

Phytochemicals and Antibacterial Activities of Garlic and Ginger Extracts Against *Klebsiella pneumoniae* and *Streptococcus pneumoniae*

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ABSTRACT

The emergence and evolution of multidrug-resistant pathogens such as *Streptococcus pneumoniae* and *Klebsiella pneumoniae* has elicited an intensified search for plant-based alternatives. Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) are widely used medicinal plants known for their antimicrobial properties. This study investigated the phytochemical composition, pH characteristics, and antibacterial activities of their ethanol and aqueous extracts against *S. pneumoniae* and *K. pneumoniae*. Garlic and ginger samples were collected in Wukari, Taraba State, Nigeria, processed, and extracted using water and ethanol by maceration. Phytochemical profiles were determined using high-performance liquid chromatography (HPLC). Antimicrobial activity was assessed by agar well diffusion and broth microdilution to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Antibiotic susceptibility testing was conducted for comparison. All extracts were slightly acidic (pH 5.46-5.67). HPLC revealed chlorogenic and ferulic acids in both plants, and tannic acid in ginger. Aqueous extracts showed no activity (MIC >1000 mg/mL; no MBC). Ethanol ginger extract inhibited both pathogens with concentration-dependent activity: *S. pneumoniae* (MIC = 500 mg/mL; MBC = 1000 mg/mL) and *K. pneumoniae* (MIC = 750 mg/mL; MBC = 1000 mg/mL). Ethanol garlic extract showed strong inhibition of *S. pneumoniae* (MIC ≤ 250 mg/mL; MBC = 500 mg/mL) but no effect against *K. pneumoniae* (MIC >1000 mg/mL). Antibiotic tests showed *K. pneumoniae* was broadly susceptible, while *S. pneumoniae* exhibited multidrug resistance, with only ceftriaxone showing moderate inhibition (15 mm). Ethanol extracts of garlic and ginger demonstrated potent antibacterial activity, particularly against multidrug-resistant *S. pneumoniae*. Solvent selection significantly influenced plant extract activity, with ethanol outperforming water. Further research should focus on isolating active compounds, exploring synergistic effects with antibiotics, and validating efficacy in vivo.

Keywords: *Klebsiella*, *Streptococcus*, Antimicrobial, Ethanol, Ginger

INTRODUCTION

Traditional medicine (TM) is deeply rooted in the cultural, historical, and territorial knowledge of societies across all six regions of the World Health Organization (WHO). When adapted to local contexts, TM is often referred to as “complementary medicine”

(WHO, 2023). The WHO has described TM as a cost-effective way of achieving universal health coverage and has encouraged its rational integration into national healthcare systems. Herbs, generally considered safe, have been proven effective against a wide range of human ailments, and their use is steadily increasing in both developing and developed nations (WHO, 2023).

In Nigeria, as in many other parts of the world, medicinal plants remain highly valued natural resources and play a central role in traditional medicine. It is estimated that herbal medicine provides the primary source of healthcare for nearly 80% of people living in rural Africa, largely due to its accessibility and affordability (Okaiyeto and Oguntibeju, 2021). The ethnomedicinal potential of these indigenous plants is attributed to their phytochemicals, which exhibit diverse therapeutic properties. The growing interest in medicinal plants as sources of pharmacologically active compounds is further fueled by the rising failure of antibiotics and the spread of multidrug-resistant pathogens (Rukundo *et al.*, 2023; Imarenezor and Gaina, 2025).

