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Antimicrobial and Antioxidant Properties of Aqueous Garlic (*Allium sativum*) Extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Authors' contributions

This work was carried out between all authors. Author MJ designed the study, collected the samples and the microorganisms used, executed laboratory experiments, literature review, performed statistical analysis and prepared the manuscript. Authors ONO and OSK were involved with the microbial analysis. Authors ONO and OSK read and corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the antimicrobial and antioxidant properties of aqueous garlic (*Allium sativum*) extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*
Methods: Determination of Proximate Composition, DPPH radical-scavenging activities, qualitative and quantitative analysis of the aqueous garlic extract (AGE) were carried out using standard methods. The antimicrobial activity was evaluated by agar well diffusion method. MIC, MBC and

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other microbial analyses were also determined using standard methods.
Results: The proximate composition of the garlic indicate that they contain carbohydrate (66.8%), oil (2.6%), moisture (14.5%), total ash (1.3%) and protein (14.8%). The Phytochemical analysis of aqueous extract of *Allium sativum* shows the presence of secondary metabolites like tannins, terpenoids, steroids, saponins, phenol, protein and reducing sugar. The amount of total phenol content present was 0.285±0.0226 mg/ml while that of flavonoid content was 28.74±8.23 mg QUE/ml. The DPPH scavenging activity of the extract range from 4.47% to 92.44% for the garlic extract with corresponding to concentration range from 3 to 40 mg/ml while the IC_{50} value was 25.3 mg/ml. Agar well diffusion method was used to determine the antimicrobial activity of the garlic extract. It was characterized by inhibition zones of 25.6±2.4 mm for *S. aureus* and 28.1±1.8 mm for *P. aeruginosa*. *Pseudomonas aeruginosa* was tested against 10 standard antimicrobial agents, of these; chloramphenicol, ciprofloxacin, septrin, pefloxacin, streptomycin, gentamicin, amoxacillin, and sparfloxacin were sensitive while augmentin and tarivid were resistant to the tested organism. *Staphylococcus aureus* were susceptible to pefloxacin, amoxacillin, ciprofloxacin, streptomycin, and septrin. Resistance to gentamicin, ampiclox, zinnacef, rocephin and erythromycin were also observed with the same organism. The minimum inhibitory concentration (MIC) for *P. aeruginosa* was 40 mg/ml while *S. aureus* has a value of 80 mg/ml. The extract has a minimum bacteriocidal concentration (MBC) of 88 mg/ml for *P. aeruginosa* and 104 mg/ml for *S. aureus.* The extract has strong potency against these microorganisms with *P. aeruginosa* being the most susceptible.

Keywords: Antimicrobial activity; antioxidant; aqueous garlic extract (AGE); minimum inhibitory concentration; minimum bactericidal concentration; Pseudomonas aeruginosa and Staphylococcus aureus.

1. INTRODUCTION

Medicinal plants continue to provide valuable therapeutic agents, in both modern medicine and in traditional system, *Allium sativum* have been documented and valued for their spicy and medicinal qualities by many cultures around the globe for many years [1]. Garlic (*Allium sativum L*.) is the edible bulb of the lily (liliaceae) family which is widely used as a spice in flavouring, as foodstuff, condiment and is used by the traditional medicine practitioners in the treatment of bacterial related diseases such as pile, cough and rheumatism. *Allium sativum* for many years have been medicinally used to alleviate tumor, cardiovascular diseases and ageing [1]. It contains aromatic sulphur based compounds which contributes to its odour and taste. Allicin is the key component to which the antimicrobial activity of garlic is attributed; it is a volatile molecule that gives it its characteristic odour. Allicin has also been found to be effective as an anti fungal, antibacterial, antiviral and anti parasitic agent [1,2]. The health properties of garlic depend on its bioactive compounds and especially on phenolic compounds [3,4], which have interesting pharmacological properties and are present in relatively high amounts [5]. The biological responses of garlic have been largely attributed to (i) antimicrobial effect (ii) reduction of risk factors for cardiovascular diseases and cancer, (iii) stimulation of immune function, (iv) enhanced detoxification of foreign compound, (v) hepatoprotection and (vi) antioxidant effect [6].

