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In-vitro assessments of the effects of garlic (*Allium sativum*) extract on clinical isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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ABSTRACT

The main objective of this study was to assess the effects and mode of action of crude preparation of garlic (Allium sativum) on clinical isolates of Pseudonomas aeruginosa and Staphylococcus aureus from Nigeria. An experimental study was conducted in Department of Medical Microbiology & Parasitology, University College Hospital, Ibadan, Nigeria by agar dilution technique. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of garlic to control strains of Staphylococcus aureus ATTC 25923 and to clinical isolates of S. aureus and P. aeruginosa were determined using agar dilution method. All the tested organisms were inhibited by 134mg/ml for P. aeruginosa and 201mg/ml for S. aureus of the crude preparation of garlic except control organism and clinical isolates of S. aureus, which were inhibited by 201mg/ml of crude garlic extract. The study showed that in the absence of the extract, the cells grew to high densities within 1 h 30 min at 37 ^{O}C. Cells treated with garlic extract reduced in number and died. Percentage of viable at 201 mg/ml was 0% for both bacteria. Sucrose and $MgSO_4$ stabilized and protected the cells. At 67, 134 and 201 mg/ml of the extract in the presence of this Sucrose and $MgSO_4$, 47, 4 and 0% of P. aeruginosa cells were viable. Microscopic examination of carbol fuschin and Giemsa stained cells showed that the garlic treated cells were bigger in size than those of untreated ones; and intact and definite nuclei were lacking. The differences could be attributed to the genetic differences among the organisms and differences in the modes of action of the garlic extracts. No isolates were resistant to garlic, making it a promising antimicrobial agent. From the findings, it appears that the cell wall of these test bacteria was the target of attack and the extract was bacteriolytic in action. It also appears that the garlic extract interfere with DNA and RNA syntheses, this could constitute an effective partner in the synergic effect of garlic currently being investigated worldwide. Further studies are also recommended to investigate the detail steps involved in the

mechanism of action of garlic extracts. Crude preparation of garlic could be used as an effective antibacterial agent for the tested bacteria. We hoped that this study would lead to the establishment of some new and more potent antimicrobial drugs from natural origin and native plants. Nevertheless, clinical trial on the effect of garlic is essential before advocating large-scale therapy.

Keywords: Antibacterial activity, Extracts, garlic, Liliaceae, minimum inhibitory concentration (MIC), *Pseudonomas aeruginosa*, *Staphylococcus aureus.*

INTRODUCTION

Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents [1]. Literature reports and ethno-botanical records suggest that plants are the sleeping giants of pharmaceutical industry and provide natural source of antimicrobial drugs that provides novel compounds that may be employed in controlling some infections globally [1]. Different extracts from traditional medicinal plants were tested and some natural products were approved as new antibacterial drugs. However, there is still an urgent need to identify novel substances active against pathogens with higher resistance [1-3].

Garlic belonging to family Liliaceae is well known for having antibacterial effects [1, 4]. Garlic is a perennial bulb-forming plant that belongs to the genus *Allium* in the family Liliaceae [4]. For several centuries, garlic has been known to possess dietary and medicinal properties [5]. Garlic (Allium sativum) has come to be seen as an all rounded treatment for preventing wound infection, common cold, malaria, cough and lung tuberculosis, hypertension, sexually transmitted diseases, mental illness, kidney diseases, liver diseases, asthma, diabetes [6]. *Allium* is the largest and important representative genus of the Alliaceae family and comprises 450 species, widely distributed in the northern hemisphere [7]. These species are characterized by a specific flavour and are used for cooking [8].

For several centuries, garlic has been known to possess dietary and medicinal properties [4-5]. Increased consumption of *Allium* vegetables decreases the risk of gastric cancer possibly because of the effect of garlic on *Helicobacter pylori*, as this organism is associated with gastric cancer [4, 9]. In addition to their nutritional effects, the antibacterial and antifungal activities against the variety of Gram-negative and Gram positive were and continue to be extensively investigated [10]. Garlic (*Allium sativum)* has traditional dietary and medicinal applications as an antiinfective agent [11].

