population. The bottom of the agar plate was labelled with relevant information, including sample identification number, specimen source, and date. Starting at one edge of the agar plate, the swab was streaked over the surface of the agar in a zigzag motion while rotating the plate slightly. After completing the first streak, without touching the initial streak, a second quadrant of the plate was streaked

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by spreading the bacteria from the first streak into the second quadrant. The streaking process was repeated for the third and fourth quadrants. The agar plate lid was closed immediately after streaking to prevent contamination. The inoculated agar plate was then placed in an incubator for 24 hours. Bacterial isolates were identified based on standard biochemical characteristics, employing both microscopic and macroscopic analyses.[18] Gram staining and motility tests were conducted. For gram-negative identification, biochemical tests such as indole, citrate, oxidase, catalase, H_2S production, lysine decarboxylase, lactose fermentation, urea hydrolysis, and gas production were employed. Gram-positive bacteria were identified using catalase, coagulase tests, and observing haemolysis patterns on blood agar. The sterility of culture media was verified by incubating 3–5% of the batch at 37°C overnight and observing for bacterial growth. The media that produced growth were examined for color, shape, elevation and pattern of growth.

Preparation and Standardization of Test Microorganisms

The test organisms that were employed in this study were bacterial isolates of *Staphylococcus aureus, Streptococcus viridans, Escherichia coli* and *Pseudomonas aeruginosa.* The micro-organisms obtained from the conjunctival swabs were identified and cultured at the laboratory. They were inoculated in the petri-dishes using sterile wire loop and the spreading method to enable them grow in the nutrient agar at 37°C for 24 hours. The test organisms were then picked up by a sterile loop from the culture and transferred and suspended into a tube containing sterile normal saline. This was then placed in an incubator for 5-10 minutes until it achieved turbidity. Turbidity was reached when the test organism reached McFarland 0.5 turbidity standard. A McFarland standard is taken as a reference to adjust the turbidity of bacterial suspensions as bacterial suspensions could cause potential bias in the result if they vary in turbidity. Turbidity implies the presence of the organism.

Test for Antibacterial Activity of *Zingiber officinale Roscoe* Extracts

The antimicrobial activity of the ethanolic and aqueous extracts of *Zingiber officinale Roscoe* was measured using agar-well diffusion method.[19] A stock solution of extract was prepared by dissolving 10 grams of the extract in 100 ml of their respective solvents (distilled water and ethanol) to produce a concentration of 100 mg/ml. The stock solution was then prepared at concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25mg/ml by dissolving 10 grams of the extract in 200 ml, 400 ml, 800 ml and 1600 ml of the solvent respectively. Sterile paper discs were impregnated with *Zingiber officinale Roscoe* extracts (ethanol and aqueous) at varying concentrations and placed onto the agar plates inoculated with bacterial cultures using sterile forceps. The set up was incubated aerobically at 37°C for 24 hours. At the end of the incubation period, inhibition zones formed on the agar were measured in millimeter (mm) using a ruler and the results were recorded.

Antibiotic Sensitivity Testing

Antibiotic sensitivity testing was carried out with Ciprofloxacin which served as control in this study. Ciprofloxacin 500 mg tablets were bought from a reputable pharmacy in Owerri. It was diluted in distilled water to prepare different concentrations. The 500 mg was dissolved in 5 ml distilled water to produce 100 mg/ml. To produce other concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml, the 500 mg was dissolved in 10 ml, 20 ml, 40 ml and 80 ml of distilled water respectively. Antibiotic sensitivity testing was done using the disc diffusion method.[19] By plating out, the test organism was seeded on Mueller Hinton agar. Sterile paper discs were impregnated with the different concentrations of ciprofloxacin. The antibiotic sensitivity disc was then placed onto agar plates inoculated with bacterial cultures with sterile forceps. The set-up was incubated aerobically at 37°C for 24 hours. After 24 hours, the zones of inhibition around the antibiotic agar plates were measured in millimeter using a meter rule and the results were recorded.

