

Phytochemical Screening and Antibacterial Properties of Ethanol and Aqueous Extracts of *Allium sativum* (Garlic) on *Staphylococcus aureus* (ATCC 9144), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC BAA 1705)

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Abstract: The misuse of antibiotics has led to antibiotic resistance which is a major challenge in controlling of infectious diseases despite efforts towards discovery and production of new antibiotics. The search for novel antimicrobial agents to combat pathogens has become crucial. This study aimed at investigating antibacterial activity of ethanol and aqueous extracts of garlic (*Allium sativum*) against typed cultures of *Staphylococcus aureus* (ATCC 9144), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC BAA 1705). Qualitative phytochemical composition of the extracts were determined according to standard procedures, while agar well diffusion method was used for antibacterial assay. The results of phytochemicals revealed the presence of tannins, terpenes, flavonoids, anthraquinones, alkaloids, sterols, glycosides, while phenols and reducing sugars were absent. The two extracts at concentration of 20 mg/ml showed activity against the three clinical isolates with minimum inhibitory concentration (MIC) between 0.1325 mg/ml and 5 mg/ml and minimum bactericidal concentration (MBC) between 0.625 mg/ml and 10 mg/ml. Ciprofloxacin used as the standard drugs showed activity at 250 mg/ml. The findings therefore, has confirmed the use of garlic in treatment of infectious diseases in folklore.

Key word: *Allium sativum*, Garlic, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

INTRODUCTION

Antimicrobial resistance (AMR), the consequence of the evolutionary response and evasive strategies which bacteria exhibit in response to external insult, such as exposure to antibiotics necessitate the search for alternative antibacterial agents from other sources with lead compounds that are effective and safe in humans and animals (Reygaert, 2018; Circella *et al.*, 2022). Medicinal and aromatic herbs are loaded with demonstrable therapeutic properties and are therefore, used in traditional practice for treatment of various ailments (Mann *et al.*, 1997). Scientific evidences on medicinal plants indicate that phytochemical compounds with promising medicinal properties capable of producing physiological changes in human bodies can be developed to solve health problems ((Dhiman *et al.*, 2012). Many plants have been used to treat infectious diseases

because of their antimicrobial constituents, which are synthesized during secondary metabolism of the plants (Nabeel *et al.*, 2014).

Garlic (*Allium sativum*) has been used as a spice in cooking worldwide especially in Italy and Southeast Asia and has its origin from Central Asia and grown in India and China, but presently grown all over the world. More importantly, garlic has been an ingredient in folk and traditional medicine since ancient times (Iotsor *et al.*, 2019; Bhatwalkar *et al.*, 2021). Garlic (commonly called ‘aayu’ in Yoruba, ‘ayo-ishi’ in Igbo and ‘tafarunua’ in Hausa) in Nigeria, is a perennial bulb-forming plant that belongs to the genus *Allium* in the family Liliaceae (Alli *et al.*, 2011) along with onions, chives and shallots (Harris *et al.*, 2001). It is the largest and most important representative genus of the Alliaceae family and comprises of 450 species which can grow up to 2 feet in height (Lanzotti, 2006). The bulb is the

main part of the plant that is used for medicine (Steven, 2015).

In traditional practice, garlic is used for the managements of cardiovascular diseases, as antibiotics, anticancer and anti-inflammatory. It is also used for treatment of common cold, malaria, cough, lung tuberculosis, hypertension, sexually transmitted diseases, mental illness, kidney diseases, liver diseases, asthma among others (Tessema *et al.*, 2016). Its antibacterial activity is based on allicin, which is produced by enzymatic hydrolysis of alliin after cutting and crushing the cloves (Ross *et al.*, 2001; Onyeagba *et al.*, 2004). The need for continuous development of novel antibiotics with diverse chemical structures and varied mechanisms of actions is important because of the threat pose by antimicrobial resistant microbes and re-emerging infectious agents (Nair and Chanda, 2008; Basavaraja, *et al.*, 2009). This study aims at determining the antibacterial effect of extracts of garlic sold in Abuja, Nigeria.