In vitro evidence of the antimicrobial activity of fresh and freeze dried garlic extracts against many bacteria [1, 5, 12-14], fungi [15] and viruses [16] supports these applications. Several studies have proved that garlic has antimicrobial effects [11, 17-23]. It inhibits the growth of both gram-negative and gram-positive bacteria, the same as molds and yeasts [5, 24]. Increased consumption of *Allium* vegetables decreases the risk of gastric cancer possibly because of the effect of garlic on *Helicobacter pylori*, as this organism is associated with gastric cancer [4, 9].

This broad spectrum of activity has been attributed to the over 100 phytotherapeutic sulfur compounds present in varying concentrations in garlic. They include allicin and thiosulfinates,

which are formed by crushing-induced metabolic action of the enzyme allicinase (a cysteine sulfoxide lyase) on the odorless amino acid allicin [25]. Variations in composition of garlic and genetic disparity among bacteria and fungi of the same or different species have been found responsible for the few inconsistencies in the antibacterial and antifungal properties of garlic extract, necessitating the need for local antimicrobial testing of garlic [11, 20, 26]. This study aimed at assessing the antibacterial effect and mode of action of crude preparation of *Allium sativum* (garlic) on bacterial isolates (*Pseudonomas aeruginosa* and *Staphylococcus aureus*) from Nigeria.

MATERIALS AND METHODS

This experimental study was conducted in Department of Medical Microbiology & Parasitology, University College Hospital, Ibadan, Nigeria by agar dilution technique. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of garlic to control strains of *Staphylococcus aureus* ATTC 25923 and to clinical isolates of *S. aureus* and *P. aeruginosa* were determined using agar dilution method.

Collection of plant material

Allium sativum examined in this study were purchased from Bodija market in Ibadan, Nigeria and their scientific names were determined in the herbarium.

Test organism

Standard strains of *Staphylococcus aureus* ATTC 25923 and the clinical isolates of *Pseudonomas aeruginosa* and *Staphylococcus aureus* were obtained from the Department of Medical Microbiology & Parasitology, University College Hospital, Ibadan, Nigeria, sub-cultured on nutrient broth and nutrient agar (Lab M, Topley, England).

Preparation of Garlic extracts

Plant material including peeled bulbs was removed from *Allium sativum* under investigation. All samples were collected from original locations and dried in darkness. The air-dried and finely ground samples were extracted [27-28]. A 5g dry weight sample of different parts of each sample was washed, mined and added adequate amount of sterile distilled water to a concentration of 12.5% (w/v), respectively, then ground in a blender previously surface sterilized with 96.0% ethanol for 90 min. The products were squeezed through gauze cloth to remove the larger particles and the extracts were passed through a 0.2 μm filter (Millipore, Spa, Italy). The procedures of extraction and filtration were operated at room temperature and then the sterilized filtrates were stored at 4°C and used in antibacterial assay.

Antibacterial assays and the mechanism of action of garlic (*Allium sativum)* extract

Antibacterial activity of garlic (*Allium sativum)* extracts was evaluated by diffusion test [29]. Method of Park [30] was used with little modification to determine the mode of action of the garlic at 67, 134 and 201 mg/ml. Minimum inhibitory concentration (MIC) of extracts was determined using twofold dilutions method [31]. For determination of MIC, the plant organs having the highest antibacterial effect in each case were employed. The mechanism of action of *Allium sativum* extract was studied at the minimum inhibitory concentrations of 134mg/ml for *P. aeruginosa* and 201mg/ml for *S. aureus* [27]. A set of eleven tubes received additions as shown

in Table 1. Turbidity of the contents of each tube was measured as optical densities using a spectrophotometer at a wave length of 670nm. Before inoculation, the optical densities of the 18h old cultures were taken at 670nm and it showed 0.635 for *P. aeruginosa* and 0.106 for *S. aureus*. An 18 h old culture at OD_{670} of 0.635 was used. Tubes 1, 2 (a, b, c), 3 and 4 (a, b, c) were incubated at 37°C for 11/2 h and 5 (a, b, c) for 6 h. Contents of each test tube prepared for this experiment are shown in Table 1. Controls were prepared for each tube which excluded the cultures substituted with dimeneralized water. Tubes 2c, 4c, and 5c were not prepared for *P. aeruginosa* (Table 1).