Minimum Inhibitory Concentration (MIC) Determination

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent required to inhibit visible growth of a microorganism. This was assessed using broth dilution, with clear endpoints indicating microbial inhibition.[20] To determine the MIC, increasing concentrations (5% - 100% with 5% intervals) of each extract were prepared in 9 ml tubes of sterile nutrient broth. Exactly 100 µl of each standardized test organism was then introduced into each tube of extract. A tube containing only nutrient broth and bacteria without extract served as negative control while another

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tube containing just the extract and broth without bacteria served as positive control. Each tube was incubated for 18 hours and then examined for visible growth or turbidity. The concentration of the extract in the tube in which no visible growth was observed when compared with the controls was taken as the MIC.

Minimum Bactericidal Concentration (MBC) Determination

The Minimum Bactericidal Concentration **(**MBC) refers to the lowest concentration of an antimicrobial that kills 99.9% of the initial bacterial population. MBC was determined by sub culturing samples from tubes showing no visible growth in the MIC test onto a growth medium without the antimicrobial agent. The absence of bacterial colonies confirms the bactericidal endpoint.[21] To determine the MBC for each extract, samples from the test tubes used in MIC test that showed no visible growth after the period of incubation were inoculated on sterile nutrient agar plates (which had no antimicrobial incorporated) in them using sterile swab sticks. The plates were incubated at 37 ^{o}C for 18-24 hours and were then observed for growth. The concentration at which absence of growth was observed (bactericidal activity) was taken as the MBC.

Statistical Analysis

Data collected from this study were uploaded onto the Statistical Package for Social Sciences (SPSS) version 23 software for analysis. The One-way Analysis of Variance (One-way ANOVA) was used to compare the antibacterial activities of the ethanolic and aqueous extracts of *Zingiber officinale Roscoe* and Ciprofloxacin at a significance level of 0.05.

Results

Table 1 shows the distribution of the mean zones of inhibition of *Zingiber officinale Roscoe* and Ciprofloxacin on the selected bacteria for the study which include *Staphylococcus aureus*, *Streptococcus viridans*, *Escherichia coli* and *Pseudomonas aeruginosa*. Ciprofloxacin served as a control in this study. The zones of inhibition of *Zingiber officinale Roscoe* ethanolic and aqueous extracts and Ciprofloxacin on the selected bacterial isolates were measured at concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25mg/ml. The selected concentrations allow for the evaluation of concentration-dependent effects on bacterial growth and viability. It also enables the determination of MIC and MBC values which are essential for understanding the antibacterial efficacy of ginger. Similar concentration ranges have also been used in previous studies.[9-11] The ethanolic extract of *Zingiber officinale Roscoe* produced zones of inhibition against all the bacteria isolated at 100 mg/ml, 50 mg/ml and 25 mg/ml concentrations. Against *S. aureus*, there was a mean (± standard error mean) zone of inhibition of 18.63± 0.24 mm with 100 mg/ml concentration, 12.13 ± 0.42 mm with 50 mg/ml, 4.18 ± 0.12 mm with 25 mg/ml, and 1.70 ± 0.12 mm with 12.5 mg/ml. There was no zone of inhibition with 6.25 mg/ml. Against *S. viridans*, the mean (± standard error mean) zone of inhibition was 17.75± 0.48 mm with 100 mg/ml concentration, 10.75 ± 0.63 mm with 50 mg/ml, 2.75 ± 0.25 mm with 25 mg/ml, and 0.65 ± 0.24 mm with 12.5 mg/ml. There was no zone of inhibition with 6.25 mg/ml. Against *E. coli*, the mean (± standard error mean) zone of inhibition was 15.50 ± 0.65 mm with 100 mg/ml concentration, 7.63 ± 0.38 mm with 50 mg/ml, 0.83 ± 0.12 mm with 25 mg/ml. There was no zone of inhibition with 12.5 mg/ml and 6.25 mg/ml concentrations. Against *P. aeruginosa*, the mean (± standard error mean) zone of inhibition was 17.00 ± 0.71 mm with 100 mg/ml concentration, 8.03 ± 0.34 mm with 50 mg/ml, and 0.78 ± 0.13 mm with 25 mg/ml. There was no zone of inhibition with 12.5 mg/ml and 6.25 mg/ml concentrations. The aqueous extract of *Zingiber officinale Roscoe* produced zones of inhibition at a higher concentration of 100 mg/ml with all the bacteria isolated. They were however lower in diameter when compared to the ethanolic extract of *Zingiber officinale Roscoe*. Against *S. aureus*, there was a mean zone of inhibition of 7.25 ± 0.48 mm with 100 mg/ml concentration, and 0.83 ± 0.12 mm with 50 mg/ml. There was no zone of inhibition with 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml concentrations. Against *S. viridans*, the mean zone of inhibition was 8.25 ± 0.25 mm with 100 mg/ml concentration, and 0.88 ± 0.13 mm with 50 mg/ml. There was no zone of inhibition with 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml concentrations. Against *E. coli*, the mean zone of inhibition was 4.25 ± 0.25 mm with 100 mg/ml concentration, and 0.28 ± 0.16 mm with 50 mg/ml. There was no zone of inhibition with 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml concentrations. Against *P. aeruginosa*, the mean zone of inhibition was 2.63 ± 0.24 mm with 100 mg/ml concentration. There was no zone of inhibition with 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml concentrations. With Ciprofloxacin which served as a