One such pathogen of global concern is *Streptococcus pneumoniae*, a capsulated, lancet-shaped, Gram-positive bacterium. It is the leading cause of pneumonia worldwide and contributes significantly to childhood mortality and morbidity. *S. pneumoniae* causes other diseases such as otitis media, nasosinusitis, bacteremia, and meningitis (Rukundo *et al.*, 2023; Li *et al.*, 2023). To address this burden, pneumococcal conjugate vaccines (PCVs) have been developed and widely implemented across the globe (Li *et al.*, 2023). Nevertheless, antibiotic resistance in *S. pneumoniae* remains a serious challenge. Increasing resistance to commonly used antibiotics has led WHO to classify penicillin-resistant *S. pneumoniae* (PRSP) as a priority pathogen (Song *et al.*, 2025) and, more recently, to list it under the Bacterial Priority Pathogens List (BPPL) of 2024 as a moderate-priority pathogen due to rising macrolide resistance (WHO, 2024). The inappropriate and excessive use of antibiotics continues to be one of the major drivers of resistance in *S. pneumoniae* (Daningrat *et al.*, 2020 ; Oh *et al.*, 2021; Sharew *et al.*, 2021).

Similarly, *Klebsiella pneumoniae* (*K. pneumoniae*), a Gram-negative opportunistic pathogen, poses a major public health threat. Traditionally, it has been associated with bacteremia, pneumonia, and urinary tract infections, particularly among immunocompromised and hospitalized patients (Abbas *et al.*, 2024). Of special concern are strains belonging to the carbapenem-resistant Enterobacteriaceae (CRE) family, which exhibit resistance to carbapenems, critical, broad-spectrum antibiotics. These strains have been identified by the WHO as a “critical concern” requiring urgent research and the development of new therapeutic options (Gholizadeh *et al.*, 2024; WHO, 2024). The virulence of *K. pneumoniae* is strongly associated with its ability to produce polysaccharide capsule, which shields it from host immunity (Gholizadeh *et al.*, 2024).

Given these challenges, medicinal plants such as ginger (*Zingiber officinale*) and garlic (*Allium sativum*) have attracted scientific interest as alternative therapeutic agents. Both plants are widely used in Nigeria and across the world as spices and flavoring agents, and their extracts have demonstrated promising antibacterial activities.

Ginger (*Zingiber officinale*), a perennial plant belonging to the family Zingiberaceae, is well known for its culinary and medicinal value (Zhukovets and Özcan, 2020). Its rhizome contains numerous bioactive phytochemicals including gingerols, shogaols, and paradols which are responsible for its antimicrobial, antioxidant, anti-inflammatory, antidiabetic, cardioprotective, and neuroprotective effects (Singh *et al.*, 2022; Zhang *et al.*, 2022; Igwe *et al.*, 2024). Traditionally, ginger has been used to relieve pain, digestive disorders, fever,

hypertension, and infections. Recent studies reported both ethanolic and aqueous extracts of ginger effective against *S. pneumoniae* and *Pseudomonas aeruginosa* in a dose-dependent manner, with ethanolic extracts showing stronger inhibitory activity (Rukundo *et al.*, 2023).

Garlic (*Allium sativum*), a perennial plant of the family Liliaceae, is another widely consumed spice with extensive ethnomedicinal applications. Its health benefits, attributed to bioactive sulfur compounds, include anticancer, anti-inflammatory, antifungal, antiviral, and antibacterial properties (Moses *et al.*, 2024). Several studies have highlighted its antimicrobial efficacy against drug-resistant bacteria using crude extracts, powders, and solvent-based preparations (Bhatwalkar *et al.*, 2021; Sasi *et al.*, 2021; Jafari *et al.*, 2023). Abidullah *et al.* (2021) demonstrated garlic's antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *K. pneumoniae* in vitro.

MATERIALS AND METHODS

Study Area

This study was done in Wukari. Wukari is a Local Government Area in Taraba State, Nigeria and is situated at latitude 7.85°North, longitude 9.78°East and 152 meters' elevation above the sea level in the northern part of Nigeria. The LGA is divided into sixteen traditional administrative districts and Wukari Local Government Area (LGA) is one of the sixteen LGAs in Taraba State. As of the 2006 census, the area had a population of 241,546 with 1205mm of annual precipitation and 26.80°C average temperature. Wukari is dominated by the Jukuns, majority of which are farmers and hunters (NPC, 2010; Imarenezor and Gaina, 2025).

