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Original Article

Assessing the antibacterial efficacy of Garlic (*Allium sativum*) extracts against multidrug-resistant pathogenic bacteria

OPEN ACCESS

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ABSTRACT

The increasing prevalence of antibiotic resistance among pathogenic bacteria has necessitated the exploration of alternative antimicrobial agents. *Allium sativum* (Garlic), commonly known as garlic, has been recognised for its rich phytochemical profile and traditional medicinal uses, including its potential antibacterial properties. This study investigated the activity of garlic solvent extracts against three bacteria isolates of clinical origin. Garlic samples were extracted by maceration using ethyl acetate, acetone, and methanol as solvents, following standard procedures. Qualitative and quantitative phytochemical profiles were performed using established analytical techniques. The antibacterial activity of the test extracts and standard drugs was assessed against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* using the Agar disc diffusion method, with the zones of inhibition estimated in millimetres. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the garlic extracts were determined using the broth dilution technique. Phytochemical screening and quantification revealed the presence of tannins (0.139 µg/g), alkaloids (55.00 mg/g), saponins (11.70 mg/g), steroids, flavonoids (15.00 mg/g), cardiac glycosides, and anthraquinones. Antimicrobial studies indicated that the methanolic extract of garlic exhibited the highest zone of inhibition, particularly against *S. aureus* (20.66 mm) and *P. aeruginosa* (25.23 mm), which was compared favourably with standard antibiotics. The lowest MIC (50 mg/ml) was produced by the methanol extract against *P. aeruginosa*, while the acetone extract yielded the lowest MBC (100 mg/ml) against *S. aureus*. These findings suggest the potential effectiveness of garlic extracts at specific concentrations against pathogenic clinical isolates.

K E Y W O R D S : *Allium sativum, Antibacterial, Antibiotics, Phytochemical, Solvents*

INTRODUCTION

Traditional medicinal plants play a vital role in alternative therapies for numerous diseases including antimicrobials. Studies have identified various plant-derived compounds, including alkaloids, saponins, flavonoids, and tannins, known for their antimicrobial properties (Das *et al*., 2010; Srivastava *et al*., 2013). The plant-derived medicines, available in various forms like powders and liniments, contribute significantly to herbal medicines widely used by traditional healers for treating infections (Sofowora, 1993).

Allium sativum (garlic), a member of the Liliaceae family, is a globally consumed food and medicinal plant with substantial health benefits. Garlic's nutrient-rich

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composition includes essential minerals and vitamins, along with sulfur-containing compounds like allicin and allisatin, which have been linked to its antibacterial and antioxidative properties (Shobana *et al.*, 2009; Sunanta *et al*., 2023; Melguizo-Rodríguez *et al*., 2022). The presence of many biologically active substances in garlic, especially in its underground bulbs makes the plant suitable for us as a nutraceutical and in alternative medicine (Awan *et al.,* 2019). It has been reported to exhibit a wide range of therapeutic effects, such as antifungal, anti-atherosclerotic, and antihypertensive activities, making it an important natural remedy in alternative medicine (El-Saber Batiha *et al*., 2020). Many studies have indicated that *Allium sativa* extract demonstrated antibacterial activity against pathogens found in health settings. For instance, studies by Magryś *et al.,* (2021) using fresh garlic extracted with 70 % ethanol revealed that the garlic extract successfully inhibited the growth of multidrug-resistant bacteria. Allicin in garlic has been shown to inhibit the growth of various bacterial strains, including *Staphylococcus aureus* and *Escherichia coli* (Choo *et al.,* 2020).

Given the increasing prevalence of antibiotic resistance, as highlighted by the World Health Organization (WHO, 2023), garlic's potent antimicrobial activity against drug-resistant pathogens, including *Staphylococcus aureus* and *Escherichia coli*, has garnered significant research interest (Wallock-Richards *et al*., 2014; Bhatwalkar *et al*., 2021; Magryś *et al.,* 2021). The use of different solvents, such as ethyl acetate, acetone, and methanol, allow for the extraction of various phytochemicals with varying polarities (Awotedu *et al.,* 2020) and helps isolate a diverse range of bioactive components in garlic. This study, therefore, explores the antibacterial efficacy of garlic extracts against selected clinical isolates to assess their potential application in treating bacterial infections.