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control, the mean zone of inhibition against *S. aureus* was 29.00 ± 0.71 mm with 100 mg/ml concentration, 17.63 ± 0.24 mm with 50 mg/ml, 9.38 ± 0.38 mm with 25 mg/ml, 2.13 ± 0.13 mm with 12.5 mg/ml, and 0.88 ± 0.13 mm with 6.25 mg/ml. Against *S. viridans*, the mean zone of inhibition was 24.13 ± 0.13 mm with 100 mg/ml concentration, 16.76 ± 0.25 mm with 50 mg/ml, 7.75 ± 0.25 mm with 25 mg/ml, 1.38 ± 0.24 mm with 12.5 mg/ml, and 0.50 ± 0.12 mm with 6.25 mg/ml. Against *E. coli*, the mean zone of inhibition was 27.75 ± 0.25 mm with 100 mg/ml concentration, 15.75 ± 0.25 mm with 50 mg/ml, 8.28 ± 0.16 mm with 25 mg/ml, 1.88 ± 0.31 mm with 12.5 mg/ml, and 0.28 ± 0.16 mm with 6.25 mg/ml. Against *P. aeruginosa*, the mean zone of inhibition was 25.25 ± 0.63 mm with 100 mg/ml concentration, 17.25 ± 0.48 mm with 50 mg/ml, 8.75 ± 0.25 mm with 25 mg/ml, 1.60 ± 0.36 mm with 12.5 mg/ml, and 0.23 ± 0.13 mm with 6.25 mg/ml. Figures 1 to 4 show a comparison of the mean zones of inhibition of ethanol and aqueous extracts of *Zingiber officinale Roscoe* and ciprofloxacin on the bacterial isolates. The standard errors of the mean values are indicated by the error bars. Statistical comparison of the mean zones of inhibition of ethanol and aqueous extracts of *Zingiber officinale Roscoe* and ciprofloxacin on the selected bacterial isolates was performed using one-way ANOVA at a significance level of 0.05. Among all the bacterial isolates, ciprofloxacin showed a significantly higher mean zone of inhibition [p(0.00) <0.05] than the ethanolic and aqueous extracts of *Zingiber officinale Roscoe*. Post-hoc analyses with Tukey's test showed that the ethanolic extract of *Zingiber officinale Roscoe* showed significantly higher mean zones of inhibition [p(0.00)<0.05] than the aqueous extracts of *Zingiber officinale Roscoe* at 100, 50, and 25 mg/ml. At lower concentrations of 12.5 mg/ml and 6.25 mg/ml, there was no difference [p(1.00)>0.05] between the ethanolic and aqueous extracts of *Zingiber officinale Roscoe*.