Table 1: Contents of test tubes used for in vitro study on the antibacterial activity of garlic (*Allium sativum)* extract

	Volume (ml)										
Parameters/Tubes	1	2a	2b	2c	3	4a	4b	4c	5a	5b	5e
Double strength nutrient broth (lml)	4	4	4	4	4	4	4	4	4	4	4
2 M sucose (ml)	0	0	0	0	2	2	2	2	0	0	0
0.1ml $MgSO_4$ (0.9ml)	0	1	1	1	0	1	1	1	0	0	0
Garlic extract (ml) (0.0067gl/ml)	0	1	0	0	0	1	0	0	1	0	0
Garlic extract (ml) (0.0201gl/ml)	0	0	0	1	0	0	0	1	0	0	1
Dimineralized water (lml)	4	2	2	2	2	0	0	0	3	3	3
Culture (ml)	2	2	2	2	2	2	2	2	2	2	2
Total volume (ml)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Microscopic examination of wet preparations

Wet samples were prepared by using methylene blue to stain the cells in the medium in the various test tubes in order to observe the cellular structure. The observation was carried out with X40 objective. During the microscopic examination living cells of *Pseudomonas aeruginosa* were differentiated, living from the dead ones. *Staphylococcus aureus* is not motile. Therefore to test the viability of *Staphylococcus aureus* was inoculated (0.1ml) into nutrient agar by pour plating and incubated at 37^0C for 24 h.

Determination of growth of test bacteria

Turbidity of the cultures in the tubes was read at 670 nm. Cells were stained with methylene blue on a glass slide and examined microscopically at x40. Living and dead cells were counted. An aliquot (1 ml) of the *Staphylococcus aureus* culture was pour-plated in nutrient agar and incubated as before in order to determine its viability since the bacterium is not motile (it is not possible to see its motility microscopically).

Test for effect of garlic (*Allium sativum)* extract on cell walls of test bacteria

Formalin (0.2 mL) was added to each tube, left for 5 min and 2 mL of the content was centrifuged at $12{,}168x10^3$ g (MSE Minor 35 Centrifuge) for 15 min. The cells in tubes 1-4 were resuspended in 0.1 ml demineralized water. Smears were made on glass slides, dried and stained with dilute carbol fuschin for 30 sec, rinsed in water, air-dried, examined under the microscope and photomicrographs were taken at x400.

Acid-Giemsa Staining Method of DNA of test bacteria

Dense smear of formalin treated cells in each of the tubes labelled 5 (a, b, c) were made on a coverslip, air-dried and heated in 1 M HCl at 55°C for 10 min. The coverslip was rinsed in tap water for 30 sec. Three millilitres of dilute Giemsa dye was added to a slide and the smeared

coverslip was turned upside down to dip the smear in Giemsa stain for 30 min. It was rinsed in water and blotted dry. Vaseline was applied to edges of the coverslip and placed on a slide. It was observed under oil immersion lens and photomicrographs were taken.

RESULTS

Garlic (*Allium sativum*) extracts possessed antimicrobial activity against the two tested organisms at the minimum inhibitory concentrations (MICs) of 67, 134 and 201mg/ml. Results showed antibacterial activity of garlic (*Allium sativum*) against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) of plant extract was also determined. Table 2 shows the effect of Garlic extract on the growth and viability of *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The spectrophotometer reading at 670nm showed a decrease in turbidity of the contents (cultures) of the test tubes (Tube 1) with introduction of garlic extract and at a higher concentration a much more decrease in absorbance was noted (Table 2). Tube 1 showed the highest absorbance reading. This was followed by Tube 2a and there was subsequent decrease in absorbance reading with introduction of garlic extract for *P. aeruginosa*. In the case of *S. aureu*s, Tube 2a showed the highest absorbance reading. There was decrease in optical density of the culture with increase in the concentration of garlic extract, except for Tube 2a, which showed a higher optical density reading than Tube 1 with no garlic extract (Table 2). All the cells in tubes 1 and 3 were living because there was no garlic extract added to them (Table 2). Table 2 also showed that the number of living cells in Tubes 2, 4 and 5 reduced.

