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Antimicrobial Activities of Extracts of Ginger and Garlic on Some Selected Bacterial Pathogens

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ABSTRACT

Original Research Article

The rising incidence of antibiotic resistance has heightened the quest for alternative and supplementary antimicrobial agents, especially those originating from medicinal plants and dietary spices. Ginger (Zingiber officinale) and garlic (Allium sativum) contain bioactive phytochemicals, which have been proven to exhibit antibacterial activities. The investigation was conducted to determine the in-vitro antibacterial properties of aqueous and ethanolic extracts of ginger and garlic against some selected clinical bacterial pathogens viz. Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. The plant extracts were generated by water and ethanol extraction procedures and were analysed at different concentrations by disc diffusion and agar well diffusion methods. Inhibition zones were examined and the minimal inhibitory concentrations (MICs) were obtained at the values of 100, 50, 25, 12.5 and 6.25 mg/mL. Both ginger and garlic extracts showed concentration dependent antibacterial activity although the degree of inhibition was depending on the plant, extraction solvent, bacterial type and concentration. The highest zones of inhibition against S. aureus (31.0 ± 1.8 mm) and P. aeruginosa (28.0 ± 1.8 mm) were seen with the ethanolic garlic extract using the disc diffusion method. Ethanolic ginger extract also showed considerable effectiveness with highest inhibition zone of 29.0 ± 1.8 mm against P. aeruginosa. The MIC of the ethanolic extracts was as low as 6.25 mg/mL for some of the species tested. The aqueous extract of ginger was required 50 mg/mL for the inhibition of P. aeruginosa, but the garlic extract showed MIC values of 6.25 mg/mL against S. aureus and E. coli and 12.5 mg/mL against P. aeruginosa. In general garlic showed more antibacterial activity than ginger, particularly when extracted with ethanol. The studied extracts were sensitive to different degrees against Gram-positive and Gram-negative bacteria. The results imply that ginger and garlic contain bioactive elements with potential in vitro antibacterial action. Further studies on phytochemical characterisation, standardised extraction processes, toxicity assessment, mechanistic investigations and in-vivo evaluation are needed to identify their potential application as sources of antimicrobial compounds.

Keywords: Allium sativum, Zingiber officinale, antimicrobial activity, antibacterial activity, garlic, ginger, minimum inhibitory concentration, clinical pathogens.

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INTRODUCTION

Bacterial infections continue to be a significant public health threat globally, especially in areas with inadequate access to proper diagnosis and efficient antibiotic treatment. There are a number of bacterial species that can be pathogenic to humans. Some organisms that usually colonise the body as part of the commensal microbiota can become opportunistic pathogens if host defences are impaired, or if normal physiological barriers are breached. It is favoured by factors such as immunosuppression, tissue damage, changes in the usual microbiota and unfavourable environmental conditions [1]. The treatment of bacterial infections has traditionally depended mainly on the use of antimicrobial medicines. But the emergence and global spread of antimicrobial-resistant microbes have steadily eroded the efficacy of many conventional antibiotics. Antimicrobial resistance can be acquired by microbes through genetic modifications or by acquiring resistance factors that enable them to withstand antimicrobial substances that would otherwise inhibit or kill them. The emergence of multidrug-resistant (MDR) organisms has posed major problems to infection prevention and treatment and has driven the quest for novel antimicrobial medicines [2].

Natural products, such as medicinal plants, herbs and spices, constitute a major source of structurally varied bioactive chemicals. Plants have been used in

traditional medicine since ancient times and are rich in secondary metabolites with biological activities, which received a great deal of scientific attention on their therapeutic potential. These chemicals include phenolics, flavonoids, terpenoids, alkaloids, tannins, saponins and organosulfur compounds, many of which have exhibited antibacterial effects [3]. The diversity of phytochemicals in plants also may provide the opportunity to uncover molecules with novel antibacterial modes of action. Herbs and spices have been used for a long time as traditional medicines and food preservatives. Various phytochemicals and volatile constituents have been associated with antimicrobial activities and the level of activity could be influenced by several factors such as plant species, extraction method, concentration, bacterial species, pH, environmental conditions and the chemical composition of the test material [4]. Hence, extraction methods can significantly affect the yield and characteristics of bioactive chemicals obtained from plant sources.