Table 1: Distribution of Mean Zones of Inhibition (mm) of *Zingiber officinale Roscoe* and Ciprofloxacin on selected bacteria isolates

Bacteria	***Zingiber officinale Roscoe*** **Ethanolic extract**									
	100 mg/ml		**50 mg/ml**		**25 mg/ml**		**12.5 mg/ml**		**6.25 mg/ml**	
	n	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**
Staphylococcus aureus	25	18.63 ± 0.24[a]	25	12.13 ± 0.42[a]	25	4.18 ± 0.12[a]	25	1.70 ± 0.12[b]	25	0.00 ± 0.00[b]
Streptococcus viridans	24	17.75 ± 0.48[a]	24	10.75 ± 0.63[a]	24	2.75 ± 0.25[a]	24	0.65 ± 0.24[b]	24	0.00 ± 0.00[b]
Escherichia coli	21	15.50 ± 0.65[a]	21	7.63 ± 0.38[a]	21	0.83 ± 0.12[a]	21	0.00 ± 0.00[b]	21	0.00 ± 0.00[b]
Pseudomonas aeruginosa	20	17.00 ± 0.71[a]	20	8.03 ± 0.34[a]	20	0.78 ± 0.13[a]	20	0.00 ± 0.00[b]	20	0.00 ± 0.00[b]
	Zingiber officinale Roscoe **Aqueous extract**									
	100 mg/ml		**50 mg/ml**		**25 mg/ml**		**12.5 mg/ml**		**6.25 mg/ml**	
	n	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**
Staphylococcus aureus	25	7.25 ± 0.48[a]	25	0.83 ± 0.12[a]	25	0.00 ± 0.00[b]	25	0.00 ± 0.00[b]	25	0.00 ± 0.00[b]
Streptococcus viridans	24	8.25 ± 0.25[a]	24	0.88 ± 0.13[a]	24	0.00 ± 0.00[b]	24	0.00 ± 0.00[b]	24	0.00 ± 0.00[b]
Escherichia coli	21	4.25 ± 0.25[a]	21	0.28 ± 0.16[a]	21	0.00 ± 0.00[b]	21	0.00 ± 0.00[b]	21	0.00 ± 0.00[b]
Pseudomonas aeruginosa	20	2.63 ± 0.24[a]	20	0.00 ± 0.00[b]	20	0.00 ± 0.00[b]	20	0.00 ± 0.00[b]	20	0.00 ± 0.00[b]
	Ciprofloxacin (Control)									
	100 mg/ml		**50 mg/ml**		**25 mg/ml**		**12.5 mg/ml**		**6.25 mg/ml**	