Staphylococcus aureus is a gram positive bacteria, an important pathogen of humans and animals and is implicated in a wide variety of infections. It is a pathogen of greater concern because of its virulence [7], its ability to cause a diverse array of life threatening infections and its ability to adapt to different environmental conditions. *Pseudomonas aeruginosa* is a gram negative, rod shaped, a sporogenous, and monoflagellated bacterium that has incredible nutritional versatility. Antibiotics are naturally occurring or synthetic organic compounds which inhibit or destroy selective bacteria generally at low concentrations. The increasing resistance of bacteria and fungi to currently marketed antimicrobial agents is becoming a world-wide medical problem [8]. This has lead to the exploration for alternative, safe and efficient antimicrobial compounds from natural resources like plants. In recent years, many bacteria have developed antimicrobial drug resistance, these include but not limited to *Staphylococcus aureus* and most of the *Enterobacteriaceae*, such as *Klebsiella pneumonia* [8]. Statistics indicate that more than 70% of the bacteria causing infections are resistant to at least one of the drugs most commonly used to treat them [8].

Antioxidants are substances that are capable of neutralizing the harmful effects of the reactive oxygen species (ROS) through the endogenous enzymatic defense system. The antioxidant effect of plant is mainly due to phenolic components like flavonoids, phenolic acids and phenolic diterpenes [9]. The antioxidants capacity of phenolic compounds is mainly due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen and decomposing peroxides [10]. Garlic possesses potential health-promoting effects due to its high phenolic secondary metabolite content and is a source of natural antioxidants [11]. Kim et al. [12] show that, the total phenolic acid content of a local garlic cultivar grown near Namhae-gun, Korea was 17.86 mg/kg of dry matter (dm) and the total flavonoid content was 29.95 mg/kg dm. The total phenolic content varied from 3.4 mg gallic acid equivalents (GAE)/g dm to 10.8 mg GAE/g dm in different garlic cultivars grown at four locations in Andalusia, Spain [5]. The content of phenolic compounds in garlic thus varies greatly with agronomic, genetic and environmental factors. It is well known that cultivar is the primary factor that determines this variation [13]. The present study demonstrated the in-vitro evaluation of antioxidant and antimicrobial properties of aqueous garlic extract (AGE) against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

2. METHODOLOGY

2.1 Collection and Identification of Plant Extract

The garlic was purchased from Ikorodu market and was authenticated by Momoh Johnson from Department of Science Laboratory Technology (Biochemistry unit), Lagos State Polytechnic.

2.2 Determination of Proximate Composition

Garlic peel was analyzed for moisture, protein, carbohydrate, and ash in triplicate using standard methods [14].

2.3 Preparation of Garlic Extract

Garlic extract was prepared according to the methods described by Zahira and Al-Delaimy, [15] and Kumar and Berwal, [16], as follows, the garlic bulbs were cleaned with water to remove any adhering soil on their surfaces. 100 g of garlic were taken after removal of their outer skin surfaces and cut into small pieces by sterile scalpel. The small pieces were blended with 200 ml sterile distilled water using sterile blender for 5 min at medium speed. The macerates were filtered using sterile funnel and Whatman filter paper. The filtered extract was used for studies within 4 hours of extract preparation. Two-fold serial dilutions were prepared from the extract previously prepared.

2.4 Qualitative Phytochemical Analysis of *Allium sativum*

Phytochemical analysis for phytochemical constituents were carried out on the aqueous peeled garlic extract using standard phytochemical procedures [17,18].

2.5 Quantitative Phytochemical Analysis of the Aqueous Garlic Extract (AGE)

2.5.1 Determination of total phenol

Total phenol of sample was determined using the modified method of Singleton et al. [19]. The calibration curves of aqueous gallic acid solutions of known concentrations were prepared. Folin– Ciocalteu's phenol reagent (5 ml) and 20% sodium carbonate solution (15 ml) were added to each 1 ml of gallic acid standard solution. The solutions were kept at room temperature for 90 min before measuring their absorbance at 760 nm by UV spectrophotometer. About 0.1 ml aliquot of the extract was prepared and mixed with Folin–Ciocalteu's phenol reagent and 20% sodium carbonate. The mixtures were kept at room temperature for 90 min before measuring their absorbance at 760 nm. For blank, the distilled water was added to replace the standard solution. The total phenolic content in the extracts were calculated from the calibration curve and determined as gallic acid equivalent (micromol gallic acid equivalent per milligram dried weight of crude extract).

