Antimicrobial Activity of *Allium sativum* (Garlic) Extract against Some Selected Pathogenic Bacteria

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Abstract: This study investigated the antimicrobial activity of garlic (*Allium sativum*) extract on six pathogenic microorganisms using the agar well diffusion method. These bacteria include; *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Bacillus subtilis* and *Salmonella typhi*. Four different extracts were obtained from the bulbs of garlic (water-soluble and ethanol-soluble extracts). There were zones of inhibitions around the wells which indicate that the organisms were sensitive to both water and ethanol extract of garlic. The result showed that the isolates behaved differently in their sensitivity to the different extracts added to their growth medium. Ethanol extracts of the garlic was absolutely effective against four pathogenic bacteria. *Escherichia coli* and *Bacillus subtilis* were resistant to the extracts. Comparison of the inhibitory activity of the extracts with both gram-positive and gram-negative antibiotics revealed that gentamycin and chloramphenicol has the highest zone of inhibition against the susceptible bacterial strains used. Gram-negative antibiotic discs used recorded significantly higher activity antimicrobial activity against *Pseudomonas aeruginosa* and *Salmonella typhi* when compared to the ethanol and water extracts of the plant. The quantitative and qualitative phytochemical analysis indicates that the extract of *Allium sativum* (garlic) constitutes antimicrobial activity. This investigation indicates that though plant had antimicrobial and greater inhibitory effect thus confirming its use in folk medicine.
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1. Introduction

The use of higher plants and their extracts to treat infection is an old age practice in traditional African medicine. Traditional medicinal practice has been known for centuries in many parts of the world. Numerous plants and herbs are used all over Nigeria by traditional medicine practitioners. It observed that these practices vary from one country to another (Sofowora, 1984). Extracts from the root, bark and leaves of various plants are used in herbal medicine production (Sofowora, 1983, 1984, 1993). It is an established practice that plant extracts are given singly or as concoctions for various ailments. In fact more than 70% of the people living in Nigeria depend on these various forms of concoctions and herbal decoctions for the treatment of some diseases.

Allium sativum, commonly known as garlic is a species in the onion family *Alliaceae* and belongs to the plant order *liliales* (Rocio, 1982). Other members of the garlic family include *Allium cepa* (onion), *Allium ascalanicum* (shallot) and *Allium porrum* (Heeks). Of all the *Allium* species, garlic is the most important (Alli et al., 2011). Garlic is commonly called 'Tantanwa' in Hausa. Therapeutical applications of garlic have been known for many ages. The plants are broadly used as antibiotics and are effective against diabetes, artherosclerosis and cancer (Augusti 1996; Chiba *et al.*, 1998; Ghazanfari *et al.*, 2002). This plant is also known to reduce blood plasma cholesterol and blood pressure. It also inhibits platelet mass formation (Mayeux *et al.*, 1997).

Research by Moore and Atkins (1977) had revealed that garlic stimulates the activity of the defensive cells of the body such as the lymphocytes and macrophages. These blood cells protect us from pathogens. They are also able to destroy cancerous cells in the initial stage of cancer formation. Garlic is currently used with some degree of success, as a complement in the treatment of AIDS (Tamer, 2009). It is also active against ascarids and certain oxyuroids which are the most frequent types of intestinal parasites (Willis, 1973). It has been proved by Sofowora (1993) that garlic prevents malignant tumors, especially digestive cancers.

The aim of this study is to determine the antimicrobial activity of *Allium sativum* (garlic) extract on different pathogenic bacteria and to carry out phytochemical screening of the extract so as to evaluate the bioactive constituents responsible for their antimicrobial activity.

2. Materials and Methods

2.1. Collection of Plant Material

The fresh forms of *Allium sativum* (garlic bulbs) were purchased from Aleshiloye market, Ibadan.

2.2. Collection of the Test Organisms

The test organisms used in this research consisted of two Gram-positive and four Gram-negative bacteria isolates obtained from the Department of Microbiology, University of Ibadan. Microorganisms used to assess the antimicrobial properties of the plant extracts are as shown in Table 1. The test organisms were cultured on agar slants and stored in the refrigerator at 4°C. Subcultures were made at two-week intervals.

Table 1: Microorganisms used to assess the antimicrobial properties of *Allium sativum* (Garlic) extracts

Microorganisms	Relevant Properties
Staphylococcus aureus	Gram-positive bacteria
Bacillus subtilis	Gram-positive bacteria
Proteus mirabilis	Gram-negative bacteria
Pseudomonas aeruginosa	Gram-negative bacteria
Escherichia coli	Gram-negative bacteria
Salmonella typhi	Gram-negative bacteria

2.3. Preparation of Water Extract of *Allium sativum* (Garlic)

The method of Olayemi and Opaleye (1999) was adopted for the extraction of the plant. This was carried out by measuring 20g of each of fine grounded powder of bulb of *Allium sativum* on an electronic weighing balance. This was dispensed into two beakers, each containing 80ml of distilled water. These were soaked for 72hours after which the solution was carefully filtered with muslin cloth into a sterilized conical flask of 100ml and the filtrates obtained were stored in the refrigerator at a temperature of 4°C until required.