Table 2: Effect of Garlic (*Allium sativum*) extract on the growth and viability of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

	Turbidity Reading [Optical Density (OD_{670})]		Viable cells (%)	
Tube	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
1	0.730	0.318	100.0	100.0
2a	0.615	0.368	39.0	19.0
2b	0.415	0.290	02.0	10.0
2c	NA	0.203	00.0	00.0
3	0.349	2.457	100.0	100.0
4a	0.329	0.186	47.0	25.0
4b	0.210	0.105	04.0	03.0
4c	NA	0.055	00.0	00.0
5a	0.442	0.218	07.0	04.0
5b	0.189	0.139	03.0	01.0
5c	NA	0.039	00.0	00.0

Treatments 1, 2a, 2b, 2c, 3, 4a, 4b, 4c, 5a, 5b and 5c: are as explained in the materials and methods. Tube 1= Contains the broth, culture and dimineralized water. Tube 2= Contains the broth, magnesium sulphate, culture dimineralized water and garlic extract at different concentrations. Tube 3= Contains the broth, sucrose, dimineralized water and the culture. Tube 4= Contains the broth, sucrose, magnesium sulphate, culture and garlic extract at different concentrations. Tube 5= Contains the broth, dimineralized water, culture and garlic extract at different concentrations. NA= not applicable.

Figure 1 shows the effect (mechanism of action) of garlic (*Allium sativum*) extract on the bacterial cells of *P. aeruginosa*. It showed the monograph of bacterial cells treated and untreated

with garlic extract. Numbers 1, 2 (a, b), 3 and 4 (a, b) are number of tubes as recorded in materials and methods (Table 1). Figure 1 shows that the cells in tubes 1 and 3 have intact cell walls. Cells in other tubes (2 and 4) appeared bigger than those in tubes 1 and 3.