2.5.2 Determination of total flavonoid

Flavonoid content of the garlic was determined based on the aluminium chloride calorimetric assay method as described by Edeoga et al. [20]. Approximately 1 ml of extract was mixed with 1 ml of 2% $AlCl_3$ in methanol. The mixture was then diluted with methanol to 25 ml. The absorbance was then read at 415 nm. Blank

samples were prepared from 1 ml of peel extract and one drop of acetic acid and diluted to 25 ml. The absorbance of rutin solutions was prepared from 50 mg. The amount of flavonoids in peel extract in rutin equivalents was calculated by the following formula;

Antioxidant capacity = (Afinal / slope)(Vf / Vs) × r × (Vcup / m)

Where r = dilution factor, Vcup = Volume used for the extraction, m = Mass of initial sample
Vf = Final reaction volume, Vs = Sample volume, Afinal = Final absorbance of sample

2.5.3 DPPH radical-scavenging activity

DPPH radical-scavenging activities of aqueous extracts were determined by the method of Jeong et al. [21]. Different concentrations (0.1–40.0 mg/ml) of samples, ascorbic acid and α-tocopherol (as positive control) were prepared. Each sample or standard solution (80 µL) was mixed with DPPH solution (0.3 mM in methanol, 80 µL). Then, the mixture was shaken vigorously and left to stand for 30 min in the dark. Absorbance of the solution was read at 517 nm against the blank. Controls were prepared in a similar way as for the test group except for the replacement of the test sample with the corresponding extraction solvent. DPPH radical-scavenging activity was calculated by using the following equation:

Scavenging activity (%) = (1 − Absorbance of sample/Absorbance of control) × 100

2.6 Test Organisms

The two bacterial strains used in this investigation were obtained from Microbiology Department, University of Lagos, Nigeria. The organisms are *Staphylococcus aureus*, a Gram-positive bacteria and *Pseudomonas aeruginosa* a Gram-negative bacteria. The microorganisms were maintained at 4℃ on Nutrient Agar slant in the Department of Science Laboratory Technology (Microbiology Unit), School of Pure and Applied Science, Lagos State Polytechnic, Ikorodu, Lagos, Nigeria and fresh subcultures were made before use.

2.6.1 Inoculum preparation

A loopful of isolated colonies was inoculated into 4 ml of peptone water, incubated at 37℃ for 4 hours. This actively growing bacterial suspension was then adjusted with peptone water so as to obtain a turbidity visually comparable to that of 0.5 McFarland standard prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate ($BaCl_2$. $2H_2O$) with 99.5 ml of 1% (v/v) tetraoxosulphate (vi) acid (H_2SO_4). This turbidity is equivalent to approximately 1 x 10^8 colony forming units per ml (CFU/ml).

2.6.2 Antibiotic susceptibility testing

Susceptibility of organisms to different antibiotics were tested using disk diffusion method as described by Kirby-Bauer diffusion technique [22] on freshly prepared Mueller Hinton agar and standardized by the method of National Committee for Clinical Laboratory Standard (NCCLS) 2000 [23] using some selected antibiotics namely: Erythromycin (10 ug), Amoxicillin (30 ug), Ciprofloxacin (5 ug), Gentamicin (10 ug), Ampicillin (25 ug), Streptomycin (25 ug) etc. For each combination of the antibiotics and the bacterial strains, the experiment was performed in triplicate. The bacteria with a clear zone of inhibition of more than 17 mm were considered to be sensitive.