MATERIALS AND METHODS

Study location: This experimental study was conducted in Department of Microbiology Baze University, Abuja, Nigeria by agar dilution technique. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration of garlic to isolates of *Staphylococcus aureus* (ATCC 9144), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC BAA 1705) was determined using agar dilution method according to Kirby-Bauer (1996).

Collection of plant material: Garlic used in this study was purchased from Deidei Market in Abuja, Federal capital of Nigeria voucher specimen was prepared and deposited in the herbarium and voucher number (NIPRD/H/7981) collected from Herbarium unit of National Institute for Pharmaceutical Research and Development, Idu-Abuja. The garlic sample was minced and dry under room temperature (20-25°C) for two weeks.

Test organisms: The clinical isolates of *Staphylococcus aureus* (ATCC 9144), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC BAA 1705) were obtained from the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.

Preparation of garlic extracts: The aqueous and ethanol extractions of garlic were carried out in the department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development Idu-Abuja as described by Ekundayo and Ezeogu (2006). A 500 g dry weight sample of minced and dried garlic was soaked in 2500 ml of ethanol for 48 hours at 25°C The extract was filtered and dried by evaporation using rotary evaporator (Infitec REV-3000AX Series 07). For the aqueous extract, 500 g of the minced garlic was soaked and boiled for twenty minutes in 800 ml of distilled water. The extract was cooled to 25°C and the supernatant was decanted and centrifuged at 5400 g for 10 minutes. Supernatant was filtered using glass microfibre filter paper and freeze dried. The samples were kept in a desiccator until ready for testing.

Phytochemical screening of the extracts: Qualitative phytochemical screening was carried out to determine the presence of secondary metabolites. Nine phytochemicals were determined using standard methods, which include alkaloids by Singh *et al.* (2004), steroids, glycosides carbohydrates by Sofowora (1993), phenols, terpenoids by Evans (2009), flavonoids Kale *et al.* (2010), anthraquinones Banso and Adeyemo (2006) and tannin by Amorim *et al.* (2008).

Preparation of inocula: Stock cultures of the organisms were maintained on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to Mueller-Hinton broth (MHB) to reactivate the test organisms by culturing overnight at 37°C. The overnight cultures were diluted using freshly prepared MHB and compared with 0.5 McFarland standards to achieve

values corresponding to 1.5×10^8 colony forming unit of bacteria (Mohankumar and Savitha, 2019).

In-vitro Antimicrobial Susceptibility Assay of the Extracts: Antibacterial susceptibility testing of the extracts against the typed cultures was carried out using Kirby-Bauer agar diffusion method (Kirby-Bauer, 1996). Mueller-Hinton agar (MHA) was used for the antimicrobial activity test. The appropriately labeled plates were seeded with 100 μ l of standardized bacterial cultures equivalent to 0.5 McFarland (1.5×10^8 cfu/ml) using micropipette in a biosafety hood. Twenty-three millilitres (23 ml) of sterilized MHA maintained at 45°C was added to the Petri dishes and swirled gently for even distribution of the organisms. Using a sterile metallic cork borer, wells of 6 mm in diameter were bored and labeled appropriately on the agar. The bases of each of the wells were sealed using 20 μ l of molten MHA. Two-fold serial dilution of the extract was performed to obtain different concentrations. A 250 μ l of different concentrations of the extracts were dispensed using sterile micropipette into each well and left in the safety hood for adequate diffusion of the extracts into the agar and then incubated at 37°C for 24 hours. The plates were observed for zones of inhibitions and measured using a translucent meter ruler in millimeter (mm) diameter. The experiment was carried out in duplicates. The control experiments included organism viability control (OVC), extract sterility control (ESC) and media sterility control (MSC). Ciprofloxacin 10 μ g was used as standard drug. All the controls were treated as samples.

