

ANTIBACTERIAL ACTIVITY OF DIFFERENT CONCENTRATIONS OF GARLIC (*ALLIUM SATIVUM*) EXTRACT ON SOME BACTERIA ISOLATED FROM CLINICAL SPECIMENS

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ABSTRACT

Background: Garlic has been recognized not only as a spice but also as a substance which exhibit antimicrobial properties. There are several reports on the broad spectrum antimicrobial activity of garlic extract against many genera of bacteria, fungi, parasites and viruses. These pharmacological properties have been ascribed to the presence of the bioactive principle (allicin) in garlic. Because many of the microorganisms are susceptible to garlic extract, garlic holds a promising position as a broad-spectrum therapeutic agent **Objectives:** The present study was aimed to determine the invitro activity of garlic extract on some bacterial pathogens. **Materials and methods:** Garlic plants were bought from a popular vegetable market (Farin gada

market) in Jos, Nigeria. The plant identification was done at the Federal college of Forestry, Jos, Nigeria. The garlic extracts were prepared by cold maceration using methanol and distilled water. The antibacterial activity of garlic extracts was determined by agar well diffusion method. The minimum inhibitory concentration (MIC) for garlic extracts was obtained by tube dilution method. **Result:** The antibacterial activity of aqueous and methanol garlic extract revealed *Escherichia coli* as the most susceptible organism with zone diameter of 24.2 ± 14.2 mm. *E.coli* also exhibit highest sensitivity to positive control drug (ciprofloxacin) with zone diameter of 24.6 ± 15.6 mm. *Pseudomonas aeruginosa* appears to be resistance to all the garlic extract except for sundried garlic extract that showed activity at 100mg/ml with zone diameter 2.6 ± 3.6 mm. The MIC of aqueous and methanol extracts were 5.6mg/ml to 40mg/ml and 6.5mg/ml to 15.0mg/ml respectively. **Conclusion:** The present study has demonstrated the antibacterial activity of garlic extract on some bacterial isolates. In addition, the use of garlic for infection and control appears to be justified.

KEYWORDS: Antibacterial activity, minimum inhibitory concentration, garlic extract, *Escherichia coli*.

INTRODUCTION

Garlic (*Allium sativum*) is one of the oldest spices and has been used not only for flavouring, but also as a medicinal herb due to its diverse biological activities. These activities including anti carcinogenic, antiatherosclerotic, antithrombotic, antimicrobial, anti-inflammatory and antioxidant effects ^{[5][33][9][20][23]}. Recently scientists have commenced proper investigation and clinical trials to confirm these activities. However, there are several reports on garlic in the light of its antibacterial, antifungal, antiviral ^{[16][6]}, anticancer.^[29] and antiparasitic properties^{[32][18]}.

Historically, Louis Pasteur was the first to describe the antibacterial activity of garlic (1822–1895), he used garlic juice to treat infections, and Albert Schweitzer (1875–1965), treated amoebic dysentery with garlic ^{[28][4]}. Since the discovery of penicillin by Alexander Fleming in 1928, there was a gradual decline in garlic regarding its medical usage. However, interest in garlic has been revived by the discovery of its antibacterial principle alkenyl thiosulfinates (allicin) by ^[11]. Indeed the antimicrobial activity of garlic has been attributed to the presence of allicin and thiosulphonates ^{[11][31][19]}. Although the precise interaction between allicin and bacteria is not well understood, its antibiotic properties are thought to be attributable to either the oxygen atom or allyl thio moiety released from allicin.^{[27][34]}

It has been found that garlic has high antibacterial activity on a wide spectrum of Gram-positive and Gram-negative bacteria including species of *Escherichia*, *Staphylococcus*, *Klebsiella*, *Proteus*, *Bacillus*, *Proteus*, *Pseudomonas*.^{[3][17][8][13]} Therefore the present study has demonstrated the activity of garlic preparations on bacterial isolates obtain from different sources.

The objective of this study was to investigate the antibacterial activity and minimum inhibitory concentration of different garlic preparations against some selected strains of bacteria.

MATERIALS AND METHODS

Plant source and identification

The test plant *Allium sativum* (garlic) was bought from a popular vegetable market (Farin gada market) in Jos, Nigeria. Identification was done at the Federal college of Forestry Jos, Nigeria.

