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Phytochemical screening and antibacterial potential of *Allium sativum* (garlic) against clinically relevant bacterial isolates

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ABSTRACT: The prevalence of counterfeit or substandard drugs is a widespread issue in the country today, particularly concerning antibiotics. This contributes significantly to the growing problem of antimicrobial resistance among organisms that were once susceptible to these drugs. Consequently, evaluating the antibacterial properties of natural alternatives such as garlic becomes essential to justify their incorporation into herbal medicine formulations. This study investigated the activity of garlic ethanol and chloroform extracts against clinically important bacterial isolates, namely Citrobacter sp., *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, using the agar well diffusion method. Ciprofloxacin (1 mg/ml) served as the standard control, while the test concentrations of the extracts were prepared at 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. Phytochemical analysis revealed the presence of tannins, flavonoids, saponins, cardiac glycosides, steroids, alkaloids, volatile oils, balsams, and terpenoids. The results showed the least activity against *Citrobacter spp*. with 6.30 mm at 100 mg/ml. The garlic ethanol extract had an MIC value of 50 mg/ml, while the garlic chloroform extract had an MIC value of 100 mg/ml. The garlic ethanol and chloroform extracts had MBC values only at 100 mg/ml. The result of this study shows that the extracts had activity against the test organisms and as such could be a potential therapeutic against the tested organisms.

Keywords: *Allium sativum* (garlic), antibacterial, phytochemicals.

INTRODUCTION

Antibiotic resistance has become a serious problem and affects almost every bacterial species. Resistance to multiple antibiotics has developed among many common pathogens, such as *Staphylococcus, Pneumococci, and Pseudomonas* organisms, and this problem is steadily increasing worldwide. Around 90-95% of *Staphylococcus aureus* strains is penicillin-resistant worldwide. In Asian countries, 70-80% of the same strains are methicillin-resistant (Mohamad Farook *et al*., 2022). Sometimes antibiotics are associated with adverse effects on the host, which include depletion of beneficial gut, mucosal microorganisms, immune suppression, hypersensitivity and allergic reaction. Some drug-resistant bacteria have complicated the treatment of infectious diseases in immunocompromised AIDS and cancer patients (Duhaniuc *et al.,* 2024). One way to beat this downside of drug resistance is by getting new molecules from natural resources.

Plants are known to produce a variety of compounds and medicinal properties to prevent infections from a wide range of microorganisms, including plant pathogens and environmental organisms (Mickymaray, 2019). Therefore, alternative antimicrobials are used from botanical sources, which provide flexibility and diversity. In many developing countries large portion of the population depends on the traditional system of medicine to treat a variety of diseases (Holzinger *et al*., 2023). The World Health Organisation (WHO) reported that 80% of the world population relies

chiefly on traditional medicine, which involves the medicinal plant extracts or their active constituents (Süntar *et al*., 2020). Subsequently advancement of common antimicrobial agents from plant sources would serve as a promising approach.

Garlic, *Allium sativum* (commonly called 'aayu' in Yoruba, 'ayo-ishi' in Igbo and 'tafarnua' in Hausa), is a perennial bulbous plant that initially came from middle Asia and is at present grown globally. It belongs to the family *Alliaceae*. Garlic can grow up to 2 feet in height or more. The bulb is the main part of the plant, which is used for medicine (Khokhar, 2023). Each garlic bulb is made up of 4 to 20 cloves. Each garlic clove may weigh about 1 gram in weight. Fresh, aged, or dried garlic can be used as a garlic supplement. Each of the supplements may have different effects on the body (AL-Mufarrej *et al*., 2019). It is commonly used as a seasoning. Its close relatives include the onion, shallot and leek. The head of garlic (the most commonly used plant part) comprises numerous discrete cloves, whereas the leaves and stems are sometimes eaten, particularly while immature and tender. The medicinal potency of garlic is due to glycosides, vitamins B, C, and D, allisatin II and I. It also contains volatile sulphur oil, which has a vermifugal action (El-Saber Batiha *et al.,* 2020). It has been used throughout recorded history for both culinary and medicinal purposes. It has a characteristic pungent, hot, flavour that mellows and sweetens considerably with cooking. Its typical pungent odour antibacterial activity depends on allicin, which is produced by enzymatic (alliin-lyase) hydrolysis of alliin after cutting and crushing of the cloves (Li *et al.,* 2022).