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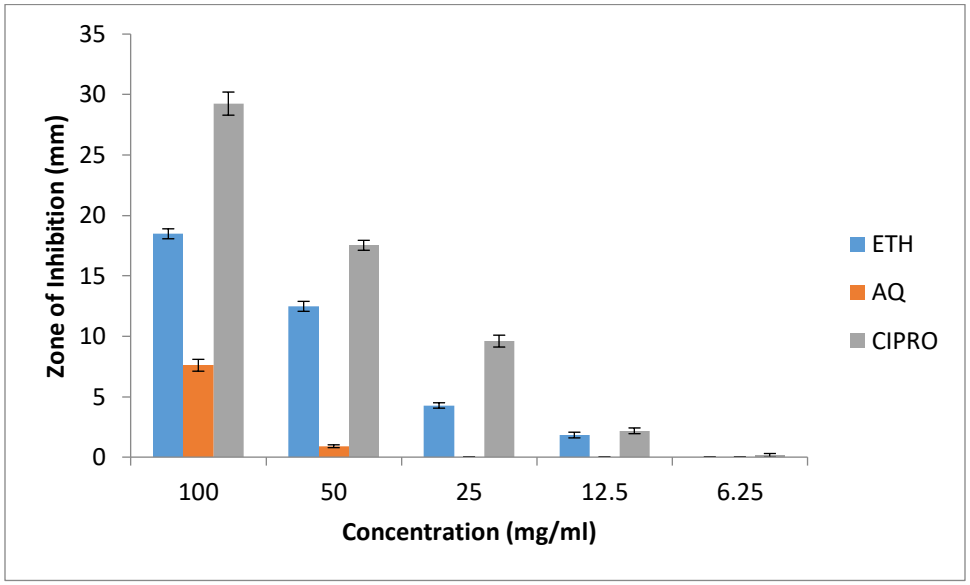
Bacteria	*Zingiber officinale Roscoe* Ethanolic extract											
	100 mg/ml			50 mg/ml			25 mg/ml			12.5 mg/ml			6.25 mg/ml		
	n	Mean ± SE (mm)		n	Mean ± SE (mm)		n	Mean ± SE (mm)		n	Mean ± SE (mm)		n	Mean ± SE (mm)	
Staphylococcus aureus	25	29.00 ± 0.71[a]		25	17.63 ± 0.24[a]		25	9.38 ± 0.38[a]		25	2.13 ± 0.13[a]		25	0.88 ± 0.13[a]	
Streptococcus viridans	24	24.13 ± 0.13[a]		24	16.76 ± 0.25[a]		24	7.75 ± 0.25[a]		24	1.38 ± 0.24[a]		24	0.50 ± 0.12[a]	
Escherichia coli	21	27.75 ± 0.25[a]		21	15.75 ± 0.25[a]		21	8.28 ± 0.16[a]		21	1.88 ± 0.31[a]		21	0.28 ± 0.16[a]	
Pseudomonas aeruginosa	20	25.25 ± 0.63[a]		20	17.25 ± 0.48[a]		20	8.75 ± 0.25[a]		20	1.60 ± 0.36[a]		20	0.23 ± 0.13[a]	

SE: Standard Error of Mean; a=Values for corresponding rows in each table indicate a significant difference ($p<0.05$); b=Values for corresponding rows in each table indicate no significant difference ($p>0.05$)



ETH: Ethanol extracts, AQ: Aqueous extracts, CIPRO: Ciprofloxacin

Figure 1: Comparison of mean zones of inhibition of Ethanol and Aqueous extracts of *Zingiber officinale Roscoe* and Ciprofloxacin on *Staphylococcus aureus*