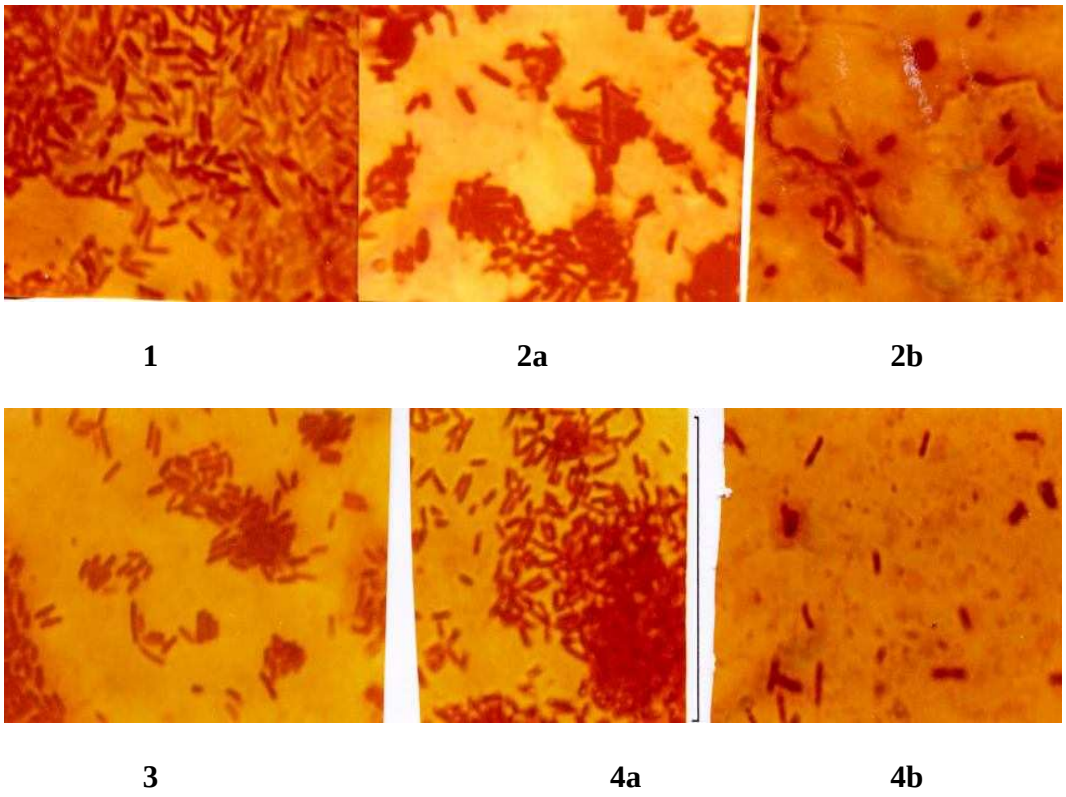


Figure 1: The effect (mechanism of action) of garlic (*Allium sativum*) extract on the bacterial cells of *P. aeruginosa*.

Figure 2 shows the effect (mechanism of action) of garlic (*Allium sativum*) extract on bacterial cells of *S. auerus*. It showed a monograph of bacterial cells treated and untreated with garlic extract. Numbers 1, 2 (a, b, c), 3 and 4 (a, b, c) are number of tubes as recorded in materials and methods.

Figure 3 and 4 shows the effect (mechanism of action) of garlic (*Allium sativum*) extract on nucleus (DNA) of *P. aeruginosa* and *S. aureus* respectively. It shows the acid-giemsa staining of the test organisms. It showed a photomicrograph of cells stained with Giemsa dye. Numbers 5a, b, and c are number of tubes as recorded in materials and methods. Pictures of the dilute carbol fushion cells of the pathogenic microbes showed that there was no difference between the cell wall structures of the organism to which the garlic extract was added and those that did not contain any extract (Figure 3 and 4). The acid-giemsa stained cells from the tubes contain only the culture and the garlic extract at different concentrations without sucrose or magnesium sulphate showed a pink powder like cytoplasmic content and a dot like nucleus containing the DNA which was stained deep blue (Figure 3 and 4). Also, cells in tube 5 have lysed as shown in Figure 3 and 4.

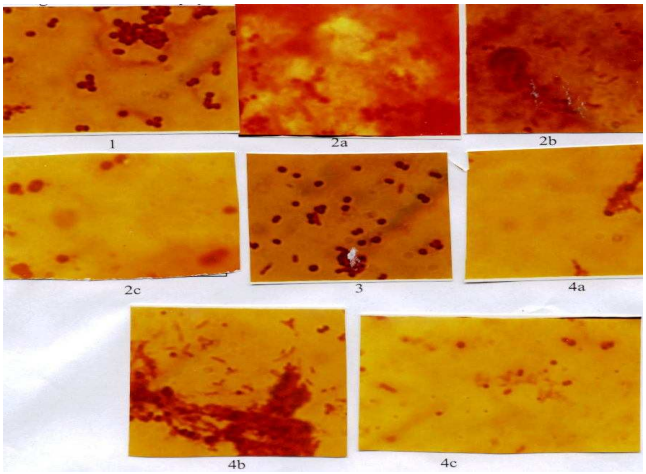
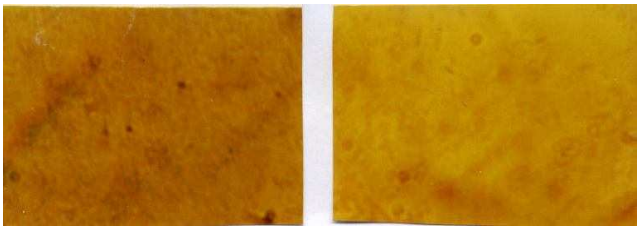


Figure 2: The effect (mechanism of action) of garlic (*Allium sativum*) extract on bacterial cells of *S. auerus*.