Dietary spices like ginger and garlic are gaining much interest due to their antibacterial activities. Ginger (Zingiber officinale Roscoe) is a herbaceous perennial plant of the family Zingiberaceae, whose rhizome is commonly used as a spice and in traditional medicine. It is widely cultivated in tropical and subtropical areas, including some portions of Asia, Africa and South America. In Nigeria, ginger is mainly grown in the northern section of the country. It has several phytochemical substances like gingerols, shogaols, zingerone and different volatile chemicals, which are responsible for its biological actions. Some of these substances have shown antibacterial and other biological properties in experimental tests [5]. Garlic (Allium sativum L.) is a member of the Amaryllidaceae family and is closely related to onions and other Allium spp. It is often used as a spice in cooking and has long been used in traditional medicine. The biological actions of garlic are mostly connected with organosulfur compounds formed after disruption of garlic tissue. Of these chemicals, allicin is of special importance and has been widely studied for its antibacterial characteristics. Other sulphur compounds and phytochemicals may also be involved in the biological effects of garlic [6]. Garlic has been shown to possess antibacterial action against a wide spectrum of Gram-positive and Gram-negative bacteria. Antimicrobial susceptibility testing methods, such as disc diffusion and agar well diffusion assays, can be used to assess the activity of plant extracts against bacteria. These methods provide qualitative or semi-quantitative proof of antibiotic action by determining the zone of growth inhibition around the test material. The determination of the minimum inhibitory concentration (MIC) gives additional information by the determination of the lowest concentration of an antibacterial agent capable of inhibiting observable microbial growth under certain experimental circumstances.

Important bacterial pathogens include Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. S. aureus is a Gram-positive, coccoid, facultative anaerobic bacteria that is normally found in clusters. It is a common coloniser of the skin and mucosal surfaces of humans but may produce a wide variety of illnesses when host barriers are impaired. It is of major clinical value also, given that it is able to acquire resistance to several antimicrobial treatments [7]. Escherichia coli is a rod-shaped, gram-negative, facultatively anaerobic bacterium belonging to the family Enterobacteriaceae. Many strains are innocuous members of the intestinal microbiota, while pathogenic strains can cause gastrointestinal, urinary tract, bloodstream and other extraintestinal infections. Its ability to acquire and spread antimicrobial-resistance determinants has made it a significant organism in antimicrobial-resistance surveillance and research [8].

Pseudomonas aeruginosa is a ubiquitous, Gram-negative, aerobic, rod-shaped bacteria found in soil, water and other natural environments. It is a major opportunistic pathogen particularly in hospitalised and immunocompromised patients. The pathogen's capacity to build biofilms, thrive in different environments, and express different antimicrobial resistance pathways contributes to its persistence and therapeutic challenges. These properties make P. aeruginosa an important target for alternative antimicrobial drugs study. The antibacterial activity of ginger, garlic and other medicinal plants and spices has been demonstrated in previous studies [9]. However, the level of antibacterial activity may vary significantly depending on the extraction solvent, concentration, plant part, geographical origin, phytochemical makeup and bacterial species studied. The mechanisms of antimicrobial activities of plant extracts are also complicated and may include rupture of bacterial cell membranes, interference with critical metabolic processes, enzyme inhibition, modification of cellular permeability, and oxidative or other intracellular impacts. The increasing burden of antimicrobial resistance, particularly among clinically significant Gram-positive and Gram-negative bacteria, necessitates the identification and characterisation of potential sources of novel antimicrobial chemicals.

Plant-derived chemicals may give important leads for the development of antimicrobials and may act as complementary sources of bioactive molecules. However, demonstrated in-vitro activity should not be taken as indication of clinical efficacy without additional pharmacological, toxicological and clinical assessment [10]. The antimicrobial activities of aqueous and ethanolic extracts of ginger (Zingiber officinale) and garlic (Allium sativum) against selected clinical bacterial pathogens Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa were investigated in the present study. The study evaluated the inhibitory effects of the extracts at various doses and identified the

minimum inhibitory concentrations. These findings may help to comprehend the scientific potential of these commonly consumed spices as antibacterial agents and may give a basis for additional inquiry into their bioactive ingredients.

MATERIALS AND METHODS

Study Area

The study was conducted in Orlu Local Government Area (LGA) of Imo State, southeastern Nigeria, between March and July 2025. Orlu is located within the Orlu Senatorial Zone of Imo State and shares boundaries with Orsu, Njaba, and Nkwerre Local Government Areas.

Collection and Identification of Plant Materials and Test Organisms

The equipment and laboratory materials used for the study were obtained from a medical equipment supplier at Relief Market, Owerri, Imo State. Fresh ginger rhizomes and garlic bulbs were purchased from vendors at Relief Market, Owerri.