Sample Collection and Processing

Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) used in this study were purchased from the New market and Old market Wukari Local Government Area of Taraba State, Nigeria. The fresh garlic cloves and ginger rhizomes were washed, sliced and shade air-dried for two weeks. After drying, garlic and ginger slices were grinded to fine powder separately using clean mortar and pestle (Allen *et al.*, 2023).

Preparation of Extracts

The extraction of the garlic and ginger were carried out using the cold Maceration method. 10g powder of each was soaked in 100 ml of distilled water and ethanol respectively (Allen *et al.*, 2023). The flasks were incubated at room temperature for 72 hours. The ethanol extracts were evaporated at 50°C while the aqueous extracts were evaporated to dryness at 60°C using water bath. The extract solutions were stored at 4°C.

Phytochemical Analysis

The plant extract was first diluted using the same solvent that had been employed during the extraction process to ensure compatibility with the mobile phase. A 2 mL portion of the diluted sample was then filtered through a 0.45µm syringe filter directly into a clean High Performance Liquid Chromatography (HPLC) vial to remove particulates and prevent column blockage. Similarly, the mobile phase solvent was filtered through a 0.45µm membrane using a vacuum flask to maintain purity and prevent interference during analysis. Each vial was appropriately labeled with its sample identification code to avoid mix-up during analysis.

The prepared vial was loaded into the autosampler of the HPLC system, and its position was noted. The phytochemical analysis method previously stored in the system application was retrieved, and the relevant phytochemical calibration file was also loaded to allow for

quantitative and qualitative assessments. An analysis sequence was then created according to the experimental design. The chromatographic conditions were set with a wavelength of 214nm and a run time of 30 minutes. Separation was performed using a Reprosil 100, C8, 5µm, 4.6 × 150mm column. The sequence was executed, and the chromatogram was monitored for retention times and peak patterns. The resulting chromatographs were printed for further interpretation and documentation.

Culture Preparation

Clinical isolates of *Klebsiella pneumonia* and *Streptococcus pneumoniae* were obtained from the central laboratory, Federal University, Wukari, Taraba state. These were inoculated in 10ml nutrient broth and allowed to grow overnight at 37°C separately before performing antimicrobial assay. The 50 µl of overnight culture of each bacterial strain was transferred separately into 10 ml of nutrient broth (pH 7.2) aseptically and placed in shaking water bath at 37°C for 8hours.

Preparation of 0.5 MacFarland Standards

To ensure that the appropriate number of microbial organisms were allocated to the agar plates, a broth standard known as the 0.5 McFarland standard (with a Concentration of 1.5×10^8 CFU/mL) was used. The McFarland Standard was prepared by weighing 85 ml of 1% sulphuric acid, which was then poured into a conical flask. With the use of a volumetric pipette, 0.5 ml of 1.1175% barium chloride was added to the conical flask. Soon after, 100 ml of 1% sulphuric acid was added to the conical flask, and it was stirred with the stirring rod until no clumps were visible. Following that, 10 ml of the barium sulphate precipitate was poured into two test tubes and covered with cotton wool. The test tubes were securely wrapped in aluminum foil and kept at room temperature to minimize evaporation (Cheesbrough, 2005).

Standardization of Inoculum

A 24-hour culture of the isolate was transferred into a sterile test tube containing normal saline using a sterile wire loop and adjusted to a turbidity equivalent to the 0.5 McFarland standard (1.5×10^8 cells/ml) (Awujo *et al.*, 2025).