MATERIALS AND METHODS

Sample collection and processing

The plant materials (Garlic) were obtained from Kabuga market, Kano, Kano State, Nigeria. They were placed in separate polythene bags and transported immediately to the laboratory of the Department of Microbiology, Bayero University, Kano. The samples were identified and authenticated by comparing them with known samples. The plant material was washed with clean water to get rid of sand particles. They were chopped and partially allowed to air-dry in the shade at room temperature for five days in order to remove excess moisture. The garlic bulbs were separated into cloves. The cloves skins were peeled off, and the cloves were sliced and also air dried at ambient temperature for about four weeks. After drying, pieces of *Allium sativum* were ground to fine particles, each, utilising a suitable sterile electric blender to obtain a homogenous sample.

Preparations of the extract

25 g of the powdered samples each was extracted with 250 mL of ethanol and chloroform by the cold maceration method as described by Akullo *et al.* (2022), with some slight modifications. The containers were left at 25ºC for 4 days (96 hours). The suspensions were filtered using Whatman no.1 filter paper. The filtrates were concentrated at 90ºC using a water bath and delivered into sterile, clean containers with suitable labelling and were kept at 4ºC in a refrigerator until further use. The percentage yield of each extract obtained was calculated using the formula.

$W_e/W_p \times 100$

Where: W_e= weight of the extract, W_p = weight of the powdered material used for the extraction.

Sterility of the extracts

One (1) ml of the extract was added separately to two test tubes containing 5 ml of sterile nutrient broth. They were incubated at 37 ºC for 24 hours. The extracts were cleared after incubation, indicating the absence of a contaminant which could have caused a turbid appearance in the tube (Iotsor *et al.,* 2019).

Qualitative phytochemical screening

Phytochemical screening of the extracts was conducted qualitatively as stated by Tafinta *et al*. (2020). Tanins, flavonoids, saponins, cardiac glycosides, reducing sugars, steroids, alkaloids, volatile oils, balsams and terpenoids were determined accordingly.

Test microorganisms

The test organisms were collected from the Microbiology Department, Bayero University, Kano. The isolates were subjected to Gram's staining and other biochemical tests according to standard procedures and identified as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Citrobacter sp*. (Dimri *et al.*, 2020). The pure isolates were then stored on Nutrient agar slant bottles at a temperature of 4ºC until further use.

Preparation of the extract concentration

The concentration was prepared by dissolving 0.1 g of crude extract into one millilitre of dimethylsulphuroxide (DMSO) in a clean grease free vial bottle, to obtain a stock of 100mg/ml concentration. Using double serial dilution methods the following concentrations of 50 mg/ml, 25 mg/ml and 12.5 mg/ml were made. 1mg/ml of ciprofloxacin was used as a positive control (Afolabi *et al.,* 2020).

Antibacterial assay

The agar well diffusion method was used to investigate the antimicrobial properties of the extracts as described by

Schumacher *et al*. (2018). All media were prepared and sterilised according to the manufacturer's instructions. Within 15 minutes of adjusting the turbidity of the inocula suspension, a sterile swab stick was used to inoculate the inocula onto the dried surface of sterile prepared Mueller Hinton agar plates. In each case, streaking was repeated two more times, rotating the plate approximately 60° each time to ensure an even distribution of the inoculums. The inoculated plates were allowed to stay for about 3-5 minutes for the surface of the agar to air-dry. While the plates were drying, four various concentrations of the extracts were prepared. A sterilized cock borer of an internal diameter of 4mm was then used to punch five holes in the inoculated medium, the bottom of the wells were then closed using 1 ml of sterile Mueller Hinton agar and the various concentrations of the Prepared extracts were dispensed into the respective labeled holes (100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml). 1 mg/ml of Ciprofloxacin was used as a positive control. Four plates were made for each test organism, and the procedure was repeated for the other organisms. The plates were kept in a refrigerator for about 4 - 5 hours for complete diffusion of the extracts and incubated at 37°C for 24 - 48 hours. After the incubation period, the diameter of each zone of inhibition was measured with a handheld vernier calliper in mm and the results were recorded.