The plant materials were submitted to the Herbarium of the Department of Plant Science and Biotechnology, Kingsley Ozumba Mbadiwe University, Ogboko, Ideato, Imo State, for botanical identification and authentication.

Pure cultures of the selected bacterial pathogens, namely *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, were obtained from the Microbiology Laboratory of Imo State University Teaching Hospital, Orlu, Imo State.

Preparation of Plant Extracts

Aqueous and ethanolic extracts of ginger and garlic were prepared separately. Fresh garlic cloves and ginger rhizomes were washed thoroughly, sliced, and air-dried for two weeks. The dried materials were separately pulverized into fine powders using an electric blender.

Ten grams (10 g) of each powdered plant material was separately soaked in 100 mL of distilled water and 100 mL of ethanol. The preparations were maintained at room temperature for 72 h. Following extraction, the ethanolic extracts were concentrated by evaporation at 50°C, while the aqueous extracts were concentrated at 80°C using a rotary evaporator. The resulting extracts were stored at 4°C until required for analysis.

Preparation and Sterilization of Culture Media and Glassware

The culture media used in the study were prepared according to the manufacturers' instructions. The prepared media were sterilized by autoclaving at 121°C for 15 min. Following sterilization, the media were allowed to cool before being aseptically dispensed into sterile Petri dishes.

The workbench was disinfected with 70% alcohol before laboratory procedures were commenced. Glassware was washed with detergent, thoroughly rinsed with tap water, and subsequently rinsed with distilled water. The glassware was air-dried, wrapped in aluminium foil, and sterilized in a hot-air oven at 170°C for 2 h.

Agar media were dispensed into conical flasks, plugged with cotton wool, wrapped with aluminium foil, and sterilized by autoclaving at 121°C for 15 min. Standard aseptic techniques were maintained throughout the laboratory procedures to minimize contamination.

Preparation of Extract-Impregnated Discs

Sterile 6-mm discs were prepared from Whatman filter paper and sterilized in a hot-air oven at 160°C for 1 h. The aqueous and ethanolic extracts of ginger and garlic were prepared at concentrations of 100, 50, 25, 12.5, and 6.25 mg/mL.

Each sterile 6-mm disc was impregnated with 20 µL (0.02 mL) of the respective extract concentration and allowed to dry overnight before use. Levofloxacin at a concentration of 50 µg/mL was used as the positive control and similarly applied to sterile 6-mm discs. The prepared discs were subsequently used for the antibacterial susceptibility testing.

Laboratory Analysis and Procedures

Confirmation of Test Organisms

Before antimicrobial testing, the bacterial isolates were subcultured from the stock cultures onto appropriate culture media to confirm purity and identity. The cultures were examined for characteristic colony morphology, followed by Gram staining and relevant biochemical tests.

The isolates were considered pure when uniform colony characteristics were observed. Identification was based on the combined findings from colonial morphology, microscopic examination, Gram-staining characteristics, and biochemical reactions.

Antibacterial Screening

Disc Diffusion Method

The disc diffusion method was used to evaluate the antibacterial activity of the aqueous and ethanolic ginger and garlic extracts. The test organisms were inoculated onto nutrient agar plates using an appropriate inoculation technique. Sterile forceps were used to aseptically place the extract-impregnated discs onto the inoculated agar surfaces.

The plates were incubated at 37°C for 24 h. Following incubation, the diameters of the zones of inhibition surrounding the discs were measured in millimetres (mm) using a transparent ruler. The antibacterial activities of the extracts were evaluated against *S. aureus*, *E. coli*, and *P. aeruginosa*.

Agar Well Diffusion Method

The agar well diffusion method was employed to further evaluate the antibacterial activity of the plant extracts. The method was performed with reference to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Standardized bacterial suspensions were used to inoculate the surfaces of sterile nutrient agar plates using sterile cotton swabs. Wells measuring 8 mm in diameter were aseptically bored into the solidified agar using a sterile cork borer. The bases of the wells were sealed with approximately 1 mL of sterile nutrient agar, after which the wells were filled with the respective plant extracts at concentrations of 100, 50, 25, 12.5, and 6.25 mg/mL.

The inoculated plates were allowed to stand at room temperature for 3 h to facilitate diffusion of the extracts before incubation at 37°C for 24 h. Antibacterial activity was assessed by measuring the zones of inhibition surrounding the wells. Levofloxacin (50 µg/mL) was used as the positive control. Extract sterility controls, medium sterility controls, and organism viability controls were included to assess extract sterility, medium sterility, and viability of the test organisms, respectively.