2.6.3 Determination of diameter of zone of inhibition (mm) using agar well diffusion method

Agar well-diffusion method was employed to determine the antimicrobial activity. Eighteen hour broth culture of the test microorganisms were suspended into sterile Nutrient broth. It was standardized according to National Committee for Clinical Laboratory Standard 2000 [23] by gradually adding 9% normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1x10^8 colony forming units per ml (CFU/ml). Nutrient agar plates were swabbed with the 18 hours old-broth culture of respective bacteria. Wells (6mm in diameter and about 4cm apart) were made in each of these plates using sterile borer. The aqueous extracts of the *Allium sativum* was tested as follows; Stock solution from the extract was prepared. About 100 µl of extracts were added into the wells using micropipette and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 37ºC for 18-24 hours for bacterial pathogens. The diameter of the inhibition zone (mm) and diffusion rates were measured. The experiment was repeated thrice, for each replicate the readings were taken in three different fixed directions and the average values recorded.

2.6.4 Minimum Inhibitory Concentration (MIC)

The minimum inhibition concentration (MIC) is defined as the lowest extract concentration that inhibited the growth of the test organisms as indicated by absence of visible turbidity in the tube compared with the control tubes. MIC of plant extracts were determined according to the method previously described by Chung et al. [24]. MIC of the garlic peel extract was assayed using the tube serial dilution method. 100 mg/ml was dissolved in water and diluted with Nutrient broth in two fold serial dilutions in test tubes. An overnight broth culture of the test organism was adjusted to McFarland turbidity standard and 50 µl (0.05 ml) of the cell suspension was added to each of the tubes. The tubes were incubated aerobically at 37℃ for 18 hours.

2.6.5 Minimum Bactericidal Concentration (MBC)

The MBC of the aqueous peeled extract of *Allium sativum was* prepared by modification of the method of Spencer and Spencer 2004 [25]. 0.1 ml aliquots of Samples taken from the non-turbid tubes of the MIC assay tubes were sub-cultured onto Nutrient agar plates. The resulting plates were then incubated aerobically at 37℃ for 24 hours. The lowest concentration of the extract at which no colonies of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were seen was taken as the MBC. The results were compared with that of control using sterilized distilled water. The experiment was performed in triplicate. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates.

2.7 Data Analysis

All analyses were carried out in triplicate and results expressed as mean ± SEM. The data analysis was done using the Graph Pad prism computer software version 6. Student s -test and one-way analysis of variance (ANOVA) were used for comparison. A *P*-value < 0.05 was considered significant.

3. RESULTS AND DISCUSSION

In the present study, aqueous extract of *garlic* were evaluated for exploration of their antimicrobial properties against Gram positive *(Staphylococcus aureus)* and Gram negative bacteria (*Pseudomonas aeruginosa*). Allium foods have been shown to reduce risks and modulate metabolism to favor the prevention of diseases. Bianchini and Vainio [26] reported in their research work that the biological response of garlic have been largely attributed to stimulation of immune function, antimicrobial effect, enhanced detoxification of foreign compounds, hepatoprotection, and antioxidant effect.

The results of the proximate composition of garlic peel are shown on Table 1 below. The result showed that the *Allium sativum* is very high in carbohydrate (66.8%) with oil content of 2.6%. The moisture content was 14.5% while the total ash content and the protein content were 1.3% and 14.8% respectively. The values of the different parameters analyzed from peel garlic were very similar to the values obtained by Gulfraz et al. 2014. In their result, the concentration of carbohydrate was between 67.5 to 68.5%, total protein (17.5 – 17.6%), moisture (9.6 – 10.2%), ash content (0.81 – 0.9%) and oil content (3.6 – 3.8%) were similar to the result obtained in our result [27].

Table 1. Proximate composition of aqueous garlic (*Allium sativum*) extract

Test	Result
Oil content	2.6%
Carbohydrate	66.8%
Protein	14.8%
Total ash content	1.3%
%Moisture	14.5%

Phytochemical analysis of plant material is an important aspect, especially if there are some ethnopharmacological claims on the use of the particular plant to cure some illness.