Determination of minimum inhibitory concentration (MIC)

The results of antibacterial activity were used for the determination of MIC. Using the single serial dilution method using dimethylsulphuroxide (DMSO) to obtain a stock of 200 mg/ml. the following concentrations of 100 mg/ml, 95 mg/ml, 90 mg/ml and 85 mg/ml were prepared, but depending on the least concentration that had reasonable activity against the test organisms. If reasonable activity starts from 50 mg/ml then the MIC concentrations start from 50, 45, 40 and 35 mg/ml. The test organism was standardised using 0.5 McFarland standards. Six test tubes were used; each test tube was levelled according to concentration, while the last two test tubes served as positive and negative controls. 2 ml of nutrient broth was dispensed into sterile test tubes and autoclaved at 121ºC for 15 minutes. The following volumes (1 ml, 0.95 ml, 0.9 ml, and 0.85 ml) were removed from the autoclaved nutrient broth and replaced with appropriate concentrations from the stock solution. One hundred microliters (0.1 ml) of standardised organisms were added to five test tubes, respectively. Tubes containing broth and extracts serve as positive control, while tubes containing broth and inocula serve as negative control. This procedure was repeated for the remaining extracts and test organisms. The tubes were observed after 24 hours of incubation to determine minimum inhibitory concentration, i.e the lowest concentration that showed no evidence of growth or turbidity (Belanger *et al.,* 2021).

Determination of minimum bactericidal concentration (MBC)

Mueller-Hinton agar was prepared, and the petri plates were separately inoculated with a sample from each of the test tubes that showed no evidence of growth. The plates were further incubated at 35 ºC for 24 hours and observed. The least concentration at which the organism did not grow was taken as the minimum bactericidal concentration (Iotsor *et al*., 2019).

RESULTS

Table 1 shows the physical properties of garlic extracts. Garlic extract was a golden-yellow, sticky residue with a harsh, disagreeable scent. The initial weight of the garlic was 25 g each, and after the extraction process, the weight of the extract yield was 0.9 g in the ETH, and 0.6 g in the CC14. The percentage yield of the ethanol garlic extract shows the highest amount of 3.6% and the garlic chloroform extract with 2.4%. The higher percentage yield of the ethanol garlic extract compared to the chloroform garlic extract could be due to the higher polarity of ethanol, which allows it to extract more bioactive compounds from garlic than chloroform.

Table 2 shows the phytochemical constituents found in both the ethanol garlic extract and the garlic chloroform extract. The (+) in the table indicates the presence of the phytochemical constituent in the extract, while the (-) indicates the absence of the phytochemical constituent in the extract. That is, tannins, saponins, and alkaloids were present in the ETH. Garlic and absent in the CC14. Garlic. Flavonoids, reducing sugar, steroids, balsams and terpenoids were absent in both extracts, while cardiac glycosides and volatile oil were present in both extracts.

Table 3 shows the zone of inhibition of 16.60 mm at the 100 mg/ml concentration for the garlic ethanol extract, measured in millimetres, with *Staphylococcus aureus* having the highest zone of inhibition and *Escherichia coli* having the lowest zone of inhibition of 4.00 mm across concentrations ranging from the lowest 12.5 mg/ml to the highest 100 mg/ml. This differential activity is likely due to the protective outer membrane of Gram-negative bacteria, which limits penetration of phytochemicals present in the extract, or intrinsic resistance mechanisms in *E. coli*.