MATERIALS AND METHODS

Collection, Identification and Processing of Plant Samples and Test Organisms

Bulbs of garlic (*Allium sativum)* were purchased from a local market in Ibadan, the sample was identified at the taxonomy unit of the Forestry Research Institute of Nigeria (FRIN) Ibadan, Oyo State. The garlic bulbs were washed and air dried at room temperature (25 ^{o}C) before milling using the electric grinder into powdered form, it is then stored in tight dry plastic containers till further analysis.

Clinical isolates were obtained from the University of Ilorin Teaching Hospital, Oke-Oyi, Kwara State, Nigeria. The isolates used for this study were *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* which were confirmed by biochemical tests such as gram staining carried out according to the method of Fawole and Osho (2004); catalase, citrate, and oxidase tests done according to the methods described by Cheesebrough (2005). Also, catalase, citrate and oxidase tests were carried out according to the methods of Cheesebrough (2005).

Preparation of Plant Extracts and Phytochemical Analysis

Fifty grams of milled garlic (*Allium sativum)* bulb was soaked in 200 ml of ethyl acetate, acetone and methanol (extracting solvents) respectively. This was stirred with a glass rod and left on the shaker for 12 hours. It was then filtered using sterile double Whatman's filter paper. The solution was concentrated using the Rotary evaporator (Gull *et al.,* 2012). The semi-solid mass left was then kept in the desiccator to get the dried crude extracts of the plant.

Qualitative phytochemical screening was performed using the methods previously described in other studies (Sofowora, 1993; Senthilkumar and Reetha, 2009). The samples were screened for cardiac glycosides, alkaloids, flavonoids, steroids, phenols, Tannins, saponins, and anthraquinones.

In the quantitative determination, alkaloid, saponin and flavonoid were determined by gravimetry (Bohm and Kocipai-Abyazan, 1994; Obadoni and Ochuko, 2001); while tannin was measured spectrophotometrically (Bohm and Kocipai-Abyazan, 1994).

Antibacterial Sensitivity Testing of the Extract and Antibiotic Sensitivity Testing against the Clinical Isolates

Using the agar disc diffusion method (James & John, 1999), *A. sativum* extracts were tested at concentrations of 200, 100, 80, 60, 40, and 20 mg/ml. Discs impregnated with each concentration were placed on inoculated Mueller Hinton Agar plates, allowed to diffuse for 30 minutes, and then incubated at 37 °C for 24 hours. Zones of inhibition were measured to assess antibacterial activity, with zones classified as resistant (<7 mm), mildly sensitive (8-10 mm), or sensitive (>11 mm) following Assam *et al*., (2010). Each test was conducted in triplicate under aseptic conditions, and inhibition was reported as the mean zone diameter in millimetres. Absence of zones indicated no antibacterial activity (Mathabe *et al*., 2006).

Commercial antibiotics discs (Ampicillin (AMP- 10 ug), tetracycline (TET-10 ug) and chloramphenicol (C-10 ug)) were used to assess the drug sensitivity and resistance pattern of bacteria. These discs were placed on the plates inoculated with different strains of approximate cell density corresponding to 1×10^{8} CFU/ml (0.5 McFarland standard). These served as positive control while a disc

containing the solvents of extraction served as a negative control.

Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal (MBC) Concentration

The broth dilution method was conducted following ESCMID guidelines (2003) to determine the MIC. Ethyl acetate, acetone, and methanol extracts of *A. sativum* were prepared at concentrations of 200, 100, 50, and 25 mg/ml using a two-fold dilution approach. Standardized test organisms in Mueller Hinton and Sabouraud Dextrose broth were used, with 9 ml of broth, 0.5 ml of standardized organism, and 0.5 ml of extract dispensed per tube, followed by a 24-hour incubation at 35°C. Turbidity indicated microbial growth, with the lowest concentration showing no turbidity recorded as the MIC. Each test was performed in triplicate, with ampicillin as a positive control and a blank (no extract or antibiotic) as the negative control.

MBC was done using the method of Andrews (2006). Plates were incubated for 24 hours at 35 ^{o}C. Plates showing no further growth of organisms were taken as the MBC. Ampicillin served as positive control while the extracting solvents served as negative control.