Measurement of Zones of Inhibition

The antibacterial activity of each extract was determined by measuring the diameter of the clear zone of inhibition surrounding each extract-impregnated disc or agar well. Measurements were taken in millimetres (mm) using a transparent ruler placed against the underside of the Petri dish.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of each plant extract was determined using serial dilution. For each test organism, sterile tubes containing the respective extract preparations were arranged in a test-tube rack. The aqueous and ethanolic extracts of ginger and garlic were serially diluted to obtain the required concentrations.

The diluted extracts were thoroughly mixed, inoculated with the respective test organisms, and incubated at 37°C for 24 h. Following incubation, the tubes were examined for visible microbial growth. The MIC was defined as the lowest concentration of the extract at which no visible growth was observed.

Biochemical Identification of Bacterial Isolates

Gram Staining

Gram staining was performed to differentiate Gram-positive from Gram-negative bacteria based on differences in the structure and composition of their cell walls. Gram-positive bacteria possess a thick peptidoglycan layer that retains the crystal violet–iodine complex, whereas Gram-negative bacteria have a thinner peptidoglycan layer and are decolorized during the alcohol treatment, subsequently taking up the counterstain.

A smear of each bacterial isolate was prepared on a clean, grease-free glass slide and allowed to air-dry. Crystal violet was applied as the primary stain for approximately 60 s and gently rinsed with water. Lugol's iodine was then applied, followed by decolorization with 95% alcohol. The smear was subsequently counterstained with neutral red for approximately 60 s, rinsed, and allowed to air-dry. A drop of immersion oil was applied, and the preparation was examined microscopically using the ×100 oil-immersion objective.

Capsule Staining

Capsule detection was performed using the India ink negative-staining technique. A loopful of 10% India ink was placed on a clean microscope slide, and a small portion of the bacterial culture was emulsified in the ink. A clean coverslip was placed over the preparation, and the edges were sealed with paraffin wax. The preparation was subsequently examined microscopically for evidence of capsular material.

Flagella Staining

Flagella staining was performed using Loeffler's silver staining method. A thin smear of the bacterial culture was prepared and gently heat-fixed. The smear was treated with Loeffler's flagella mordant for 5 min, rinsed with distilled water, and subsequently stained with heated Loeffler's flagella stain for 3 min. The preparation was rinsed, dried, mounted, and examined microscopically.

Coagulase Test

The coagulase test was performed to assist in the identification of *Staphylococcus aureus*. Coagulase is an enzyme that promotes the conversion of fibrinogen to fibrin, resulting in plasma clotting.

For the slide coagulase test, two separate drops of sterile saline were placed on a clean glass slide. Colonies of the test organism were emulsified separately in the two drops to produce thick suspensions. A small amount of plasma was mixed with one suspension, while the second suspension served as a negative control. The preparation was examined for immediate coarse agglutination, which was interpreted as a positive reaction.

Catalase Test

The catalase test was used to detect the production of catalase by bacterial isolates. Catalase catalyzes the decomposition of hydrogen peroxide into water and oxygen:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The production of oxygen was demonstrated by the formation of visible bubbles following exposure of the bacterial culture to hydrogen peroxide.

A few colonies of the test organism were emulsified in distilled water on a clean glass slide. Two drops of hydrogen peroxide were added, and the preparation was observed for the immediate production of gas bubbles. Bubble formation was interpreted as a positive catalase reaction.

Citrate Utilization Test

The citrate utilization test was performed to determine the ability of bacterial isolates to utilize citrate as a carbon source. A light suspension of the test organism was prepared and inoculated onto Simmons citrate agar using a sterile straight wire. The medium was incubated under appropriate conditions, and a change in the medium from green to blue, accompanied by growth, was interpreted as a positive citrate utilization reaction.

Motility Test

Motility was assessed using semisolid motility medium. The test organism was inoculated into the medium by a straight stab using a sterile straight wire and incubated at 37°C for 48 h.

Following incubation, motility was assessed by examining the pattern of bacterial growth along and away from the inoculation line. Diffuse or hazy growth extending from the stab line was interpreted as evidence of motility.

Indole Test

The indole test was performed to determine the ability of the test organism to degrade tryptophan with the production of indole. The organism was inoculated into 3 mL of sterile tryptone water and incubated for 48 h. Following incubation, 0.5 mL of Kovac's reagent was added and gently mixed. The formation of a red colour in the surface layer within 10 min was interpreted as a positive indole reaction.