Phytochemical screening of aqueous extract of *Allium sativum* shows the presence of secondary metabolites like tannins, terpenoids, steroids, saponins, phenol, protein and reducing sugar (Table 2). The presence of these secondary metabolites in *Allium sativum* may be responsible for the antimicrobial activity of the extract. The result is in agreement with the work of Lawal et al. [28] who reported that aqueous garlic extract revealed the presence of alkaloids, saponins, tannins, flavonoids, glycosides, cardiac glycosides, volatile oils and steroids. It has been reported that tannins are responsible for anti-diarrheal activity. Alkaloids are known to possess a lot of pharmacological properties used as antidepressant (morphine), stimulants

(caffine), anaesthetic (cocaine), antitumor (vinblastine), antimalaria (quinine) and amoebicide (emetine) [29,30].

Table 3 shows the total phenolic (0.285±0.02 mg/ml GAE) and flavonoid content (28.74±8.23 g/ml QUE) of aqueous garlic extracts. Phenolic compounds with strong antioxidant properties are prominent components of many food plants, including allium species like onion and garlic which are used to enhance the sensory quality of foods [31]. Researchers have shown that flavonoid is one of the most diverse widely spread group of natural products and probably the most important natural phenolic compound in spices. Amagase et al. [32] findings have shown that garlic bulb contained flavonoids (allixin), essential micronutrients (selenium) and macronutrients such as lectins, that has been shown to exhibit antiperoxide properties in the liver, kidney and heart of rats. Phenolic compounds are considered to be bacteriostatic against such bacteria like *E. coli* and *S. aureus* and also fungistatic [33,34]. These compounds caused swelling of hyphal tips, plasma seeping around hyphae, leaking of plasma, cell wall distortions, abnormal branching or fusion of hyphae surface [34,35]. The biological function of flavonoids includes protection against allergies, inflammatory, free radical scavenging, platelets aggregation and microbes. The presence of saponins and flavonoids explain the reasons why garlic is used traditionally for the treatment of bacterial and related diseases. It has been reported that garlic extract has two main classes of antioxidant components, namely, flavonoids and sulfur-containing compounds diallyl sulfide, disulfide, and allyl–cysteine. Allicin (allyl-2-propenethiosulfinate or diallyl thiosulfinate) is thought to be one of the principal bioactive compounds present in aqueous garlic extract or raw garlic homogenate [32]. When garlic is chopped or crushed, allinase enzyme is activated and acts on alliin (S-allylcysteine sulfoxide) to produce allicin [36]. Allicin has antioxidants properties, which provide the garlic protective role against compound toxicity, by scavenging OH and inhibiting lipid peroxidation [37].

Table 2. Phytochemical screening of aqueous extract of *Allium sativum*

Phytoconstituent	Qualitative abundance
Tannins	++
Terpenoids	++
Steroids	+++
Reducing sugar	+
Protein	+++
Anthraquinones	-
Saponin	++
Anthocyanin	-
Phenol	+

(+) present at low levels, (++) present at moderate levels, (+++) present at high level, (-) absent

Fig. 1 shows, the % DPPH radical scavenging activities of Garlic extract.

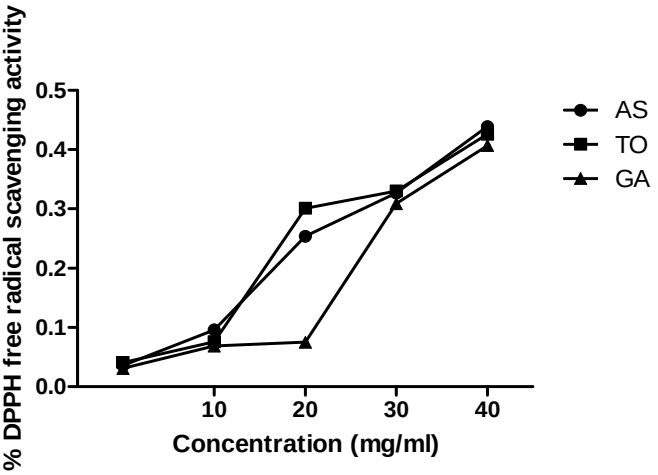

Fig. 1. DPPH radicals scavenging activities of the aqueous garlic extract(AGE), Ascorbic acid (AS) and Tocopherol (TO) at different concentrations. Each value represents mean ± SD (n=3).