2.4. Preparation of Ethanol Extract of *Allium sativum* (Garlic)

This also followed the method in 2.3 above. Twenty grams of each fine grounded powder of *Allium sativum* was dispensed into a beaker containing 80ml of 95% ethanol. They were soaked for 72hours while the resulting supernatant was decanted into a conical flask of 100ml and kept in the refrigerator for further study. The extraction of the plants was carried out according to the method of Olayemi and Opaleye (1999).

2.5. Antibacterial Tests of the *Allium sativum* (Garlic) Extracts

2.5.1. Inhibitory Test for Ethanol Extract of Allium sativum (Garlic)

The agar diffusion method of Olayemi and Opaleye (1999) was used. From the 48 old cultures of the test organisms with the aid of six sterile syringes, 0.5ml of each of the test organisms was inoculated into six different sterile Petri dishes. About 20ml of sterile media was aseptically poured into each dish. The dishes were gently rocked for proper mixture and the nutrient agar was allowed to solidify. Afterwards, wells were dug in the plates with the aid of a sterilized cork borer of 6mm diameter. Five wells were bored on each plate; two wells for the ethanol extract of garlic, two wells for the water extract and the fifth well served as the control. Ethanol was used as the control. With the proper labeling of wells, 0.5ml of each of the ethanol extract of the plant was introduced into the first and third wells while 0.5ml of ethanol was introduced into the fifth well as the control. They were allowed to stand for one hour for proper diffusion and then incubated at 37°C for 24hours. The sensitivity of the test organisms to ethanol and water extracts of the plant was indicated by a clear zone of inhibition around the wells. The diameter of the clear zone (Zone of inhibition) was measured to the nearest millimeter using a transparent ruler. This was taken as an index of the degree of sensitivity of the test organisms to both ethanol and water extracts.

2.5.2. Inhibitory Tests for Aqueous Extract of *Allium sativum* (Garlic)

This also followed the method in 2.5.1 above. 0.5ml of each of the test organism from the 48hour-old culture was poured into six different sterile Petri dishes. About 20ml of sterile media was aseptically poured into each dish. The dishes were gently rocked together for proper mixture and the nutrient agar was allowed to solidify. Afterwards, wells were dug in the plates with the aid of a sterilized cork borer of 6mm diameter. Five wells were bored on each plate; two wells for the water extract of garlic, two wells for the aqueous extract and the fifth well served as the control. Ethanol was used as the control. With the properly labeling of the wells, 0.5ml of each of the water extract of the plant was introduced into the second and fourth well while 0.5ml of ethanol was introduced into the fifth well (control). They were allowed to stand for one hour for proper diffusion and then incubated at 37°C for 24hours. The sensitivity of the test organisms to ethanol and water extracts of the plant was indicated by a clear zone of inhibition around the wells. The diameter of the clear zone (Zone of inhibition) was measured to the nearest millimeter using a transparent ruler. This was taken as an index of the degree of sensitivity of the test organisms to both ethanol and water extracts.

2.6. Antibiotic Sensitivity Test

The Kirby-Bauer method was used to test the bacterial isolates susceptibility to antibiotics. A

sensitive disc impregnated with various broad spectrum antibiotics namely tetracycline, cloxacillin, gentamycin, erythromycin, chloramphenicol and ampicillin were employed to compare and contrast the antimicrobial activities of the garlic extracts. About 2.8 grams of nutrient agar powder was weighed and dissolved into a conical flask containing 100ml of distilled water. The solution was then sterilized in an autoclave at a temperature of 121°C for 15minutesand at a pressure of 151b/sq inch. The sterile agar which was cooled to a temperature of about 50°C was poured into different sterile Petri dishes already inoculated with test organisms. The Petri dishes were rocked gently to allow distribution of the inocula. The Petri dishes were then left for 45 minutes on the laboratory bench to solidify. Thereafter, antibiotic discs were laid on the seeded agar. The plate was incubated at 37°C for the 24hours. Appearance of clear zones around the disc, indicative of inhibition was therefore observed.