Determination of Minimum Inhibitory Concentration (MIC)

The determination of Minimum Inhibitory (MIC) Concentration was performed by micro dilution method in sterile 96-well microtiter plates using the Clinical and Laboratory Standards Institute method (CLSI, 2006). Stock solutions were prepared and diluted in Mueller-Hinton broth to obtain pre-inoculation concentrations of 2000, 1500, 1000, and 500 mg/mL, giving final well concentrations of 1000, 750, 500, and 250 mg/mL after inoculation. A 24-hour bacterial culture was standardized to 0.5 McFarland's standard and further diluted to yield an inoculum of 5×10^5 CFU/mL. Each well received 100 µL of the extract solution and 100 µL of the inoculum, while growth, sterility, solvent, and antibiotic controls were included. Plates were incubated at 37°C for 24 hours. The MIC was recorded as the lowest concentration showing no visible growth compared with the control.

Minimum Bactericidal Concentration (MBC)

Aliquots from wells from the MIC were sub-cultured onto a freshly prepared nutrient agar, and the MBC was defined as the lowest concentration yielding no growth. All assays were performed in triplicate.

Antimicrobial activity of garlic and ginger extract

Antimicrobial activity of the extracts was evaluated using the agar well diffusion assay. Standardized bacterial suspensions were swabbed uniformly onto sterile nutrient agar

plates. Wells of 10 mm diameter were aseptically prepared with a sterile cork borer, and 100 μ L of garlic extract at concentrations of 100%, 75%, 50%, and 25% was introduced into each well. Same was repeated for ginger. The crude extracts were designated as 100% concentration. Working concentrations of 75%, 50%, and 25% were prepared by diluting the crude extract with sterile distilled water in ratios of 3:1, 1:1, and 1:3, respectively. These graded concentrations were used to evaluate the concentration-dependent antibacterial activity of the extracts. The plates were incubated upright at 37°C for 24 hours, and the diameters of the inhibition zones were measured in millimeters. Inhibition zones of less than 11 mm were considered inactive.

RESULTS AND DISCUSSION

Table 1: pH values of various extracts of garlic and ginger

Sample	pH Value	
	Aqueous Extract	Ethanol Extract
Ginger	5.60	5.46
Garlic	5.67	5.53

The pH analysis as shown in table 1 above revealed that all extracts were slightly acidic, with values ranging between 5.46 and 5.67. The ethanol extract of ginger recorded the lowest pH (5.46), whereas the aqueous extract of garlic had the highest (5.67). The slight acidity can be attributed to the presence of organic acids, phenolic compounds, and other phytochemicals that are known contributors to antimicrobial activity (Adamczak *et al.*, 2019). Acidic conditions are generally less favorable for pathogenic bacteria, which tend to thrive in neutral to slightly alkaline environments. Thus, the acidic nature of these extracts may enhance their antimicrobial effects by complementing the activity of the bioactive compounds.