Methyl Red Test

The methyl red test was used to determine the ability of bacterial isolates to produce and maintain stable acidic end products from glucose fermentation. The test organism was inoculated into the appropriate medium and incubated according to the laboratory protocol. Methyl red indicator was subsequently added, and development of a red colour was interpreted as a positive reaction.

Nitrate Reduction Test

The nitrate reduction test was used to determine the ability of bacterial isolates to reduce nitrate through nitrate reductase activity. The peptone water medium was inoculated with the test organism and incubated for 24 h. Following incubation, 1 mL of the test reagent was added to 1 mL of the culture broth and mixed. The reaction was examined after 2 min. Development of a pink colour was interpreted as a positive nitrate reduction reaction, whereas absence of colour development was recorded as negative.

Urease Test

The urease test was performed to determine the ability of the test organism to hydrolyze urea through the action of the urease enzyme. Hydrolysis of urea results in the production of ammonia, which increases the pH of the medium and produces a characteristic colour change.

The entire surface of Christensen's urea agar slope was inoculated with the test organism and incubated at 37°C. The medium was examined following overnight incubation. Development of a pink-red colour was interpreted as a positive urease reaction.

Voges-Proskauer Test

The Voges-Proskauer (VP) test was performed to detect acetoin production by bacterial isolates. Some bacteria ferment carbohydrates with the production of acetoin, which can be oxidized to diacetyl under alkaline conditions. Diacetyl subsequently reacts with components of the reagent to produce a characteristic colour reaction.

Approximately 2 mL of the test medium was inoculated with the test organism and incubated at 37°C for 48 h. Following incubation, 1 mL of 10% potassium hydroxide was added, and the preparation was allowed to stand at room temperature for approximately 1 h before interpretation.

Oxidase Test

The oxidase test was performed to determine the presence of cytochrome *c* oxidase in the bacterial isolates. The test is based on the oxidation of the chromogenic reagent tetramethyl-*p*-phenylenediamine, which produces a characteristic dark purple colour in oxidase-positive organisms.

A few drops of oxidase reagent were applied to colonies of the test organism. Development of a blue to deep-purple colour within approximately 10 s was interpreted as a positive oxidase reaction.

RESULTS

Table 1: The antimicrobial activities of aqueous and Ethanol extracts of ginger and garlic on *S. aureus, E.coli and P. aeruginosa*

Isolate	Ginger Aq. Extract	Ginger Ethanol Extract	Garlic Aq. Extract	Garlic Ethanol Extract	Positive Control (AGX)	Negative Control (Water)
S.aureus	++	++	++	++	--	--
E-Coli	++	++	++	++	++	--
P.aeruginosa	++	++	++	++	++	--

Key: ++ = Antimicrobial activity
-- = No antimicrobial activity
AGX = Levofloxacin
H_2O = Water

Results revealing the various antimicrobial activities in their zone of inhibition at different concentration. Table 1 shows that the different extract of the spices has broad spectrum antimicrobial activity on the three selected clinical isolates.

Table 2: Diameter of zone of inhibition (mm) of the different concentrations of aqueous and Ethanol extract of ginger through disc diffusion method.

Clinical isolates/	Ginger Aqueous Extract					Ginger Ethanol Extract					+ve AGX	-ve H_2O
Concentration (mg/ml)	100	50	25	12.5	6.25	100	50	25	12.5	6.25	50	100
S.aureus	25.0 ±1.3	23. 0 ±1. 3	13. 0 ±0. 6	-	-	30. 0 ±1. 8	26. 0 ±1. 8	20. 0 ±1. 8	18. 0 ±0. 9	13. 0 ±0. 6	25 ±1. 3	-
E.Coli	20 ±1.3	-	-	-	24 ±1. 3	20 ±1. 3	18 ±0. 9	15 ±0. 9	11 ±0. 6	22 ±1. 3	-	
P.aeruginosa	16 ±0.9	14 ±0. 6	-	-	-	29 ±1. 8	24 ±1. 3	20 ±1. 3	16 ±0. 9	12 ±0. 6	24 ±1. 3	-

Key:
AGX = Levofloxacin
H_2O = Water

Table 2 also shows that the ethanolic extracts of ginger exhibited stronger antimicrobial activity than the aqueous extract across the selected clinical pathogens. *Staphylococcus aureus* showed the highest susceptibility (31.0±1.8ml, while *E. coli* was the least effect with the lowest diameter of inhibition of 11.0±0.6mm.

The aqueous extracts showed minimal inhibition, suggesting ethanol is a better solvent for extracting bacterial antimicrobial compound from ginger.