2.7. Determination of Minimum Inhibitory Concentration of *Allium sativum* (Garlic)

The minimum bactericidal concentration of the garlic extract was carried out by concentrating the two plant samples into the solvents 95% ethanol and distilled water used. The plant samples were measured differently into 1.3 and 1.6 grams and soaked in 8.7, and 8.4ml of ethanol and water respectively. This was soaked for 72 h while the extract obtained was used to determine the minimum inhibitory concentration of the extracts against the test organisms, using the earlier method mentioned above. The least concentration of the plant extracts that show inhibitory effects on the test organisms was taken as the minimum inhibitory concentration.

2.8. Qualitative Phytochemical Screening of *Allium sativum* (Garlic)

Simple standard chemical tests were carried out for the qualitative phytochemical screening of the extracts. These tests were used to detect the presence of bioactive agents such as the alkaloids, tannins, saponins, cardiac glycosides, phenols and phlobatanins. The phyto-constituents were assayed for using standard methods described by Trease and Evans (1978). The phytochemical analysis was carried out in the Department of Biochemistry, Lead University, Ibadan, Oyo State. Other phyto-constituents assayed include: saponins; tannins; alkaloids; phlobatannins; anthraquinones; cardiac glycosides; Legal test; Lieberman's test; Salkowskis test.

2.9. Quantitative Phytochemical Screening of *Allium sativum* (Garlic)

The quantitative phytochemical screening was carried out to determine the actual amout of the bioactive constituents present in the plant. The bioactive constituents worked on were saponins, tannins, alkaloids and flavonoids.

3. RESULTS ANALYSIS

Table 2 shows the result obtained from the antimicrobial activity of the water and ethanol extracts of the plant sample. *Allium sativum* was observed to be effective against *Proteus mirabilis*, *Salmonella typhi* and *Staphylococcus aureus*. The extracts were found to be ineffective against *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa.* The result showed that ethanol extracts of *Allium sativum* was effective against four of the tested organisms except *Escherichia coli* and *Bacillus subtilis*. Ethanol extracts of the plant were effective against *Escherichia coli* and *Bacillus subtilis* (Table 2).

Table 2: Zone of Inhibition (mm) of Water Extract of the *Allium sativum* (Garlic) on Tested Organisms

Test Organisms	Water extract	Ethanol extract
Escherichia coli	0.00	0.00
Staphylococccus aureus	5.00	9.00
Proteus mirabilis	7.00	6.00
Bacillus subtilis	0.00	0.00
Salmonella typhi	6.00	19.00
Pseudomonas aeruginosa	0.00	7.00

Table 3 shows the zone of inhibition of the antibiotic sensitivity disc on the selected test organisms. *Pseudomonas aeruginosa* and *Salmonella typhi* were observed to be very sensitive to the Gram – negative antibiotic disc. *S. typhi* however showed high degree of sensitivity (16mm and 11mm) to gentamycin and chloramphenicol while the other tested organisms showed variation in their sensitivity to the antibiotic discs. There was no zone of inhibition among the Gram – positive bacteria. The Gram – negative antibiotic disc recorded significantly higher antimicrobial activity against *P. aeruginosa* and *S. typhi* which was very similar to the potency of the water and ethanol extracts of *Allium sativum* as shown in Table 3. This shows that the extracts of the *Allium sativum* possesses antimicrobial compounds which could be used as substitutes for the antibiotics.

Table 3: Antibiograms: Zone of Inhibition (mm) of Antibiotic Discs on Tested Organisms

Pathogens	Zone of Inhibition (mm)									
Gram Negative Bacteria	**NIT**	**AUG**	**NFL**	**TET**	**GEN**	**CIP**	**CHL**	**AMP**	**NAL**	**CEF**
Escherichia coli	0.0	0.0	0.0	0.0	5.0	4.0	0.0	0.0	6.0	0.0
Pseudomonas aeruginosa	0.0	3.0	11.0	5.0	7.0	9.0	6.0	4.0	5.0	4.0
Proteus mirabilis	0.0	0.0	0.0	0.0	6.0	0.0	8.0	0.0	0.0	0.0
Salmonella typhi	3.0	6.0	10.0	6.0	16.0	4.0	11.0	7.0	6.0	5.0
Gram Positive Bacteria	**DRO**	**CEP**	**NFL**	**GEN**	**ERY**	**CIP**	**CLD**	**SEP**	**AMX**	**AMC**
Staphylococcus aureus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bacillus subtilis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Key: NIT= Nitrofurantoin, AUG= Augmentin, NFL= Norfloxacin, TET= Tetracycline, GEN= Gentamycin, CIP= Ciprofloxacin, CHL= Chloramphenicol, AMP= Ampicillin, NAL= Nalidixic acid, CEF= Cefuroxime, DRO= Drovid, CEP= Cephalexin, ERY= Erythromycin, CLD= Clindamycin, SEP= Septrin, AMX= Amoxil, AMC= Amplicox, 0.0= No inhibition.