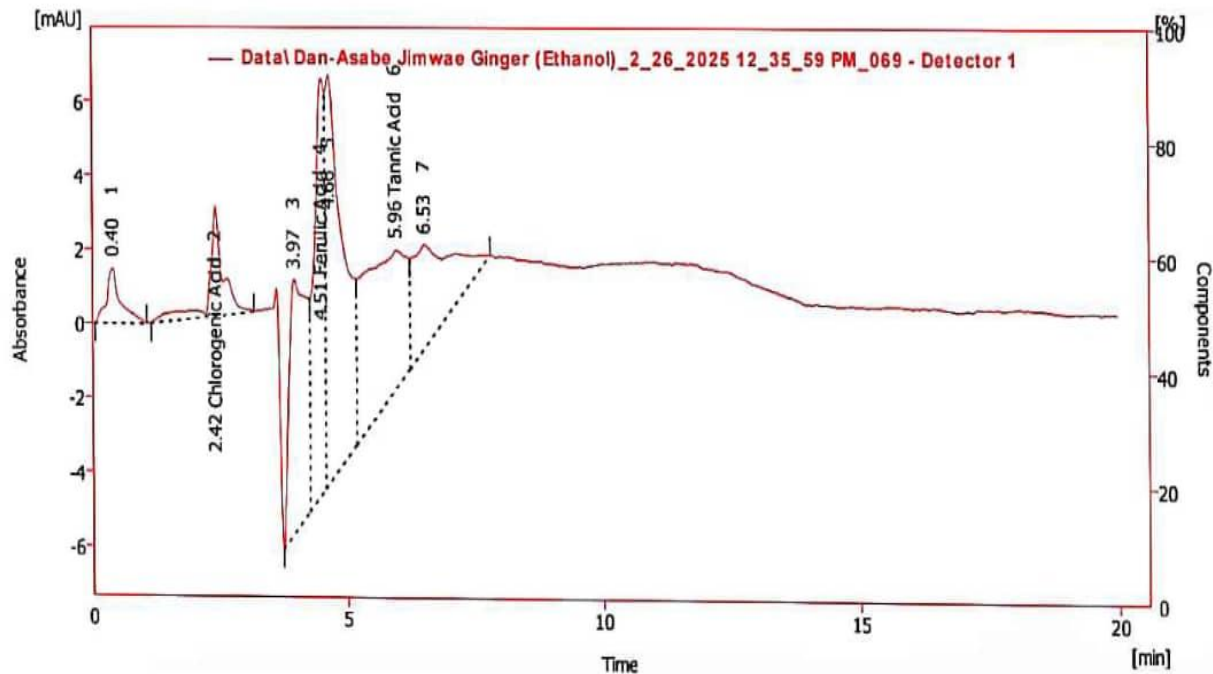


Figure 1: HPLC chromatogram of the ethanol extract of ginger

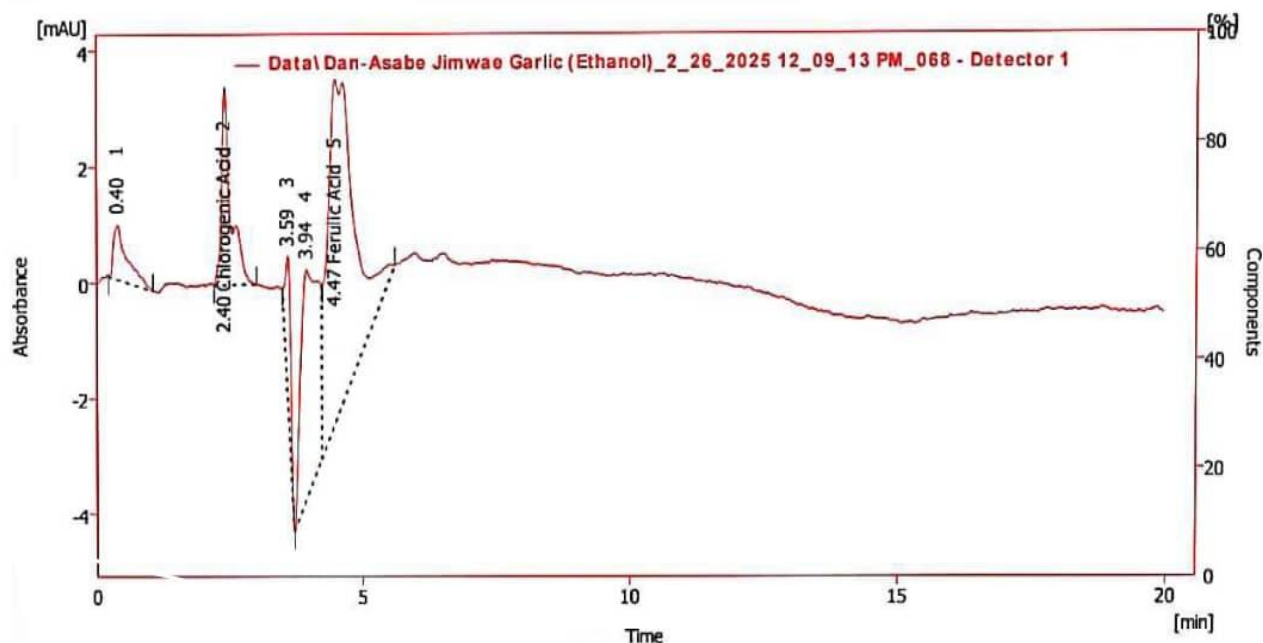


Figure 2: HPLC chromatogram of the ethanol extract of garlic

High-performance liquid chromatography (HPLC) confirmed the presence of phenolic compounds, particularly chlorogenic acid, ferulic acid, and tannic acid, in the ethanol extract of ginger (figure 1). In garlic extracts, chlorogenic and ferulic acids were also detected (figure 2). These findings agree with earlier reports by Jan *et al.* (2022) and Šnirc *et al.* (2023), who similarly identified these phenolic acids in ginger and garlic, respectively. Phenolic acids are well established for their antimicrobial and antioxidant properties, acting through mechanisms such as disrupting microbial membranes, chelating essential nutrients, and interfering with microbial metabolism. The wider diversity of compounds detected in ginger may partly explain its broader spectrum of activity compared to garlic.

Aqueous extracts of both ginger and garlic did not exhibit inhibitory activity against either *Streptococcus pneumoniae* or *Klebsiella pneumoniae* at the tested concentrations (1000-250 mg/mL). This suggests that water is not an efficient solvent for extracting the active phytochemicals responsible for antimicrobial activity. Similar observations were reported by Mehmood *et al.* (2022), who demonstrated that polar solvents such as ethanol and methanol are more effective at extracting phenolics and flavonoids with antibacterial potential. It is also possible that the concentrations of active compounds in aqueous extracts were too low to achieve therapeutic effects.

Table 2: Antimicrobial activity of aqueous extracts of ginger and garlic against *Streptococcus pneumoniae* and *Klebsiella pneumoniae*

Organism <i>pneumoniae</i>	<i>Streptococcus pneumoniae</i>					<i>Klebsiella</i>				
	100	75	50	25	Ctrl	100	75	50	25	Ctrl