Determination of Minimum Inhibitory Concentration (MIC): The broth microdilution method in 96 micro wells titre plates as described by National Committee for Clinical Laboratory Standards (2000) with little modification was used to determine the MIC of the two extracts. Using a micropipette, 50 μ l volume of the extracts was dispensed into first row and the same volume of the sterile MHB was

dispensed into all the wells except the first row. A two-fold dilution was carried out from row 2 by taking 50 μ l of the extract to the next row, mixed well and the serial dilution continued to row 7 where 50 μ l from the wells was discarded away. Again, 50 μ l of 0.5 McFarland of 2 h bacterial culture was added to each well in row 1-7. The rows 8 and 9 were the organism viability and media sterility controls. The microwell plates were sealed and incubated at 37°C for 24 hours. Thereafter, 50 μ l of tetrazolium dye was added into each well and further incubated for 2 h at 37°C and observed for colour change. The microbial growth is indicated with a reddish pink colour change. The concentration of the first well without colour change was taken as the minimum concentration of the dilutions that inhibited the growth of the organisms.

Determination of Minimum Bactericidal Concentration (MBC): The minimum bactericidal concentration (MBC) was determined by sub-culturing the organisms from all the wells that did not show evidence of growth according to the method by Banso and Adeyemo (2006). The mixture was streaked on MHA in duplicates and incubated for 24 hours at 37°C. The plates with minimum concentration of the extract that do not allow the growth of the organisms were considered as minimum bactericidal concentration of the extracts.

RESULTS

The phytochemical screening of both ethanol and aqueous extracts revealed the presence of various constituents. The phytochemical constituents are carbohydrates, alkaloids, tannins, free anthraquinones, terpenes, glycosides sterols and flavonoids and phenols (Table 1).

Table 2 is a summary of the antibacterial activity of ethanol and aqueous extracts of garlic (*Allium sativum*) against *Staphylococcus aureus* (ATCC 9144), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC BAA 1705) were studied. The findings of the test revealed that the two extracts had activity at

20 mg/ml with ethanol extracts having higher antimicrobial potency in the following order; *S. aureus* > *P. aeruginosa* > and *K. pneumonia*, while the standard drug however, had activity at 10 µg/ml. The findings of minimum inhibitory concentrations and minimum bacterial concentrations showed that the extracts had

varied MIC ranging from 5 mg/ml to 0.3125 mg/ml, while the MBC ranges from 10 mg/ml to 10 mg/ml of the extracts (Table 3). The inhibitory capacity of garlic is predominantly due to its bacteriostatic rather than bactericidal properties, as MIC values are generally below the minimum bactericidal concentration (MBC).

Table 1: Qualitative phytochemical constituents of the ethanol and aqueous extracts of *A. sativum*

Phytochemicals	Solvents	
	Ethanol	Aqueous
Carbohydrates	-	-
Alkaloids	+	+
Tannins	+	+
Free anthraquinones	+	+
Terpenes	+	+
Glycosides	+	+
Sterols	+	+
Flavonoids	+	+
Phenols	-	-

Key: + = present, - = absent

Table 2: Antibacterial activity of aqueous and ethanol extracts of *A. sativum* at 20 mg/ml measured as zones of inhibition (mm)

Organisms	Zone of Inhibition (mm)		
	Aqueous	Ethanol	Ciprofloxacin (10µg)
<i>Staphylococcus aureus</i>	20.4±1.08	24.1±2.31	26.2± 0.11
<i>Pseudomonas aeruginosa</i>	19.3±0.47	21.3±0.31	-
<i>Klebsiella pneumonia</i>	13.8±0.54	17.2±0.1	-

Key: - =No activity

Table 3: Minimum inhibitory concentration and minimum bactericidal concentration of the aqueous and ethanol extracts of *A. sativum*

	MIC and MBC of the extracts in mg/ml					
	<i>Staphylococcus aureus</i>		<i>Pseudomonas aeruginosa</i>		<i>Klebsiella pneumonia</i>	
	Aqueous Extract	Ethanol Extract	Aqueous Extract	Ethanol Extract	Aqueous Extract	Ethanol Extract
MIC	1.25	0.1325	2.5	1.25	5	2.5
MBC	2.5	0.625	2.5	1.25	10	2.5

DISCUSSION

The variety of different natural chemical compounds from plants and plant products such as saponins, tannins, alkaloids, terpenoids, cyanoglycosides, flavonoids, stearic acids and essential oils have been found to have antimicrobial properties

(Aboaba and Efuwape 2001; Raji *et al.*, 2019). Therefore, presence or absence of certain phytochemicals could be used to explain some of the biological activity of certain plant extracts. Antimicrobial properties of a number of tannins, flavonoids, alkaloids have been reported.