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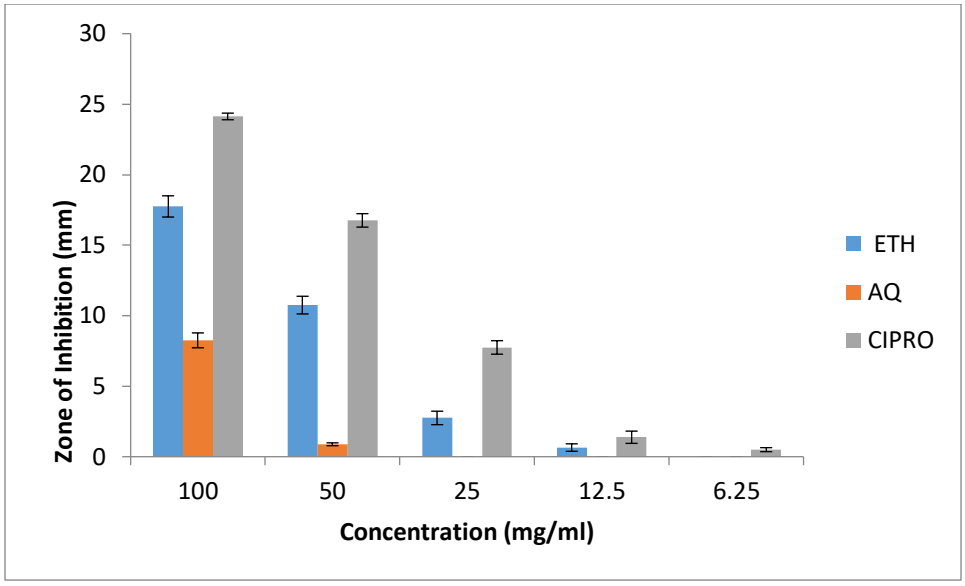
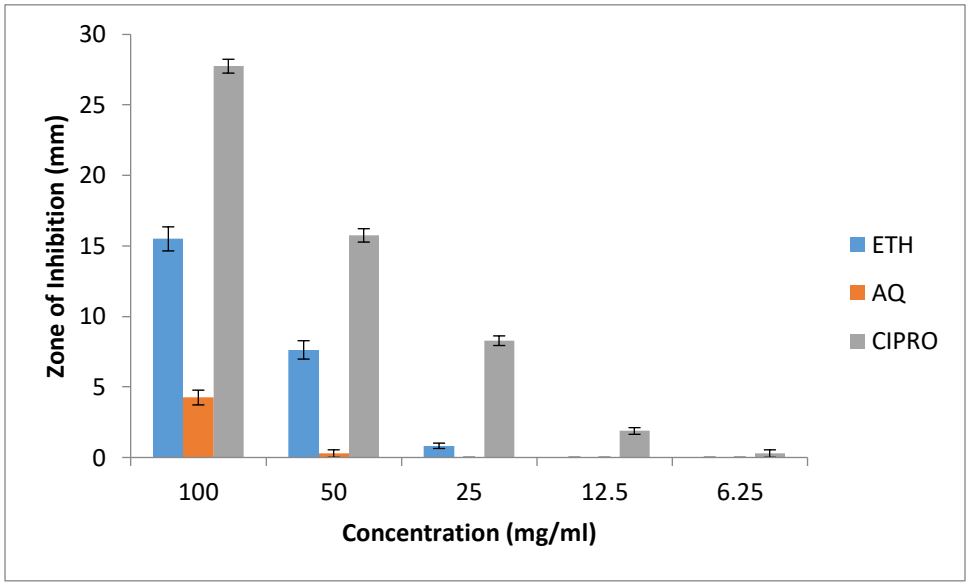


ETH: Ethanol extracts, AQ: Aqueous extracts, CIPRO: Ciprofloxacin

Figure 2: Comparison of mean zones of inhibition of Ethanol and Aqueous extracts of *Zingiber officinale Roscoe* and Ciprofloxacin on *Streptococcus viridans*



ETH: Ethanol extracts, AQ: Aqueous extracts, CIPRO: Ciprofloxacin

Figure 3: Comparison of mean zones of inhibition of Ethanol and Aqueous extracts of *Zingiber officinale Roscoe* and Ciprofloxacin on *Escherichia coli*

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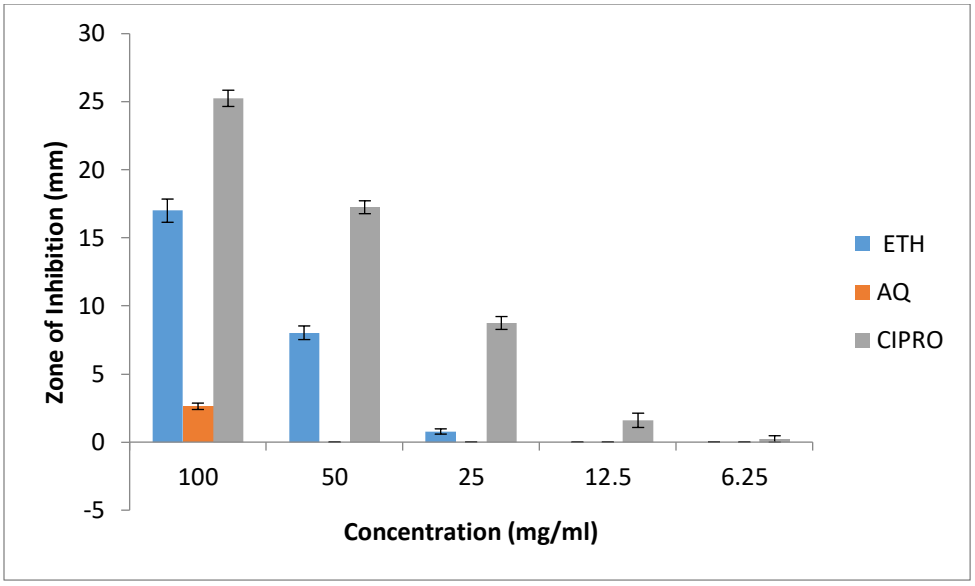