Table 4 shows the zone of inhibition for garlic chloroform extract measured in millimetres with *Citrobacter spp.* Having the 6.30 highest zone of inhibition, *Escherichia coli* and *Staphylococcus aureus* had the 4.00 mm lowest zones of inhibition. This differential effect is likely due to a combination of bacterial intrinsic resistance mechanisms (outer membrane permeability, efflux) and the physicochemical properties of the chloroform-extracted constituents (nonpolar, poor agar diffusion).

Table 5 shows the minimum inhibitory concentration (MIC) of ethanol and chloroform of garlic extracts on test organisms. It presents zero growth of *Citrobacter spp.,*

Table 1. Physical properties of the garlic extracts.

Characteristics	ETH. Garlic	CC14. Garlic
Weigh	25 g	25 g
Odour	Harsh scent	Harsh scent
Volume of solvent	250 ml	250 ml
Weight of extract yield	0.9 g	0.6 g
Percentage yield	3.6 %	2.4 %

Keys: ETH. Garlic= Garlic ethanol extract, CCl4. Garlic = Garlic chloroform extract.

Table 2. Phytochemical constituents found in garlic extracts.

Phytochemical	ETH. Garlic	CC14. Garlic
Tannins	+	-
Flavonoids	-	-
Saponins	+	-
Cardiac glycosides	+	+
Reducing sugar	-	-
Steroids	-	-
Alkaloids	+	-
Volatile oil	+	+
Balsams	-	-
Terpenoids	-	-

Keys: ETH. Garlic= Garlic ethanol extract, CCl4. Garlic = Garlic chloroform extract, + = present and - = absent.

Table 3. Zone of inhibition for the garlic ethanol extract, measured in millimeters.

Test organism	Concentration/Zone of inhibition				
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Control
Citrobacter spp.	4.00	4.00	4.00	4.00	30.60
Escherichia coli	4.00	4.00	4.00	4.00	4.00
Staphylococcus aureus	16.60	13.59	4.00	4.00	4.00
Pseudomonas aeruginosa	4.00	4.00	4.00	4.00	4.19

Key: mg/ml = milligram per mil.

Table 4. Zone of inhibition for garlic chloroform extract, measured in millimeters.

Test organism	Concentration/Zone of inhibition				
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Control
Citrobacter spp.	6.30	4.00	4.00	4.00	25.19
Escherichia coli	4.00	4.00	4.00	4.00	4.00
Staphylococcus aureus	4.00	4.00	4.00	4.00	4.00
Pseudomonas aeruginosa	5.63		4.00	4.00	15.21

Key: mg/ml = milligram per mil.

Escherichia coli, Pseudomonas aeruginosa and *Staphylococcus aureus,* giving an MIC of 50 for the ethanolic garlic extract. Zero growth of *Escherichia coli, Staphylococcus aureus* and *Citrobacter spp., Pseudomonas aeruginosa* gives an MIC of 100 for the chloroform ginger extract.

Table 6 shows the minimum bactericidal Concentration (MBC) of ethanol and chloroform extract of garlic on test organisms. It presents zero growth of *Citrobacter spp., Escherichia coli* and *Pseudomonas aeruginosa*, and

Table 5. Minimum inhibitory concentration (MIC) of ethanol and chloroform extracts of garlic on test organisms.

Test organism	ETH. Garlic	CC14. Garlic
Citrobacter spp.	ND	100
Escherichia coli	ND	ND
Staphylococcus aureus	50	ND
Pseudomonas aeruginosa	ND	100

Keys: ETH. Garlic= Garlic ethanol extract, CCl4.Garlic = Garlic chloroform extract and ND = Not detected.

Table 6. Minimum bactericidal concentration (MBC) of ethanol and chloroform extract of garlic extracts on test organisms.

Test organism	ETH. Garlic	CC14. Garlic
Citrobacter spp.	ND	100
Escherichia coli	ND	ND
Staphylococcus aureus	100	ND
Pseudomonas aeruginosa	ND	100

Keys: ETH. Garlic= Garlic ethanol extract, CCl4.Garlic = Garlic chloroform extract and ND = Not detected.