DPPH is a stable free radical that accepts hydrogen radical or an electron to become a stable diamagnetic molecule. It is employed as a substrate to evaluate antioxidant activity. The dark colour of the DPPH radical solution becomes lighter when it is mixed with an antioxidant. The degree of discoloration indicated the scavenging potential of the antioxidant garlic extracts in the term of hydrogen or electron donating ability. AGE showed DPPH radical scavenging activities in a dose-dependent manner. The scavenging activity of the extract ranged from 4.47% to 92.44% for the garlic extract with concentrations from 3 to 40 mg/ml. The concentration of sample at which the inhibition percentage reaches 50% is defined as the IC_{50} value. Basically, a higher DPPH radical scavenging activity is associated with a lower IC_{50} value. IC_{50} value was determined from plotted graph of scavenging activity against the different concentrations of aqueous garlic extracts, ascorbic acid (AS) and α-tocopherol (TO). The IC_{50} value obtained was 25.3 mg/ml (Fig. 1).

Table 3. Total phenol and flavonoid content of aqueous garlic extract

Antioxidant assay	Garlic extract (0.5 mg/ml)
Total phenolic content (gallic acid equivalent in mg/ml)	0.285±0.02
Total flavonoid content (mg QUE/ml)	28.74±8.23

Table 4 shows the susceptibility of *Staphylococcus aureus* to some selected standard antibiotics.

Staphylococcus aureus were susceptible to pefloxacin (PEF), amoxacillin (AM), ciprofloxacin (CPX), streptomycin (S), and septrin (SXT), since they have zone of inhibition greater than 17 mm. The organism shows high resistance to gentamicin (CN), ampiclox (APX), zinnacef (Z), rocephin (R) and erythromycin (E). *Pseudomonas aeruginosa* were susceptible to Chloramphenicol (CH), Ciprofloxacin (CPX), Septrin (SXT), Pefloxacin (PEF), Streptomycin (S), Gentamicin (CN), Amoxacillin (AM) and Sparfloxacin (SP), but were resistant to Augmentin (AU) and Tarivid (OFX) as shown in Table 5. The zones of inhibition for the aqueous garlic extract were 25.6±2.4 mm for *S. aureus* and 28.1±1.8 mm for *P. aeruginosa* (Table 6). Fig. 2 and 3 show the zone of inhibition at 500 mg/ml concentration of the aqueous extract of *Allium sativum* against *Staphylococcus aureus* and *Pseudomonas aeruginosa* as described in Table 6.The extract exhibited strong potency against the micro-organisms used with *P. aeruginosa* being the most susceptible. The result of this analysis is similar to the result reported by Iwalokun et al. 2004 [38]. They reported the antibacterial activity of aqueous garlic extract (AGE) by well-diffusion and macrobroth dilution method characterized with inhibition zones of 20.2 to 22.7 mm for gram-positives and 19.8 to 24.5 mm for gram-negatives bacteria against 133 multidrug-resistant gram-positive and gram-negative bacteria isolates. Studies have reported antimicrobial effect of garlic against some enteropathogens [39].

Fig. 2. Zone of inhibition at 500 mg/ml concentration of the aqueous extract of *Allium sativum* against *Staphylococcus aureus*

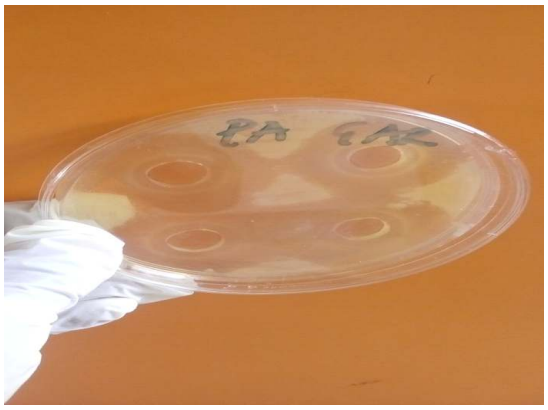

Fig. 3. Zone of inhibition at 500 mg/ml concentration of the aqueous extract of *Allium sativum* against *Pseudomonas aeruginosa*