Not only the antimicrobial properties have been ascribed to plant phytochemicals, but other biological activities including modulation of the immune system have been assigned to these compounds in plants (Vaou *et al.*, 2021). For example, saponins are a special class of glycosides which have soapy characteristics and have been reported to be active antifungal agents (Salhi *et al.*, 2017).

Terpenoids have been found to possess antimicrobial, antiparasitic, antispasmodic, anti-allergenic, anti-inflammatory, immunomodulatory and anti-carcinogenic properties (Tahfa *et al.*, 2024). Steroids are found to possess compounds like sex hormones and can be used for drug production (Dembitsky 2023). The presence of flavonoids, saponins, terpenes, alkaloids, tannins, steroids, glycosides present in the extracts were consistent with those of previous studies (Abdullahi *et al.*, 2014; Aliyu *et al.*, 2017; Iotsor *et al.*, 2019; Muhammad and Idris, 2019). However, phenol and carbohydrates were absent in the extracts. It therefore, suggests that constituents of the plant extracts could serve as source of drugs useful in the chemotherapy of some microbial infections. The findings of this study also correlates with the study by Deresse (2011) who found that garlic extracts exhibited activity against both Gram-negative and Gram-positive bacteria.

The findings of this study correlate with the finding of Gull *et al.* (2012) which found that *A. sativum* extract possessed a broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria Ross *et al.* (2001) *Staphylococcus aureus*, *Pseudomonas aeruginosa* (Kuda *et al.* (2004), *Streptococcus mutans*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Salmonella enterica*, *Klebsiella aerogenes*, Cutler and Wilson (2004), *Vibrio cholerae*, *Mycobacterium tuberculosis*, *Proteus vulgaris*, and *Enterococcus faecalis* (Wallock-Richards *et al.*, 2014). Meriga *et al.* (2012) reported that aqueous garlic extract showed antibacterial activity toward Gram-negative (*K. pneumoniae* and *E. coli*)

as well as Gram-positive (*Bacillus subtilis* and *S. aureus*) strains, while methanolic garlic extract showed antimicrobial activity against all tested strains except *S. aureus*. David *et al.* (2024) also reported activity of garlic, honey and synergistic activities against *Staphylococcus aureus* and *Escherichia coli*. Our study revealed a higher MIC values compared to the studies by Noman *et al.* (2023) which reported MIC of 0.625 mg/ml against multidrug resistant *Klebsiella* species and 0.312 mg/ml against *Salmonella* species.

It has been established that the antimicrobial activity of garlic may vary (Hyldgaard *et al.*, 2012) towards different microorganisms, as the mechanism of microbial susceptibility. The mechanism of action could be inhibition of cell wall synthesis, depolarisation of the cell membrane, inhibition of protein synthesis, inhibition of nucleic acid synthesis or inhibition of metabolic pathways. The method followed may also result in such disparity, as reported in another study in which the antibacterial activity of spices was less evident when the paper disc method was used instead of the agar well assay method (Oyinlola *et al.*, 2024).

The findings of the study indicated that the inhibitory capacity of garlic is due to its bacteriostatic rather than bactericidal properties, as MIC values are generally below the minimum bactericidal concentration (MBC), therefore, could be said that garlic is a growth inhibiting agent (GIA) against these organisms used for the experiment which is in agreement with the findings of Ziarlarimi *et al.* (2011), Mohsenipour and Hassanshahian (2015) and Circella *et al.* (2022). The antimicrobial activity of garlic is mainly attributed to the presence of allicin which was reported to have activity against wide variety of microorganisms including antibiotic-resistant, Gram-positive and Gram-negative bacteria (Batiha *et al.*, 2020).