5a 5b

Figure 3: The effect (mechanism of action) of garlic (*Allium sativum*) extract on nucleus (DNA) of *P. aeruginosa*. It showed a photomicrograph of cells stained with Giemsa dye. Numbers 5a and 5b are number of tubes as recorded in materials and methods.

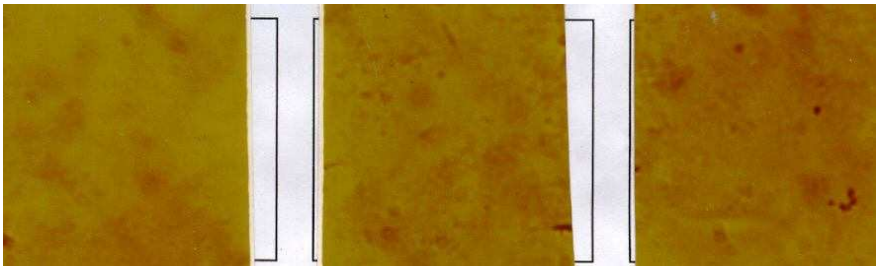


Figure 4: The effect (mechanism of action) of garlic (*Allium sativum*) extract on nucleus (DNA) of *S. auerus*. It showed a photomicrograph of cells stained with Giemsa dye. Numbers 5a, 5b, and 5c are number of tubes as recorded in materials and methods.

DISCUSSION

Results showed that the highest antibacterial activity of *Allium sativum* was against *Staphylococcus aureus*. *S. aureus* was extensively studied and its sensitivity to plant extract is reported widely [1, 32]. The minimum inhibitory concentration (MIC) of plant extract was also determined. The results indicated that the minimum inhibitory concentration (MIC) of plant extracts against the tested organisms was 67, 134 and 201mg/ml. Also, we can regard the plants that have MICs between 67, 134 and 201mg/ml as relatively good antibacterial agents [5, 20-23,

27, 32-34]. Boboye and Dayo-Owoyemi [27] also found out that garlic was effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* at minimum inhibitory concentrations of 161 and 134 mg/ml respectively. *Allium sativum* reduced chronic *Helicobacter pylori* disease at minimum bactericidal concentrations [35]; confirming the killing potential of the garlic extracts on *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Higher number of living cells was observed in tubes 2 and 4 than 5 due to the effect of sucrose and $MgSO_4$ added which were lacking in tubes of number 5 treatment. The sucrose used was meant to protect the cells from lysis and magnesium sulphate stabilizes unprotected membranes [30]. Viable count of *S. aureus* also showed a similar effect with no considerable viable cells in the third plates of each set of the tubes as shown in Table 2. This could be attributed to the action of garlic extract on the test bacteria. This has also been reported by many researchers. Groppo*et al*. [36] reported that the levels of some microorganisms were reduced after treatment with garlic and tea tree oil separately. The difference in percentage of viable cells and turbidity of culture in the tubes indicates effect of increase in concentration of the extract. This pattern of the effect of the garlic is common to the two bacteria used in this experiment.

Cultures of *S. aureus* in tubes numbered 1 and 3 lacking garlic (*Allium sativum*) extract showed considerable growth while those with the extract at concentration 201mg/ml did not show any growth. The nature of the cell wall of gram negative bacteria (*P. aeruginosa*) may be responsible for this observation. However, the cell wall of gram-negative bacteria has been reported to contain 15.0-20.0% polysaccharides and 10.0-20.0% lipid, whereas gram-positive bacteria contain 35.0-60.0% polysaccharides and only 0.0-2.0% lipid [4, 37]. Sivam [38] reported that the cell membrane of *Staphylococcus aureus* contains 2.0% lipid.