Data Analysis

Data collected for zones of inhibition was presented as Mean ± standard deviation of triplicate measurement; one way analysis of variance and post hoc test was done to assess the differences in the mean zone of inhibitions between the solvent extracts and standard drugs for each isolate respectively where $p<0.05$ was considered significant. Analysis was done using SPSS version 20.

RESULTS AND DISCUSSION

Results

The qualitative phytochemical screening of *A. sativum* revealed the presence (+) of tannins, alkaloids, saponins, steroids, flavonoids, cardiac glycosides, and anthraquinones. Phenols, however, were found to be absent (-) in the garlic samples. In the quantitative analysis, *A. sativum* exhibited the following concentrations (mean ± standard deviation): flavonoids (15.00±0.05 mg/g), alkaloids (55.0±2.66 mg/g), saponins (11.70±2.17 mg/g), and tannins (0.139±0.01 µg/g).

The results of antibacterial activities of the three solvent extracts of *A. sativum* showed that the mean zone of inhibition produced by the *A. sativum* extracts against the isolates at the tested highest concentration of 200 mg/ml ranged from 9.66-15.33 mm produced by three solvent extracts against *E. coli*, 18.00-20.66 mm produced against *S. aureus*, and 12.66-25.23 mm produced against *P. aeruginosa* (Table 1). The solvent also produced zones of inhibition based on increasing polarity. At the highest extract concentration, *S. aureus* was most sensitive to ethyl acetate extract of garlic with a zone of inhibition of 18.00 mm while *E. coli* was the least sensitive with a zone of inhibition of 9.66 mm; similarly, *P. aeruginosa* was most sensitive to acetone and methanol extracts of garlic with a zone of inhibition of 21.00 mm and 25.23 mm respectively, with *E. coli* being the least sensitive to both extracts with a zone of inhibition of 15.00 mm and 15.33 mm respectively (Table 1). The result also suggests that methanol extract of garlic was the most sensitive to the test organisms, while both methanol and acetone extracts produce comparable activity ($p < 0.05$) against *E. coli*. All other concentrations gave zones of inhibitions ranging from sensitive to mild to insensitive.

Table 1: Antibacterial Activities of the Three Extracts of Garlic against the Bacterial Isolates

Test Organisms	Solvent	Concentration (mg/ml)/Mean Zone of Inhibition (mm)					
		200	100	80	60	40	20
E. coli	Ethyl acetate	9.66 ± 8.39^{a}	7.00±6.08	4.66±4.16	3.00±1.00	2.00±0.00	1.00±1.00
	Acetone	15.00±1.00^{b}	7.33±6.43	6.00±5.19	5.33±1.53	2.33±1.16	0.33±0.58
	Methanol	15.33±13.3^{b}	11.66±0.58	6.00±5.29	3.30±2.88	2.33±2.08	1.30±0.58
S. aureus	Ethyl acetate	18.00±3.00^{a}	10.00±1.00	7.00±1.00	3.33±1.16	1.70±0.58	1.33±1.16
	Acetone	19.00±1.00^{ab}	13.00±1.00	10.00±1.00	7.00±1.00	3.00±0.00	1.00±0.00
	Methanol	20.66±4.04^{b}	16.66±1.53	12.30±0.58	8.33±1.53	4.66±1.53	1.33±1.16
P. aeruginosa	Ethyl acetate	12.66±0.58^{a}	7.33±0.58	6.33±0.58	2.66±0.58	1.00±1.00	1.00±0.00
	Acetone	21.00±1.00^{c}	14.33±1.16	10.00±2.00	5.66±0.58	2.00±2.00	1.00±1.00
	Methanol	25.23±0.58^{d}	17.70±1.53	13.33±1.53	9.30±0.58	6.70±0.58	2.70±0.58

Mean ZI with different superscript alphabet within the same column for each species are significantly different ($p<0.05$).