Preparation of crude extracts

The cloves of *Allium sativum* (garlic) was removed, the seeds were washed and rinsed thoroughly in sterile water. The seeds were chopped into tiny pieces and air-dried for 10-12 days. It was then pulverized using a pestle and mortar. Two hundred (200g) of powdered garlic was measured and dissolved in 800ml of methanol, while the same quantity was dissolved in 2000mls of distilled water in a conical flask and was left for 6-7 days. It was then agitated using mechanical shaker. The resulting suspension was filtered using sterile Whatman filter paper No 1. The filtrate obtained was evaporated at controlled temperature of 60°C to dryness in a water bath and weighed on chemical balance.^{[2][15]} The resulting extract was preserved in a refrigerator.

Phytochemical screening

The phytochemical screening of the crude methanolic and aqueous extract of garlic was carried out by standard protocols.^{[35][36]} at the Department of pharmacognosy Faculty of Pharmaceutical sciences University of Jos, Nigeria.

Source of test organisms

Bacterial isolates were obtained from the following specimens; urine, wound and sputum. The test organisms include *Staphylococcus aureus*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Escherichia coli*, Coagulase negative Staphylococci.

Culture media

Nutrient agar was used for the antimicrobial sensitivity test. And was prepared according to the manufacturers' instruction.

Standardization of inocula

The test organisms were inoculated by transferring representative organisms from fresh culture plate into sterile saline bottle. The mixture was shaken to achieve homogenous suspension. The homogenous suspension was later adjusted to 0.5 McFarland's standard.^[24]

Antibacterial activity of garlic extracts

The antibacterial efficacy of methanolic and aqueous extract of garlic was tested by agar well diffusion method ^[7]. The cultures from the standardized broth were aseptically swabbed on sterile dried Nutrient agar plates using sterile cotton swabs. Wells of 6 mm diameter were bored on the inoculated culture plates using a sterile borer, the base of each hole was filled with molten nutrient agar to seal the bottom of the plate. Aliquots of 100 µl volume of aqueous and methanolic garlic extract of different concentrations were transferred into labeled wells. The wells were also filled with 100 µl positive control (ciprofloxacin 10µg) and distilled water was used as negative control. The plates were incubated at 37°C for 24 hrs and the zones of inhibition were recorded

Determination of minimum inhibitory concentration (MIC)

The minimum inhibition concentration (MIC) is defined as the lowest concentration of extract that inhibits the growth of the test organisms as indicated by the 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 ^[12]. Briefly, dilutions of aqueous and methanol extract of garlic were made using 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 of the cell suspension was added to each of the tubes. The tubes were incubated aerobically at 37°C for 18-24hours. The MIC was indicated by the highest dilution of extract that showed no visible growth of the test organism.

RESULTS

The result of the different concentrations of aqueous garlic extract tested against bacterial isolates as shown in table 1. All concentrations of aqueous extract of room temperature-dried and sundried garlic were active against test bacterial isolates except *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The room temperature garlic extract had more antibacterial activity with a wider zone of inhibition than the sundried garlic extract. *Escherichia coli* was most sensitive to both aqueous extract, while *Pseudomonas aeruginosa* showed a complete resistance to room temperature extract but was sensitive to sundried garlic extract at 100mg/ml concentration (Table 1).

Table 2 shows the antibacterial sensitivity of methanol garlic extract. The sensitivity pattern indicates that *Pseudomonas aeruginosa* was resistant to all the concentrations of methanol garlic extract except the positive control that gave a narrow zone of inhibition.

The phytochemical components of three different extracts (room temperature, sundried and methanol) used in this study were similar as reported in (Table 3).

The aqueous garlic extract and the methanol garlic extract had similar lower minimum inhibitory concentration (MIC) of 7.5mg/ml (Table 4). The methanol garlic extract and room temperature garlic extract did not exhibit activity against *Pseudomonas aeruginosa* as indicated in (Table 4). However, the highest 40.0mg/ml and the lowest 5.6mg/ml MIC were observed in sundried aqueous garlic extract as shown in (Table 4)

Table 1. Antibacterial activities (mm) of aqueous Garlic extract (Room temperature and Sundried)