Minimum inhibitory concentration (MIC) refers to the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation [40]. MICs are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also used to monitor the activity of new antimicrobial agents [40]. MIC of 80 mg/ml was obtained by the aqueous garlic extract on *S. aureus while* 40 mg/ml was observed for *P. aeruginosa.* The extract has a minimum bacteriocidal concentration (MBC) of 88 mg/ml for *P. aeruginosa* and 104 mg/ml for *S. aureus.* The extract has strong potency against these microorganisms with *P. aeruginosa* being the most susceptible. Iwalokun et al. [38] reported MIC ranges of 15.6 to 48.3 mg/mL and 22.9 to 37.2 mg/ml for AGE against 133 multidrug - resistant gram-positive and gram negative bacteria respectively. Saravanan et al. [41] reported the antimicrobial effect of garlic against pathogenic bacterial strains. Due to geographical variation, the MICs of AGE for the organisms tested are relatively lower than values obtained by Ross et al. [42]. This antimicrobial potency disparity of garlic has been attributed to the different concentrations of individually and synergistically active bio- substances in garlic preparations. In 1996, Lawanson reported that allicin and other diallylsulfide compounds have been found at different concentrations in AGE determined by age and method of extract preparation [43].

Table 4. Antimicrobial susceptibility pattern of standard antibiotics against *Staphylococcus aureus*

Antibiotic sensitive disc	Concentration (µg)	Diameter of zone of inhibition (mm)	Interpretation
Pefloxacin (PEF)	10	18.2±1.2	S
Gentamicin (CN)	10	13.5±2.1	R
Ampiclox (APX)	30	14.4±2.9	R
Zinnacef (Z)	20	16.0±1.5	R
Amoxacillin (AM)	30	18.9±1.4	S
Rocephin (R)	30	12.7±2.4	R
Ciprofloxacin (CPX)	10	24.6±2.6	S
Streptomycin (S)	30	23.4±3.2	S
Septrin (SXT)	30	20.5±2.4	S
Erythromycin (E)	10	12.2±2.3	R

Key: S = Sensitive (zone diameter of bacterial inhibition ≥ 18mm)
R = Resistant (zone diameter of bacterial inhibition < 18mm).

Table 5. The susceptibility test result of some selected standard antibiotics against *Pseudomonas aeruginosa*

Antibiotic sensitive disc	Concentration (µg)	Diameter of zone of inhibition (mm)	Interpretation
Septrin (SXT)	30	19.5±2.1	S
Chloramphenicol (CH)	30	21.4±1.4	S
Sparfloxacin (SP)	10	21.2±1.2	S
Ciprofloxacin (CPX)	10	23.5±2.3	S
Amoxacillin (AM)	30	18.5±1.7	S
Augmentin (AU)	30	13.3±1.6	R
Gentamicin (CN)	10	20.3±2.1	S
Pefloxacin (PEF)	30	21.4±1.5	S
Tarivid (OFX)	10	11.5±2.4	R
Streptomycin (S)	30	19.8±2.3	S

Key: S = Sensitive (zone diameter of bacterial inhibition of ≥ 18mm)
R = Resistant (zone diameter of bacterial inhibition < 18mm)

Table 6. Zone of inhibition of aqueous garlic extract against *S. aureus* and *P. aeruginosa*

Test organisms	Concentration (mg/ml)	Zone of inhibition (mm)	Interpretation
Staphylococcus aureus	500	25.6±2.4	Sensitive
Pseudomonas aeruginosa	500	28.1±1.8	Sensitive

Table 7. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous extract of *Allium sativum* against *Staphylococcus aureus and Pseudomonas aeruginosa*

Organism	MIC (mg/ml)	MBC (mg/ml)
Pseudomonas aeruginosa	40	88
Staphylococcus aureus	80	104

4. CONCLUSION

This study revealed that aqueous garlic extract exerted significant ($P<0.05$) antimicrobial properties against *Pseudomonas aeruginosa and Staphylococcus aureus* and might be source of active antimicrobial agent for the development of drugs for the treatment of these infectious microorganisms.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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