Monographic pictures of DNA stained bacterial cells showed irregular distribution of cellular particles as shown in Figure 1 and 2. This indicates that cell division and DNA replication have occurred in the cells. Abnormal elongation of cells was not observed. This is because essential oils of plants including that of garlic can interact with microbial cells membranes and inhibit the growth of some gram-positive and gram-negative bacteria [39]. The acid giemsa stain shows the DNA blue in colour in pink cell content background. This however shows that the garlic extract affected the nucleus. The cell wall was also affected after staining with the dilute carbol fuschin. This is similar to the result obtained by Feldberg et al. [40] who reported a partial inhibition of DNA and protein synthesis by garlic extract but concluded that the mechanism of action was likely the complete and almost immediate inhibition of RNA synthesis. This conforms to previous reports. However, the antimicrobial activity of garlic has been attributed to the presence of thiosulfinates (*e.g.*, allicin) whose removal completely renders garlic ineffective against microorganisms [41]. Allicin is obtained by crushing or cutting garlic cloves. The ordorless amino acid, alliin, present in the garlic cloves, is metabolized by the enzyme allinase (a cysteine sulfoxide lyase) to allicin and other thiosulfinates, which besides their antimicrobial effects, produce the characteristic odor of garlic [42]. Allicin acts by totally inhibiting RNA synthesis and partially inhibiting DNA and protein synthesis, suggesting that RNA is the primary target of allicin [4, 40].

Figure 3 and 4 deviates from the findings of previous studies. Allicin, the active ingredient of garlic [41], acts by partially inhibiting DNA and protein synthesis and also totally inhibiting

RNA synthesis as a primary target [40]. Sivam *et al.* [43] noted that garlic has broad spectrum activity, and is known to act synergistically with antibiotics. Similarly, Ciprofloxacin inhibits bacterial DNA gyrase, and thus interferes with DNA transcription and other activities involving DNA [44]. Also, similar to garlic, Amplicillin inhibit cell wall synthesis, it inhibits transpeptidation enzymes involved in the cross-linking of the polysaccharide chains of the bacterial cell wall and also activates lytic enzymes [44]. Feldberg *et al*. [40] reported that there was partial inhibition of DNA and protein synthesis by garlic extract. Clear nuclei were not observed in this work because the cellular contents were released outside the cells (due to cell lysis) and scattered in the medium and thus they were observed as particles in the monographic pictures. Bacteriolytic agents like this garlic extract bind tightly to cellular target of the relevant microorganisms and induce killing by lysis which was observed as a decrease in cell numbers or in turbidity after the agent is added. They inhibit cell membrane and cell wall synthesis [45].

The turbidity which reduced with the introduction and increase in concentration of the garlic extract was due to antimicrobial effect of the garlic extract on the tested organism. Introduction of magnesium sulphate into tubes number 2 and 4 made the organism a little more tolerant to the extract. This was because the compound acts to protect the cell wall by stabilizing it. Sucrose although serve as a source of food for the organism, it is primarily osmotic and thus made the organism to tolerate the extract a little and also protect the nucleaus. Dilute carbol fuschin stained cells of both organisms which appeared intact, made it clear that the extract did not distrupt the cell wall. Bacterial susceptibility to garlic may also depend on structural differences of the bacterial strains. The polysaccharide and lipid contents of the cell wall have an effect on the permeability of allicin and other garlic constituents; this may be responsible for the difference in susceptibility to garlic between gram negative *P. aeruginosa* and gram positive *S. aureus* [4, 9, 43].