ETH: Ethanol extracts, AQ: Aqueous extracts, CIPRO: Ciprofloxacin

Figure 4: Comparison of mean zones of inhibition of Ethanol and Aqueous extracts of *Zingiber officinale Roscoe* and Ciprofloxacin on *Pseudomonas aeruginosa*

Discussion

Results obtained from this study revealed that both the ethanolic and aqueous extracts of *Zingiber officinale Roscoe* exhibited significant levels of antibacterial activity on the selected bacterial isolates. This antibacterial property of ginger can be attributed to the phytochemical and bioactive components of this plant source such as gingerols, shogaols, tanins, terpenoids, phenylbutenoids, diarylheptanoids, flavanoids, diterpenoids, and sesquiterpenoids.[22] The mechanism of action involves the disruption of biofilm formation in multidrug-resistant strains of bacteria like *Pseudomonas aeruginosa* by affecting membrane integrity and reducing the level of bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP), a key regulator of biofilm development.[23] Compounds such as gingerenone-A and 6-shogaol in ginger have demonstrated the ability to inhibit *Staphylococcus aureus* by targeting and inhibiting 6-hydroxymethyl-7, 8-dihydropterin pyrophosphokinase.[8] The higher concentrations of the aqueous and ethanolic extracts of *Zingiber officinale Roscoe* in this study exhibited greater antibacterial efficacy against *Staphylococcus aureus, Streptococcus viridans, Escherichia coli* and *Pseudomonas aeruginosa* compared to the lower concentrations of the extracts. This implies a concentration-dependent antibacterial effect of the *Zingiber officinale Roscoe* extracts. This is in line with the findings of Rukundo, *et al.*[10] who studied the antibacterial activity of ethanolic and aqueous extracts of *Zingiber officinale* on *Streptococcus pneumoniae* and *Pseudomonas aeruginosa.* In their study, both the ethanolic and aqueous extracts *Zingiber officinale Roscoe* exhibited greater antibacterial activity against *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* at higher concentrations of 100mg/ml and lesser antibacterial activity at concentrations of 25 mg/ml and 12.5 mg/ml. Similar studies[24-26] have also reported the antibacterial activity of the ethanolic and aqueous extracts of *Zingiber officinale Roscoe*. The higher concentrations of the ethanolic extract of *Zingiber officinale Roscoe* exhibited stronger antibacterial effect on all the tested bacterial isolates when compared to the aqueous extract which demonstrated a significant antibacterial activity at the highest concentration of 100mg/ml, and no antibacterial activity at concentrations of 25 mg/ml, 12.50 mg/ml and 6.25 mg/ml. This stronger antibacterial activity of the ethanolic extract of *Zingiber officinale Roscoe* over the aqueous extract could be because ethanol can extract more bioactive components from the plant than water. Hence, the ethanolic extract contains higher amounts of the bioactive compounds of *Zingiber officinale Roscoe* leading to stronger antibacterial activity than the aqueous extract. Similar studies[10,25,27] also reported a stronger antibacterial activity with ethanolic extracts over the aqueous extracts. Shuaibu, *et al.*[28] suggested in their study that this may be due to the greater extraction efficiency of ethanol since ginger components are more

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soluble in organic solvents like ethanol, possibly resulting in the higher amount of the active constituents responsible for the higher antibacterial effects.