Ginger	-	-	-	-	+	-	-	-	-	+

Garlic - - - - + - - - - +

Key: (-) = no inhibition, growth observed (+) = inhibition, (ctrl) = Control (antibiotics)

Table 3: Antimicrobial activity of ethanol extracts of ginger and garlic against *Streptococcus pneumoniae* and *Klebsiella pneumoniae*

Organism <i>pneumoniae</i>	<i>Streptococcus pneumoniae</i>					<i>Klebsiella</i>					
	100	75	50	25	Ctrl	100	75	50	25	Ctrl	
Ginger		+	+	+	-	+	+	+	+	-	+
Garlic	+	+	+	+	+	-	-	-	-	+	

Key: (-) = no inhibition, growth observed (+) = inhibition, (ctrl) = Control

Table 4: Antibacterial Activity of Ethanol Extract of Ginger Against *Streptococcus pneumoniae* and *Klebsiella pneumoniae*

Conc. (mg·mL⁻¹) <i>pneumoniae</i>	Zone of inhibition (mm)	
	<i>Streptococcus pneumoniae</i>	<i>Klebsiella</i>
1000	21.00	18.00
750	16.00	17.00
500	12.00	0.00
250	0.00	0.00

As shown in table 2 and 3, antimicrobial evaluation revealed that ethanol extracts were markedly more effective than aqueous extracts, which exhibited no activity. The ethanol ginger extract displayed concentration-dependent inhibition against both *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. Complete inhibition was observed at 100%, 75%, and 50% concentrations, while no activity occurred at 25%. Zone of inhibition measurements in figure 4 confirmed this trend, with *S. pneumoniae* showing higher susceptibility (21.00 mm at 1000 mg/mL and 12.00 mm at 500 mg/mL). *K. pneumoniae* was also inhibited but only at higher concentrations (1000 and 750 mg/mL), with no effect observed at 500 mg/mL and below. These results are consistent with Allen *et al.* (2023),

who reported stronger activity of ginger extracts against Gram-positive bacteria than Gram-negative species. The reduced efficacy against *K. pneumoniae* likely reflects structural barriers in Gram-negative bacteria, including the protective outer membrane and efflux systems that restrict phytochemical penetration.