Several studies, including those of Rees *et al*. [17] and Reuter et al. [18), had previously demonstrated the antibacterial potency of garlic against enteropathogens such as *Vibrio parahaemolyticus*, *E. coli*, *Klebsiella* spp., *Proteus* spp., and *S. aureus*. In spite of geographical variation, the MICs of garlic for our isolates are consistent with those of Boboye and Dayo-Owoyemi [27] but are relatively lower than values obtained by Ross *et al*. [5], Sivam [38] and Iwalokun et al. [20]. This antimicrobial potency disparity of garlic has been attributed to the different concentrations of individually and synergistically active biosubstances in garlic preparations coupled with their interactions with sulfhydryl agents in culture media. This phenomenon has been used to explain the stronger antimicrobial effect of allicin than garlic oil disulfides [20, 35]. Meanwhile, allicin and other diallylsulfide compounds have been found at different concentrations in garlic determined by age and method of extract preparation [20, 46].

Our results showed that all tested *Allium sativum* had antibacterial activity against tested bacteria. Broad spectrum activity of garlic against gram-positive and gram negative bacteria has been previously reported [4-5, 47]. Many prior reports showed that garlic have considerable antibacterial and antifungal effects [1, 21-23, 28, 48-49]. Our findings is comparable to what was reported by Iwalokun et al. [20] and the results of this study support the use of garlic in health products and herbal remedies in Nigeria. Since the studied plants have edible parts, our finding is important regarding using them as edible medicinal plants. It seems that this ability is due to having Allicine and *Allium* species have been reported to accumulate the higher concentration of

Allicine in their bulbs than other organs [1]. The species studied had different compounds and formulation. The differences could be inferred from genetic differences among the organisms and differences in the modes of action of the garlic extracts. It appears that garlic interfere with DNA and RNA syntheses, this could constitute an effective partner in the synergic effect of garlic currently being investigated worldwide [4].

Garlic has been known for ages to have anti-infective properties against a wide range of microorganisms [20]. Our study has further demonstrated the antimicrobial potency of garlic against local multidrug-resistant bacteria isolates (*Pseudomonas aeruginosa* and *S. aureus*) from Nigeria. The major part of the findings in this study was that raw garlic extract could be a more effective antimicrobial agent than antibiotics currently in use in Nigeria. Garlic mechanism of action is the inhibition of DNA and or RNA syntheses. Secondly, the effect of garlic extract is most pronounced on bacterial pathogens [4]. The absence of resistance to garlic enhances its ability to effectively act against even highly resistant bacterial strains, such as *Pseudomonas aeruginosa*. It therefore appears attractive that antibiotics that affect DNA and RNA syntheses could form an effective combination with garlic. Garlic extract can also cause death of microorganisms through oxidative stress as was demonstrated in *Candida albicans* with concomitant inhibition of both growth and respiration of the yeast [50].

In this study, the mode of action of garlic appeared to differ from one test organism to the other. Since the extract affected the cell wall, there is an indication of the effect of the garlic extract on the nucleus by inhibition of DNA or RNA synthesis. Mercola [50] reported that allicin from garlic blocks the action of bacterial enzymes by reacting with thiols thereby inhibiting the growth of the microbe. It could also be concluded from the findings of this study that the mode of action of garlic extract as an antibiotic is by affecting the nucleus (DNA or RNA synthesis). This study has shown that garlic extract is antagonistic to *Pseudomonas aeruginosa* and *Staphylococcus aureus* by causing their cells to rupture. Further studies are also recommended to investigate the detail steps involved in the mechanism of action of garlic extracts. More studies on the DNA, RNA and protein synthesis is also advocated to know the exact mode of action of garlic extract on pathogenic microbes.

In this study, neither of the isolates of *P. aeruginosa* nor *S. aureus* showed resistant to garlic, thus, making garlic a promising antimicrobial agent. This is part of the search for suitable antibiotics for synergism with garlic, or garlic alone against *P. aeruginosa* and *S. aureus*. No resistance has been reported but more clinical studies need to be done to assess the use of an antibiotic/garlic combination for bacteria that has proved difficult to eradicate. In line with the assertions of Iwalokun et al. [20], complementary and alternative medicine practices with plant extracts including garlic as a means of decreasing the burden of drug resistance and reducing the cost of management of diseases would be of clinical and public health importance in this country. We hoped that this study would lead to the establishment of some new and more potent antimicrobial drugs from natural origin and native plants.

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