Test organisms	Garlic extract (Room temperature)			Garlic extract (Sundried)			Control Ciprofloxacin(10ug)
	100mg/ml	50mg/ml 12.5mg/ml	25mg/ml	100mg/ml	50mg/ml 12.5mg/ml	25mg/ml	
<i>E.coli</i>	24.2±14.2	21.0±12.5 8.20±4.7	17.4±11.7	17.0±15.7	16.0±14.7 7.0±6.6	13.4±13.1	24.6±15.6 24.2±13.9 3.6±5.1 16.0± 14.9 13.4±13.1 16.4±14.3
<i>S. aureus</i>	17.6±10.0	14.60±8.7 3.60±4.9	13.0±7.9	6.0±9.6	3.6±8.1 0.0± 0.0	1.8±4.0	
<i>P. aeruginosa</i>	0.0±0.0	0.0±0.0	0.0±0.0	2.6±3.6	0.0±0.0	0.0±0.0	
CoNS	13.8±12.7	11.8±10.8	9.8±9.1	14.8±13.6	11.6±10.8	9.2±8.9	
<i>Klebsiella spp</i>		6.0±5.7			5.2±4.9		
Total	13.4±13.7	12.2±11.8 6.0±5.7	9.8±9.9	20.2±13.2	16.6±12.3 5.6±5.2	12.8±9.2	
	13.80±13.2	11.9±11.4 4.8±5.2	10.0±9.8	12.1±12.8	9.6±11.7 3.6±4.9	7.4±9.6	

Table 2. Antibacterial activities of methanol Garlic extract

Test organisms	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	control
<i>Escherichia coli</i>	16.0±14.8	14.2±13.2	13.0±12.0	6.2±5.7	24.6±15.6
<i>Staphylococcus aureus</i>	21.0±14.0	8.0±9.8	4.0±8.9	2.4±5.4	24.2±13.9
<i>Pseudomonas aeruginosa</i>	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	3.6±5.1
CoNS	16.6±15.3	12.8±11.8	10.0±9.2	6.8±6.3	16.0± 14.9
<i>Klebsiella spp.</i>	13.8±13.4	10.6±10.1	6.8±9.3	4.6±6.4	13.4±13.1
Total	13.5±13.8	9.1±10.6	6.8±9.3	4.0±5.5	16.4±14.3

Table3. Phytochemical components of extracts Of *Allium sativum* (Garlic)

Phytochemical	Methanol extract	Room temperature aqueous extract	Sundried aqueous extract
		Constituents	
SAPONIN	+	+	+
TANNINS	-	-	-
STEROID	+	+	+
GLYCOSIDE	+	+	+
ANTHRAQUINONE	-	-	-
FLAVONOID	+	+	+
ALKALOID	+	+	+
TERPENES	+	+	+

Table 4. Minimum inhibitory concentration of aqueous and methanol garlic extract

Test organisms	Aqueous garlic extract (mg/ml)		Methanol garlic extract
	Room temperature extract	Sundried extract	
<i>E. coli</i>	10.0	7.5	7.5
<i>S. aureus</i>	15.2	25	6.5
<i>P. aeruginosa</i>	Nil	40	Nil
CoNS	7.5	10.0	7.5
<i>Klebsiella spp.</i>	7.5	5.6	15.0

DISCUSSION

The antibacterial activity of garlic is widely attributed to allicin which is an important component of garlic extract. The present study was aimed to determine the antibacterial activity of methanol and aqueous preparation of garlic extract against some bacterial isolates. All the bacterial isolates used for the study were sensitive to aqueous and methanol garlic extract except for *Pseudomonas aeruginosa* which was resistant to the different concentrations of garlic extracts but only inhibited at 100mg/ml concentration of sundried aqueous garlic extract. The reason for this high resistance to garlic extract might be due to the organism natural resistance to antibiotics which is ascribed to the permeability barrier of the

cell membrane. In addition, *P. aeruginosa* has been implicated in most nosocomial infection, hence could acquire resistance trait to a wide range of antibacterial agent including garlic extract.

Regarding the non *Pseudomonas* isolates, the patterns of sensitivity are similar to one another. This can best be explained by the almost similar phytochemical component liberated by the extracting solvent used in the study.

The aqueous garlic extract inhibited test organism within MIC range of 5.6mg/ml to 40mg/ml while the range of MIC for methanol garlic extract was 6.5mg/ml to 15mg/ml. This result is in agreement with the report of ^[1] who asserted that methanol is a better extracting solvent.

CONCLUSION

This study has justified the antibacterial properties of garlic extract. The properties are ascribed to allicin which has widely been reported as the principal bioactive compound present in extracts of garlic. However, a wider scope of study on garlic extract would be useful on improve knowledge on regarding its therapeutic potentials.

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