Table 4 shows the minimum inhibitory concentration of ethanol and water extracts of *Allium sativum* on the tested organism. The results showed that at 1.3g of the ethanol extract was the least concentration that had inhibition zone of 0.70mm against *Salmonella typhi* while the water extract of the same concentration was also effective against *Pseudomonas aeruginosa* (1.30mm) as shown in Table 4.

Table 4: Minimum Inhibitory Concentration of Extracts of *Allium sativum* (Garlic) on tested Organisms

	Minimum Inhibitory Concentration (1.3gm) Zones of inhibition (mm)		**Minimum Inhibitory Concentration (1.6gm) Zones of inhibition (mm)**	
Pathogens	**Water extract**	**Ethanol extract**	**Water extract**	**Ethanol extract**
Escherichia coli	0.00	0.00	0.00	0.00
Staphylococcus aureus	2.20	2.40	5.00	3.00
Proteus mirabilis	0.00	0.00	0.00	0.00
Bacillus subtilis	0.00	0.00	0.00	0.00
Salmonella typhi	2.60	0.70	3.90	1.90
Pseudomonas aeruginosa	1.30	1.30	0.00	3.00

Table 5 shows the result of the phytochemical contents of the water and ethanol extracts of *Allium sativum*. It was observed that all the constituents screened were present in both water and ethanol extracts of *Allium sativum* except saponins and cardiac glycosides (Table 5).

Table 5: Phytochemical Compounds Present in the Extracts of *Allium sativum* (Garlic)

Phyto-Constituents	**Ethanol Extracts**	**Water Extracts**
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Phlobatannins	+	+
Anthraquinones	+	+
Cardiac glycosides		
Legal Test	+	+
Liberman's Test	+	+
Salkowski's Test	+	+

Key: + = Present; - = Absent

Table 6 show the quantitative result of the phytochemical contents of the ethanol and water extracts of garlic. It was observed that all the quantitative phytochemical constituents screened for were present in both extracts.

Table 6: Quantitative Determination of the Common Bioactive Constituents of the Extracts of *Allium sativum* (Garlic)

Bioactive Constituents (%)	Ethanol extracts	Water extracts
Alkaloids	2.81	0.34
Saponins	2.34	1.64
Tannins	5.89	3.14
Flavonoid	1.22	0.89

4. DISCUSSION

The results of this study showed that of all the extracts screened, garlic ethanol extract had a higher inhibitory activity against the test organisms than that of the water extract. This could be as a

result of better extraction with alcohol solvents. The effect of the water extract of this medicinal plant used on these organisms *in vitro* cannot be predicted from this study. This is in conformity with the work of Gomaa and Hashish (2003) in which the inhibitory property of garlic extracts on the growth of some microorganisms including *Salmonella typhi* was reported. They discovered that water extract of garlic produced higher antimicrobial reduction than the ethanol extract of the plant.

The water extracts of garlic did not show any inhibitory effect against *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Also, the ethanol extract of the plant did not produce any inhibitory activity against *Escherichia coli* and *Bacillus subtilis*. Although the reason for this variation is not clear, it could be assumed to be as a result of genetic differences between the plant and microbial strains used in this study. The antimicrobial activity of the garlic extract against *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* has also been reported by other researchers (Sofowora, 1983; Arora and Kaur, 1999; Onyeaba *et al.*, 2004; Ekwenze and Elegalam, 2005).

Furthermore, comparison of the inhibitory activity of the garlic extracts with both Gram-positive and Gram-negative antibiotics revealed that gentamycin and chloramphenicol had the highest zones of inhibition against the susceptible bacterial strains used. Gram-negative antibiotics recorded a significantly higher antimicrobial activity against *P. aeruginosa* and *S. typhi* compared to the ethanol and aqueous extracts of the plant. The quantitative and qualitative phytochemical tests carried out on garlic indicated that it constitutes antimicrobial properties. This is in conformity with the work of previous studies by some other authors.

In conclusion, the result of the present study emphasizes the usefulness of *Allium sativum* (garlic) in the treatment of diseases and the need to enhance its exploitation on this regard. This is particularly of urgent interest when the growth rate of multi-resistant drug strains of bacteria worldwide is considered (Prescott *et al.*, 2005).

5. Conclusion

The excellent performance of both the water-soluble and ethanol-soluble extracts of garlic against the test organisms brought about the suggestion of frequent input of garlic in foods and pharmaceutical products. It is therefore suggested that efforts should be made by pharmaceutical companies towards carrying out more research work especially in the development of new drugs containing more of these biochemical and biologically active compounds which are of natural origin.

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