Results obtained from the antibiotics sensitivity testing against the test organisms shows that *E. coli* was sensitive to ampicillin (AMP-10ug), tetracycline (TET-10ug) and chloramphenicol (C-10ug) with zones of inhibitions of 15.00±1.00 mm, 21.00±1.00 mm and 12.00±2.00 mm respectively (Table 2). The activity of the acetone and methanol extract was found to be comparable with the antibiotic, ampicillin however, significantly less active

compared with tetracycline ($p<0.05$). Also *S. aureus* was sensitive to ampicillin, tetracycline and chloramphenicol with zones of inhibition of 21.00±1.00 mm, 23.00±2.00 mm and 18.00±0.64 mm respectively, all the extracts compare well with the standard antibiotics, however, only tetracycline produced better activity than the extracts of *A. sativum* ($p<0.05$). Similarly, *P. aeruginosa* also showed sensitivity to ampicillin, tetracycline and chloramphenicol as shown in Table 2. This trend also shows that the acetone and methanol extract produced significantly better activity against the organism than all three standard antibiotics.

Table 2: Antibiotics Sensitivity Testing against Clinical Isolates (Positive control) and Extracting Solvents (Negative Control)

Test Organisms	Antibiotics/ Zone of Inhibition(mm)			Negative Control
	Ampicillin (AMP-10ug)	Tetracycline (TET-10ug)	Chloramphenicol (C-10ug)	
E. coli	15.00 ±1.00^{b}	21.00 ±1.00^{c}	12.00 ± 2.00^{ab}	0.00
S. aureus	21.00 ±1.00^{b}	23.00 ±2.00^{c}	18.00 ±0.65^{b}	0.00
P. aeruginosa	17.00 ±0.29^{b}	20.00 ±1.00^{c}	14.00 ±0.46^{a}	0.00

Mean ZI with different superscript alphabet within the same row for each species are significantly different ($p<0.05$); values with same alphabet as the mean ZI of the solvent extracts in Table 1 for each species are not significantly different ($p>0.05$).

The result of the minimum inhibitory concentration (MIC) showed that ethyl acetate extract of garlic gave MIC value of 100 mg/ml against *S. aureus*; the acetone extract of *A. sativum* showed MIC against *S. aureus* (100 mg/ml) and *P. aeruginosa* (100 mg/ml) respectively, furthermore, the methanol extract of *A. sativum* showed MIC against *E. coli* (200 mg/ml), *S. aureus* (100 mg/ml) and *P. aeruginosa* (50 mg/ml). however, ethyl acetate extract showed no activity against *E. coli* and *P. aeruginosa* cultures; while acetone extract also showed no activity against *E. coli* at all the tested concentration range (Table 3). Thus, acetone extract of *A. sativum* gave MBC of 100 mg/ml against *S. aureus.* Similarly, the methanol extract produced MBC against *E. coli* at 200 mg/ml (Table 3).

Table 3: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Garlic Extracts against Pathogenic Bacteria

Parameter	Solvents	*E. coli*	*S. aureus*	*P. aeruginosa*
MIC (mg/ml)	Ethyl acetate	-	100	-
	Acetone	-	100	100
	Methanol	200	100	50
MBC (mg/ml)	Ethyl acetate	-	-	-
	Acetone	-	100	-
	Methanol	200	-	-

Note: - = Absence

Discussion

The phytochemical analysis of garlic in this study identified bioactive compounds, including tannins, alkaloids, saponins, flavonoids, and cardiac glycosides, which are widely recognized for their antimicrobial properties. Similar compounds are also present in related species like onion and leek, where sulfur compounds like allicin and various flavonoids enhance antimicrobial efficacy (Effiong *et al*., 2020; Marefati *et al*., 2021). The tannins, for example, may disrupt bacterial cell walls by binding to proteins, a mechanism linked to broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria (Vu *et al*., 2017; Farha *et al*., 2020; Tong *et al*., 2022). Additionally, alkaloids contribute to garlic's antimicrobial effects by inhibiting bacterial DNA synthesis, which may prevent replication (Yan *et al*., 2021). This mode of action is consistent with findings in other *Allium* species, supporting garlic's traditional use in treating infections (Lanzotti *et al*., 2014). Saponins in garlic also promote antibacterial effects by destabilizing cell membranes, a characteristic similarly observed across a range of bacteria (Lanzotti *et al*., 2014; Moses *et al*., 2024). Furthermore, the presence of flavonoids, known for disrupting bacterial cell walls and inhibiting essential bacterial enzymes, further supports garlic's role in microbial defence mechanisms, as reported in related *Allium* species (Cushnie & Lamb, 2005; Shahrajabian *et al*., 2021).