Table 3: Diameter of zone of inhibition (mm) of the different concentrations of aqueous and Ethanol extract of ginger through well diffusion method.

Clinical isolates/ Concentration (mg/ml)	Ginger Aqueous Extract					Ginger Ethanol Extract					+ve AGX	-ve H_2O
	100	50	25	12.5	6.25	100	50	25	12.5	6.25	50	100
S.aureus	21.0 ±1. 3	18.0 ±1. 3	-	-	-	28.0 ±1. 8	22.0 ±1. 3	0.9 ±1. 3	0.9 ±1. 3	11.0 ±0. 6	26 ±1.3	-
E.Coli	18.0 ±1. 3	14.0 ±0. 6	11.0 ±0. 6	-	-	21.0 ±1. 3	18.0 ±0. 9	16.0 ±0. 9	13.0 ±0. 6	-	20 ±1.3	-
P.aeruginosa	13.0	11.0	-	-	-	27.0	20.0	18.0	14.0	11.0	23	

	±0.6	±0.6				±1.8	±1.3	±0.9	±0.6	±0.6	±1.3	

Key:
AGX = Levofloxacin
H_2O = Water

Table 3 shows the diameter zone of inhibition (mm) of the different concentrations of aqueous and ethanol extracts of ginger through well diffusion method, using the well diffusion method, the ethanolic extracts of ginger exhibited a greater antimicrobial effect than the aqueous form. The inhibition zones decreased by 1-2mm when compared to the disc diffusion method. Due to a better diffusion in the disc method *Staphylococcus aureus* remain the most sensitive bacteria.

Table 4: Diameter of zone of inhibition (mm) of the different concentrations of aqueous and Ethanol extract of Garlic through disc diffusion method.

Clinical isolates/ Concentration (mg/ml)	**Ginger Extract**	**Aqueous**				**Ginger Extract**	**Ethanol**				**+ve AGX**	**-ve H_20**
	100	50	25	12.5	6.25	100	50	25	12.5	6.25	50	100
S.aureus	29.0 ±1.8	25.0 ±1.3	20.0 ±1.3	16 ±0.9	11 ±0.6	31.0 ±1.8	22.0 ±1.3	19.0 ±0.9	15.0 ±0.9	11.0 ±0.6	22 ±1.3	-
E.Coli	25.0 ±1.3	23.0 ±1.3	20.0 ±1.3	14.0 ±0.6	11.0 ±0.6	21.0 ±1.3	19.0 ±0.9	16.0 ±0.9	13.0 ±0.6	11.0 ±0.6	21 ±1.3	
P.aeruginosa	28.0 ±1.8	24.0 ±1.3	19.0 ±0.9	15.0 ±0.9	12.0 ±0.6	26.0 ±1.8	21.0 ±1.3	18.0 ±0.9	14.0 ±0.6	-	25 ±1.3	-

Key:
AGX = Levofloxacin
H_2O = Water

Table 4 shows the diameter of zone of inhibition (mm) of the different concentrations Of aqueous and ethanol extracts of garlic through the disc diffusion method. The extracts exhibited antimicrobial activity against all selected pathogens at concentration of l00mg/ml. producing the highest zone of inhibition of 31.0±1.8mm and 29.0±1.8mm for ethanol and aqueous extract respectively.

Table 5: Diameter of zone of inhibition (mm) of the different concentrations of aqueous and Ethanol extract of garlic through well diffusion method.

Clinical isolates/ Concentration (mg/ml)	**Ginger Extract**	**Aqueous**				**Ginger Extract**	**Ethanol**				**+ve AGX**	**-ve H_20**
	100	50	25	12.5	6.25	100	50	25	12.5	6.25	50	100
S.aureus	25.0 ±1.3	20.0 ±1.3	18.0 ±0.9	15.0 ±0.9	11.0 ±0.6	21.0 ±1.3	19.0 ±0.9	16.0 ±0.9	14.0 ±0.6	11.0 ±0.6	23.0 ±0.3	-
E.Coli	20.0 ±1.3	16.0 ±0.9	12.0 ±0.6	11.0 ±0.6	-	18.0 ±0.9	16.0 ±0.9	14.0 ±0.6	12.0 ±0.6	11.0 ±0.6	21.0 ±1.3	
P.aeruginosa	23.0 ±1.3	20.0 ±1.3	19.0 ±0.9	16.0 ±0.9	12.0 ±0.6	22.0 ±1.3	20.0 ±1.3	19.0 ±0.9	15.0 ±0.6	12.0 ±0.6	18.0 ±0.9	-

Key:
AGX = Levofloxacin
H_2O = Water

Table 5 shows the aqueous and ethanol extracts of garlic through well diffusion method. The result in this table shows lower antimicrobial activity of the extracts against the selected clinical isolation when using well diffusion method.