Ciprofloxacin is a broad-spectrum bactericidal antibiotic belonging to the fluoroquinolone class that was used as a control antibiotic in this study. It works by inhibiting bacterial DNA replication through the inhibition of DNA topoisomerase and DNA gyrase.[29] Ciprofloxacin exhibited a higher antibacterial efficacy compared to both the ethanolic and aqueous extracts of *Zingiber officinale Roscoe* against *Staphylococcus aureus, Streptococcus viridans, Escherichia coli* and *Pseudomonas aeruginosa.* This could be because Ciprofloxacin specifically targets bacterial DNA gyrase and topoisomerase IV, which are essential enzymes for bacterial DNA replication leading to a bactericidal effect, directly killing the bacterial cells. In this study, there were larger zones of inhibition on all the tested bacterial isolates with ciprofloxacin compared to the ethanolic and aqueous extracts of *Zingiber officinale Roscoe*. While Ciprofloxacin inhibited bacteria growth at concentrations as low as 6.25 mg/ml; the ethanolic and aqueous extracts of *Zingiber officinale Roscoe* inhibited bacterial growth at 12.5 mg/ml and 50 mg/ml respectively. This also reaffirms the broad-spectrum nature of ciprofloxacin as it was able to inhibit the growth of both the gram-positive and gram-negative bacteria at all concentrations. In this study, the gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus viridans*, demonstrated more sensitivity to the various concentrations of the aqueous and ethanolic extracts of *Zingiber officinale Roscoe* when compared to *Escherichia coli* and *Pseudomonas aeruginosa,* the gram-negative bacteria. This is possibly due to the differences in the cell wall structure of gram-positive and the gram-negative bacteria.[30] The outer membrane of the gram-negative bacteria like *Pseudomonas aeruginosa* and *Escherichia coli* is made up of lipopolysaccharides which acts a barrier through which molecules cannot penetrate.[4] Similar studies[11,31] have also reported stronger antibacterial activity against gram-positive bacteria.

Although ciprofloxacin demonstrated stronger antibacterial activity, its potency and efficacy could decline as bacteria develop resistance to them. Due to its antibacterial effect, *Zingiber officinale Roscoe* is a potential herb for drug development. Further research is advocated using higher concentrations of *Zingiber officinale Roscoe* to understand fully its antibacterial effect. Other extraction techniques such as supercritical fluid extraction, hydro-distillation, ultrasound-assisted extraction, enzyme-assisted extraction, and microwave-assisted extraction are recommended in future research. The mechanisms of action of antibacterial activity of ethanolic and aqueous extracts of *Zingiber officinale Roscoe* is also recommended for future studies. The use of plant extracts in the treatment of infections is widely advocated due to their antibacterial properties. With the rising concerns of antibiotic resistance, there is a need to explore natural remedies as potential alternatives to conventional antibiotics. This is also applicable to the treatment of ocular infections such as bacterial conjunctivitis. There is a need for drugs free from side effects in the treatment of ocular surface infections. Plant therapies have the potential of solving this limitation.[32] The results from this study indicate that *Zingiber officinale Roscoe* can be used to make herbal remedies for bacterial infections. However, this study was a laboratory In-vitro study from which our conclusion of the antibacterial activity of *Zingiber officinale Roscoe* was derived. In-vivo studies are recommended in future studies to fully appreciate its clinical significance in the treatment of bacterial infections. Ginger is widely available in Nigeria making it relatively affordable in local markets. The potential antibacterial health benefits of ginger, combined with its affordability and availability, make it an attractive plant for promoting local healthcare.

Conclusion

Zingiber officinale Roscoe exhibited antibacterial activity against the test bacterial isolates of this study at higher concentrations. This suggests that ginger can be used as a natural therapeutic drug against certain gram-positive and gram-negative bacteria. In developing countries like Nigeria where the poverty level is high and most people, especially in the rural areas do not have access to adequate healthcare, there is need for affordable and accessible antimicrobial agents for the treatment of bacterial infections.

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performed data analysis and interpreted the data. All the authors contributed to the development of the final manuscript and approved its submission.

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