In the present study, ethanol and aqueous extracts were tested against typed cultures of *Staphylococcus Aureus* (ATCC9144), *Pseudomonas Aeruginosa* (ATCC 27853)

Klebsiella Pneumoniae (ATCC BAA 1705). The two extracts showed varied, but broad spectrum antibacterial activities against the three organisms at 20 mg/ml of the extracts. The control drug used for this study was ciprofloxacin at 10 µg/ml. The standard drug only had activity against *S. aureus* and no antimicrobial effect on *P. aeruginosa* and *K. Pneumoniae*. The antimicrobial effect of the ethanol extract was observed to be higher than that of the aqueous extract for the three organisms. This correlates with the findings of Deresse (2011) who reported that the garlic extracts exhibited activity against both Gram-negative (*E. coli*, *Salmonella* sp, and *Citrobacter Enterobacter*, *Pseudomonas*, *Klebsiella*) and Gram-positive (*S. aureus*, *S. pneumoniae*, *Streptococcus* and *Bacillus anthrax*) due to presence of some phytochemicals such as saponin and tannin. Ali *et al.* (2018) reported the activity of ethanol and aqueous extracts of *Allium sativum* against the two organisms. Chinedu (2019) reported that *S. aureus* and *P. aeruginosa* were resistant to ethanol extracts, but sensitive to aqueous extracts of *Allium sativum*. The study of Mohammed *et al.* (2021) also revealed that both aqueous and 70% ethanolic extracts of garlic had activity against methicillin resistant *S. aureus* at concentration of 25%.

The minimum inhibitory concentration of aqueous extract was between 1.25 mg/ml and 5 mg/ml, while that of ethanol extracts was between 0.1325 and 2.5 mg/ml. This is indicating that the ethanol extracts had higher antimicrobial activities compare with aqueous extracts. This is lower than the MIC from the work of Okiemute *et al.* (2020) that revealed that ethanol extracts of *A. sativum* and *Zingiber officinale* had activities against *S. aureus* with MIC of 25 µg/ml and 12.5 µg/ml respectively and Garba *et al.* (2013) with activity of aqueous and methanol extract of *A. sativum* with MIC of 100 mg/ml and 50 mg/ml respectively against *E. coli* and for *S. aureus* it was 200 mg/ml and 100mg/ml respectively. Similarly, The MIC against *P. aeruginosa* for the aqueous and methanol

extracts was 2.5 mg/ml and 1.25 mg/ml respectively. Also, the MIC of the extracts on *Klebsiella pneumoniae* were 5 mg/ml and 2.5 mg/ml. The finding finding is also in conformity with the report of Mohammed *et al.* (2021) which revealed that ethanol extract is effective compared to aqueous extract of garlic. Tsao and Yin (2001), reported higher MIC values of over 16 mg/ml for drug resistant *Pseudomonas aeruginosa* and ≥ 24 mg/ml *Klebsiella pneumoniae*.

The study showed that the three organisms were susceptible to garlic extracts, making garlic a promising antimicrobial agent. Use of extracts of garlic in complementary and alternative medicine practice, could reduce the burden of drug resistance and hence, reduce the cost of treatment of infectious diseases. This finding is therefore, is very importance to clinical and public health practice especially in the poor countries where people depend on alternative means of treatment because of high cost of orthodox medicine. It is hoped that the findings of this study would lead to the establishment of some new and more potent antimicrobial phytomedicine.

CONCLUSION

The findings obtained in this study showed that ethanol and aqueous extracts of *A. sativum* displayed varying degree of antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*) and *Klebsiella pneumoniae*. This suggests that extracts of *A. sativum* could be beneficial in developing a novel antibacterial agent that could be used in treating infections. This could be of commercial interest to pharmaceutical industries in search of new leads that are cheap with readily available raw materials especially in the present threat of resistance by many pathogenic bacteria. The antimicrobial activity of these extracts may not be unconnected to the phytochemical constituents. The findings of this study therefore, reiterates its use in folklore medicine.

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