Table 6: Minimal Inhibitory Concentration of Aqueous and Ethanol extract of ginger on *S. aureus, E-coli* and *P. aeruginosa*

Clinical Isolates	Ginger Aqueous Extract	Ginger Ethanol Extract
S. aureus	25mg/ml	6.25mg/ml
E. coli	25mg/ml	6.25mg/ml
P. aeruginosa	50mg/ml	6.25mg/ml

Table 7: Minimal Inhibitory Concentration of Aqueous and Ethanol extracts of Garlic on *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas*

Clinical Isolates	Ginger Aqueous Extract	Ginger Ethanol Extract
S. aureus	6.25mg/ml	6.25mg/ml
E. coli	6.25mg/ml	6.25mg/ml
P. aeruginosa	6.25mg/ml	12.5mg/ml

Table 6 and table 7 shows the minimum inhibitory concentration of the aqueous and ethanol extracts of both ginger and garlic. The MIC was determined by making the dilution of different extracts of ginger and garlic ranging from l00mg/ml to 6.25mg/ml. The MIC value of different ginger and garlic extract are summarized in table VI and table VII. The result showed that MIC of different extract of ginger and garlic against the clinical isolate range from 6.25mg/ml to 50mg/ml. From all MIC value of different ginger extract, lowest MIC value for *staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa* was 6.25mg/ml except for the aqueous extract for ginger that gave 50mg/ml as MIC for *Pseudomonas aeruginosa*. The ethanol extracts for ginger had lower MIC when compared with the aqueous extracts for ginger against the selected clinical isolate. The garlic and ethanol extracts showed highest MIC value of 12.5mg/ml against *P. aeruginosa* in table VII. The result showed that both gram negative and gram-positive bacteria were Sensitive to all tested extracts of ginger and garlic but gram-positive bacteria were more sensitive compared to gram negative bacteria.

DISCUSSION

The rising prevalence of antibiotic resistance in clinically significant pathogens such as Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa has escalated the hunt for new and supplementary sources of antimicrobial drugs. Plants used in medicines and spices such as Zingiber officinale (ginger) and Allium sativum (garlic) have drawn much attention because of their antibacterial capabilities and general availability [11, 12]. Previous studies have reported the antibacterial activities of ginger and garlic extracts against various bacterial pathogens, indicating that these plants may serve as a source of bioactive antimicrobial compounds [13].

In the present investigation, antibacterial activity of aqueous and ethanolic extracts of Z. officinale and A. sativum against three therapeutically significant bacterial species, S. aureus (Gram-positive), E. coli and P. aeruginosa (both Gram-negative) has been examined. The antibacterial activity was measured by disc diffusion and agar well diffusion methods and the minimum inhibitory concentration (MIC) of the extracts was also determined.

The results showed that both ginger and garlic extracts had antibacterial activity against the organisms tested but the level of inhibition depended on plant species, extraction solvent, bacterial species and extract concentration. In general, the zones of inhibition produced by the ethanolic extracts were bigger than those produced by the aqueous extracts. S. aureus and P. aeruginosa were generally more susceptible whereas E. coli was somewhat less susceptible to several of the extracts among the species examined.

The antibacterial activity was concentration-dependent with the highest zones of inhibition found with 100 mg/mL and the smallest with 6.25 mg/mL. This observation shows that higher concentration of the extracts provided more bioactive ingredients to interact with the bacterial cells. The ethanolic garlic extract showed the highest inhibitory activity against S. aureus with a zone of inhibition of 31.0 ± 1.8 mm while lower inhibition values were reported at lower extract concentrations.

The increased antibacterial activity of the ethanolic extracts could be explained by the ability of extraction solvents to extract bioactive elements from plant materials. Ethanol extracts a broad spectrum of phytochemical including significantly less polar molecules which can contribute to antibacterial action. Ginger is rich in chemicals such as gingerols and other

phenolic compounds that have been linked to antimicrobial action. Garlic includes organosulfur compounds, especially allicin, which are well known for their antibacterial activities. The relatively reduced activity of the aqueous extracts may thus be attributed to differences in the quantity and quality of antibacterial elements extracted by water. However, the extracts were not chemically characterised in the present investigation; thus, the impact of individual phytochemicals could not be clearly determined from the results.