In contrast, the ethanol garlic extract demonstrated strong and consistent antibacterial activity against *S. pneumoniae* across all tested concentrations (100-25%), with an MIC \leq 250 mg/mL, highlighting broad potency against this Gram-positive organism. However, no inhibitory effect was recorded against *K. pneumoniae* at any concentration tested. This suggests possible intrinsic resistance mechanisms, such as efflux pumps, enzymatic degradation, or cell envelope impermeability. Interestingly, this finding differs from Allen *et al.* (2023), who reported garlic extract as effective against *K. pneumoniae*. Such discrepancies may be attributed to differences in extraction methods, strain variation, or geographical diversity of bacterial isolates.

The antibiotic susceptibility tests presented in table 5 below served as a benchmark for evaluating the effectiveness of the plant extracts. *K. pneumoniae* in this study was broadly susceptible to conventional antibiotics, with inhibition zones ranging from 16.00 mm to 23.00 mm. Ceftriaxone, Gentamicin, and Ciprofloxacin showed the highest activity, suggesting the isolate was likely non-ESBL-producing. This observation contrasts with reports by Ayeni *et al.* (2024) and Neelambike *et al.* (2025), who documented high rates of resistance among *K. pneumoniae* isolates, particularly to cephalosporins, aminoglycosides, and fluoroquinolones.

On the other hand, *S. pneumoniae* exhibited multidrug resistance (MDR), with no inhibition zones against most antibiotics except for Ceftriaxone (15.00 mm). This pattern is consistent with reports by Ummilaila *et al.* (2024), Nnanna *et al.* (2023), and Anosike *et al.* (2024), who observed high levels of MDR among *S. pneumoniae* isolates, including resistance to β -lactams. This highlights the clinical challenge posed by resistant *S. pneumoniae* strains, for which therapeutic options are limited. In such contexts, the activity of ethanol extracts of garlic and ginger against *S. pneumoniae* is particularly noteworthy, suggesting their potential as alternative or adjunct therapies.

Table 5: Antibacterial Activity of Conventional Antibiotics against *Streptococcus pneumoniae* and *Klebsiella pneumoniae*

Antibiotic	Zone of inhibition (mm)	
	<i>Streptococcus pneumoniae</i>	<i>Klebsiella pneumoniae</i>
Ciprofloxacin (CIP)	0.00	22.00
Chloramphenicol (C)	0.00	18.00
Ofloxacin (OF)	0.00	20.00
Clarithromycin (CR)	0.00	19.00
Pefloxacin (PF)	0.00	21.00
Ceftriaxone (CT)	0.00	23.00
Amoxicillin (AX)	0.00	17.99

Streptomycin (ST)	0.00	16.00
Nitrofurantoin (N)	0.00	20.00
Gentamicin (GN)	15.00	22.00

This comparative finding emphasizes that ethanol extracts of garlic and ginger are superior to aqueous extracts in terms of antimicrobial activity, reinforcing the importance of solvent selection in phytochemical studies. More so, the selective activity of these extracts against *S. pneumoniae*, a multidrug-resistant organism, highlights their therapeutic potential in the management of antibiotic-resistant infections.

Future research should focus on isolating and characterizing the specific bioactive compounds responsible for the observed effects, elucidating their mechanisms of action, and investigating potential synergistic effects with conventional antibiotics. In vivo studies are also necessary to validate the safety and efficacy of these extracts, as in vitro results do not always translate directly to clinical effectiveness.

CONCLUSION

This study demonstrates that ethanol extracts of garlic and ginger possess promising antimicrobial potential, particularly against *Streptococcus pneumoniae*. Their activity against an organism with limited antibiotic treatment options emphasizes their potential role in the fight against multidrug-resistant pathogens.

CONFLICTS OF INTEREST

The Authors declares no conflicts of interest

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