The antibacterial activity of garlic extracts (ethyl acetate, acetone, and methanol) against *E. coli*, *S. aureus*, and *P. aeruginosa* showed a concentration-dependent effect, with methanol and acetone extracts exhibiting significant potency comparable to standard antibiotics like ampicillin

and chloramphenicol. This trend is similar to those previously reported by Gull *et al.,* (2012). The methanol extract, in particular, displayed higher inhibition zones than tetracycline against *P. aeruginosa*, indicating strong activity potentially useful against resistant strains. The comparable activity between *A. sativum* extracts and standard antibiotics supports garlic's traditional use as an antimicrobial agent and its potential application in treating infections where antibiotic resistance is of concern (Magryś *et al*., 2021; Bhatwalkar *et al*., 2021). For *S. aureus*, the acetone and methanol extracts produced inhibition zones comparable to those of standard antibiotics, aligning with studies showing polar solvents like methanol effectively extract potent bioactive compounds from garlic against Gram-positive bacteria (Khashan, 2014; Iotsor *et al*., 2019; Magryś *et al*., 2021). Against *P. aeruginosa*, the methanol and acetone extracts demonstrated superior activity over antibiotics, highlighting garlic's potential for treating difficult Gram-negative pathogens, consistent with earlier findings attributing garlic's efficacy to sulfur compounds that disrupt cell integrity (Magryś *et al*., 2021; Bhatwalkar *et al*., 2021).

The MIC and MBC are key indicators in evaluating antibacterial potency, with MIC representing the lowest concentration needed to inhibit visible bacterial growth, and MBC the concentration required to achieve complete bacterial eradication (Rodríguez-Melcón *et al*., 2022). Lower MIC and MBC values are indicative of strong antimicrobial effects (Shah *et al*., 2024). This study's MIC and MBC results support the potent antibacterial properties of garlic extracts, especially methanol, which demonstrated MICs of 200 mg/ml for *E. coli*, 100 mg/ml for *S. aureus*, and 50 mg/ml for *P. aeruginosa*. These values reflect higher sensitivity of *P. aeruginosa* to the methanol extract, consistent with larger inhibition zones observed for this pathogen, suggesting that methanol effectively isolates active antibacterial compounds from garlic (Garba *et al*., 2013). Furthermore, the MBC results reinforce methanol's bactericidal effects against *E. coli* at 200 mg/ml, confirming its effectiveness against Gram-negative bacteria. Conversely, the ethyl acetate extract showed limited efficacy, with no recorded MIC or MBC for *E. coli* or *P. aeruginosa* and only moderate activity against *S. aureus* (MIC 100 mg/ml), aligning with studies that find less polar solvents like ethyl acetate are less effective than polar solvents in extracting garlic's bioactive compounds (Iotsor *et al*., 2019; Bar *et al*., 2022).

Compared to standard antibiotics, while tetracycline showed greater activity against *E. coli* and *S. aureus*, garlic extracts, particularly methanol, demonstrated comparable or superior activity to ampicillin and chloramphenicol, especially against *P. aeruginosa*. These findings highlight garlic's potential, especially methanol extracts, as a practical antimicrobial option, even for infections involving antibiotic-resistant strains (Garba *et al*., 2013; Magryś *et al*., 2021).

CONCLUSION AND RECOMMENDATION

This study has demonstrated the significant antibacterial properties of *A. sativum* extracts, particularly those obtained using solvents like ethyl acetate, acetone, and methanol. The presence of various phytochemicals, including saponins, flavonoids, and alkaloids, contributes to the antimicrobial efficacy observed against clinical isolates such as *E. coli*, *S. aureus*, and *P. aeruginosa*. The findings reveal that garlic extracts exhibit comparable or greater activity to standard antibiotics like ampicillin and chloramphenicol. The MIC and MBC results further emphasise the efficacy of garlic extracts, especially methanol extract, in inhibiting and killing bacterial pathogens. These findings support the use of garlic as a potential natural alternative in antimicrobial therapy and recommends further exploration of the use of garlic extracts especially methanol-based formulations, as a natural alternative for treating bacterial infections particularly in the context of rising antibiotic resistance

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Authors' contributions

EPC carried out sample collection and preparation, carried out the methodology, managed data collection, POO handled data interpretation, data analysis and wrote the first draft of the manuscript. MBO, IBE and OSA managed the literature searches and reviewed the manuscript. All authors approved the final manuscript.

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