Part of the substantially higher susceptibility of S. aureus could be explained by structural variations between the cell envelopes of Gram-positive and Gram-negative bacteria. Gram-negative bacteria have an exterior membrane which can block some antimicrobial agents from entering the organism. Gram-positive bacteria do not have this outer membrane. However, the susceptibility of different bacterial species varies and cannot be explained purely on the basis of cell-wall construction. The antibacterial activity observed against P. aeruginosa is of particular interest as this organism has several intrinsic and acquired mechanisms that may decrease susceptibility to antimicrobial agents including restricted membrane permeability, efflux systems, biofilm formation and antimicrobial-modifying mechanisms [14].

The present study findings are in good agreement with earlier publications on antibacterial activity of ginger and garlic extracts. It has demonstrated significant antibacterial activity of garlic extracts against E. coli and S. aureus, with ethanolic preparations displaying higher activity in various experimental settings. Similarly, it was observed that ethanolic ginger extracts had higher antibacterial activity than aqueous extracts against selected clinical isolates [15]. Antimicrobial activity was also observed using A. sativum and Z. officinale by [16] and they hypothesised that the extraction techniques may affect the antibacterial activity of plant preparations. The concordance between these investigations and the present findings supports the notion that antibacterial activity of plant extracts is a key driver of extraction solvent.

Further evidence of the inhibitory potential of the extracts was obtained from the MIC results. Ethanolic ginger extract exhibited a MIC of 6.25mg/mL against the investigated species while the aqueous ginger extract needed a higher concentration especially against P. aeruginosa for which a MIC of 50mg/mL was recorded. This finding indicates that ethanolic ginger preparation has a more potent inhibitory activity under the condition of the present study. The MIC of aqueous garlic extract was 6.25 mg/mL against the tested isolates while that of ethanolic extract was 12.5 mg/mL against P. aeruginosa. These findings suggest that the correlation between extraction solvent and antibacterial effectiveness may vary depending on the plant type and bacterial organism investigated [17,18].

The disparity between the diffusion assay and MIC results further illustrates the need for the use of complementary antimicrobial testing methodologies. Diffusion based assays are impacted not only by antibacterial potency but also by a number of parameters including molecular size, diffusion characteristics, extract concentration, agar composition and the physicochemical properties of the compounds present. So, a bigger zone of inhibition doesn't necessarily mean higher antibacterial power. MIC determination offers additional quantitative data on the lowest concentration that may visually suppress bacterial growth [19, 20].

The antibacterial activity of ginger and garlic observed in this work demonstrates their potential as sources of bioactive antimicrobial molecules [21]. However, the results should be viewed in the light of an in vitro experimental investigation. Such action as observed does not prove clinical efficacy or that these extracts may substitute conventional antibiotics safely [22]. Further investigations are necessary to identify and characterise the active chemical, define its mechanism of action, evaluate toxicity, pharmacological properties and confirm its efficacy in relevant in-vivo models and finally clinical studies.

Indeed, the results indicate that both ginger and garlic exhibit measurable in-vitro antibacterial activity against the selected Gram-positive and Gram-negative microorganisms. The ethanolic extract of garlic and garlic itself shown fairly considerable action against certain of the examined species. These findings further encourage the search for ginger and garlic as possible sources of antimicrobial compounds and underscore the importance of standardisation of extraction, phytochemical characterisation, toxicity studies, mechanistic studies and in-vivo evaluation.

CONCLUSION

The outcomes of this investigation showed that the extracts of ginger (Zingiber officinale) and garlic (Allium sativum) in aqueous and ethanolic solvents have antibacterial activity against the selected bacterial pathogens such Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. The antibacterial activity was dependent on the plant extract, extraction solvent, concentration and tested bacterial type.

Garlic has better antibacterial activity than ginger against some of the species studied. Ethanolic garlic extract displayed the highest zone of inhibition against S. aureus. The generally higher activity of the ethanolic extracts indicates the importance of the solvent used in the extraction in the recovery of antibacterial elements from these plants. The MIC results also showed that the two plants possessed elements that limit the growth of bacteria at relatively low concentrations in the experimental setup.

The findings show that ginger and garlic are excellent natural antibacterial agents and may have potential for further exploration as supplementary sources in antimicrobial drug discovery. However, the results should not be construed as any evidence that these spices may replace traditional antibiotic medication. However, further investigations including phytochemical characterisation, determination of MBC, mechanism of action studies, toxicity assessment, standardised extract formulation and in-vivo evaluation are required before any therapeutic application can be advised.

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