Staphylococcus aureus gives an MBC of 100 for the ethanolic garlic extract. Zero growth of *Escherichia coli, Staphylococcus aureus,* while *Citrobacter spp.* and *Pseudomonas aeruginosa* give an MBC of 100 for the chloroform ginger extract.

DISCUSSION

The findings of this study show that solvents with high polarity yielded higher bioactive compounds. This work correlates with the findings of Wolde *et al.* (2018), who reveal that the higher the polarity of a solvent, the greater the amount of bioactive compound to be obtained.

The outcomes of the phytochemical screening of ethanol and chloroform extracts of garlic indicated that the majority of the phytochemicals were slightly present in the ethanol and chloroform extracts of garlic. Only garlic ethanol extracts contain tannins, but absent in the chloroform extract. The study findings show that the garlic ethanol and chloroform extracts contain no flavonoid, which is known to act as a potent barrier against bacterial infection (Namadina *et al.,* 2021). Ethanolic extract of garlic was found to contain saponins. High levels of saponin were shown to have structure-dependent therapeutic effects (V'kovski *et al.,* 2021). The garlic ethanol and chloroform extracts contain cardiac glycosides and volatile oil content. There was no reducing sugar in any of the extracts. Both the garlic ethanol and chloroform extracts contain no steroids. The ethanol extract of garlic contains alkaloids. Many alkaloids derived from medicinal plants exhibit biological activities such as antimicrobial, cytotoxic and pharmacological effects (Thawabteh *et al*., 2019).

In the present investigation, the garlic extracts, which showed less activity at only higher concentrations among the test organisms, *Staphylococcus aureus* and *Citrobacter sp.,* were most susceptible then followed by *P. aeruginosa,* which showed the least susceptibility to garlic chloroform extract. *E. coli* was found to be resistant to all four extracts, and this could be related to the fact that the lipopolysaccharide (LPS) layer of gram-negative bacteria in the outer membrane has a high hydrophobicity, which acts prolly as a strong barrier against the bioactive molecules. Certain molecules can pass through the cell wall of Gram-positive bacteria more easily than the Gram-negative bacteria because the cell wall of Gram-positive bacteria contains only peptidoglycan (Ruhal and Kataria, 2021). Similar finding was obtained from other researchers (Akrayi, 2014; Ababutain, 2011; Keskin and Toroglu, 2011), where they found that many extracts of spices and herbs did not have antibacterial activity against *E. coli* tested in their studies.

The least activity of garlic observed in this study was in disagreement with earlier reports that garlic is highly effective against microorganisms (Belguith *et al.,* 2010; Yin *et al.,* 2002; Bakht *et al.,* 2011). The resistivity might be a result of the heat applied during the evaporating phase of the extracts' filtrates in a water bath, which may have caused the denaturing of some bioactive compounds in the garlic used. According to Gupta and Ravishankar (2005), commercial garlic showed antimicrobial activity only at 4°C and 8°C, indicating that the antimicrobial activity of garlic is temperature dependent. Apart from temperature, it is also believed that the geographical

location of a plant, temperature, and seasonal variation of an area may have an influence on the yield of medicinal plants (Pant *et al.*, 2021). Hence, the low or no inhibition zones observed.

However, the study findings demonstrated that compared with all the extracts and ciprofloxacin as the control drug, it had a higher zone of inhibition, producing 30 mm against all the test isolates, with the exception of *Staphylococcus aureus,* which showed resistance to Ciprofloxacin. Ginger ethanol and chloroform extracts performed better than Ciprofloxacin on *Staphylococcus aureus.*

Finally, the susceptibility screening of *Citrobacter spp, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa,* on the extracts was further evaluated in order to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). All the results obtained are within the range of 12.5 to 100 mg/ml.

Conclusion

This study found that the ethanol and chloroform extracts of garlic (*Allium sativum*) displayed inhibitory action against test organisms. The garlic extract could be regarded as a potential antibacterial agent with therapeutic potential in the treatment of bacterial infections.

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CONFLICT OF INTEREST

The authors declare no conflict of interest exists.

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