Original Research Article

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Phytochemical screening and antibacterial potentials of the garlic (*Allium sativum*) extracts against clinical isolates

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Abstract

This study investigated various phytochemicals and antibacterial activity of garlic (*Allium sativum*) bulb extract on five clinical bacterial pathogens using the agar well diffusion method. These bacteria include; *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella sp, Escherichia coli,* and *Salmonella typhi*. Two different extracts were obtained from the bulbs of garlic (ethanol-soluble and methanol-soluble extracts). Phytochemical screening of the garlic bulb extracts indicated the presence of glycosides, steroids, phlobatanins, flavonoid, alkaloids, terpenoids and carbohydrate while saponin, phenolics and tannins were absent. There were zones of inhibitions around the wells which indicate that the organisms were sensitive to both ethanol and methanol extract of garlic having inhibitory strength on the test organisms ranging from 2-32 mm. The MIC and MBC revealed the isolates behaved differently in their sensitivity ranging from ranged from 20 - 76 mg/mL for ethanolic extract, while methanolic extract has ranged from 13 - 62 mg/mL. The methanol extract of the garlic bulb was absolutely more effective against five pathogenic bacteria than the ethanolic extract. This study indicates that *Allium sativum* had great antibacterial effect thus confirming its usage in herbal medicine.

Keywords: *Allium sativum*, antibacterial activity, garlic bulb, clinical isolates, phytochemical screening

1. Introduction

Plants contain numerous biologically active compounds which have the potential for development as medicinal agents. Traditional medicines already form the basis of therapeutic use in the developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world too [1]. Plants provide an alternative strategy in the search for new drugs. There is a rich abundance of plants reputed in traditional medicine to possess protective and therapeutic properties [2]. It is likely that plants will continue to be a valuable source of new molecules which may, after possible chemical manipulation, provide new and improved drugs [3].

Phytochemicals found in plants include alkaloids, glycosides, essential oil, saponins, tannins, steroids, terpenoids, resins, flavonoids, proteins and others [4]. These chemicals are bioactive compounds present in medicinal plant parts and can be used for therapeutic purposes [5]. These inherent bioactive principles differ from plant to plant as a result of their biodiversity and they produce significant physiological effects in human body. Bacterial resistance to antibiotics is a serious challenge, clinicians and the pharmaceutical industries are battling with and intensive studies are ongoing globally to address this challenge. Screening of medicinal plants is one of the most researched area with the hope of getting safer, newer, and more effective agents that can be used to fight infectious diseases [6].

Allium sativum, popularly known as garlic is one of the species of the onion family *Alliaceae* and belongs to the plant order *Liliales* [7]. Among all the *Allium* species, garlic is the most important [8]. Garlic is commonly called 'Tantanwa' in Hausa. Medicinal applications of garlic have been known for many ages [9]. The plants are broadly used as antibiotics and are effective against cancer, arthrosclerosis and diabetes [10, 11]. Garlic has been reported to be effective in reducing blood plasma cholesterol and blood pressure. It also inhibits platelet mass formation [12]. The most active components present inside fresh garlic are alliin and an enzyme called alliinase. Its medicinal claims have included cures for cold, toothaches, coughs and other viral infections, open wounds and evil demons [4]. The aim of this study was to assess the phytochemical profile and antimicrobial activities of ethanolic and methanolic extracts of *A. sativum* bulb.

2. Materials and methods

2.1 Test organisms

The bacterial species used in this study, *Salmonella typhi*, *Escherichia coli, Klebsiella sp*, *Pseudomonas aureginosa*, and *Staphylococcus aureus* were obtained from the Department of Microbiology, University of Ilorin Teaching

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Hospital (UITH), Ilorin, Kwara state. Purity of the cultures was checked at regular intervals as described by Acheampong *et al*. [13].

2.2 Plant collection, identification and processing

Samples of the bulbs were bought at Ipata Market, Ilorin, Kwara State. The plant samples were confirmed at the herbarium unit of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. The fresh samples were sundried for a week, ground into a fine powder and kept in plastic containers until further use at room temperature (28 ± 1 °C). The scales around the fresh bulbs of garlic were removed and the bulbs were washed and rinsed properly in tap and sterile distilled water, respectively. The bulbs were macerated, dried in an electric oven and milled mechanically before being stored in a plastic container.

2.3 Preparation of ethanolic extracts

One hundred grams (10 g) of each of the plant parts (seed, leaf and stem bark) were soaked into 100 ml of the solvent (95% ethanol) in different air-tight sterile jars respectively at room temperature and kept on a shaker (90 rpm) with uniform shaking for 24 hr. The solvents containing the extracts were decanted filtered with a muslin cloth and then with Whatman no. 1 filter paper respectively. Further extraction of the ground samples was done with the same volume of 95% ethanol, decanted and filtered two more times. The filtrates from each round of extraction were combined and were evaporated to dryness in small, open - mouth jars and then packed in separate clean dry bottles and stored at room temperature until required.

2.4 Methanolic Extract

The methanol extract of the garlic bulb was prepared using the procedure described by Ameh et al. [4]. Twenty-five gram (25 g) of the powdered sample was soaked in a mixture of methanol and distilled water in the ratio of 3:2 for four days and later filtered to obtain the methanol extracts. The mixture was first concentrated by evaporation using a water bath at 100°C for 1 hr.

2.5 Sterility of extracts

Each of the extracts was tested for growth of contaminants by using the procedure of Fadiji *et al*. [14]. The plates were observed for growth. Absence of microbial growth in the extract indicated their sterility. Sterile extracts were used to test for antimicrobial efficacy.

2.6 Standardization of inoculums

Standardized inoculums of each tested organism were obtained by making their respective suspension up to 0.5 McFarland standard as observed in the spectrophotometer and as described by Barry *et al*. [15].

2.7 Antimicrobial Assay of the Plant Extracts

Prepared sterile nutrient agar plate was inoculated with standardized organisms of 0.1ml of a day old culture. Glass spreader was used in spreading the inocula evenly on the surface of the agar and excess are drained off. A sterile cork borer of 5 mm diameters was used to make five (5) ditches on the plates. The bacteria were inoculated into nutrient agar with varying concentrations of the extracts 250 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml were prepared from the stock concentration of the extracts. 0.5 ml of each concentration of the extracts was dispensed into each of the ditches on the plates that are appropriately labeled. The fifth ditch in the plates was picked as control by adding 0.5 ml of appropriate solvent use for the different extraction. The plates were done in duplicates and left on the bench for few minutes for the extract to diffuse into the agar and later incubation at 37 ^{0}C for 24 hr. After incubation, the zone of clearance around each ditch was measured using a metric ruler by taking measurement of the zone of clearance around the ditch. The diameter of the cork borer was removed from the diameter of the zone of clearance and this made or represented the antibacterial activity measured or diameter of the zone of inhibition.

2.8 Determination of Minimum Inhibitory Concentration (MIC) of the Extracts

Broth dilution method was used to determine MIC. Varying concentrations of the extracts were used that ranged from 10.0 mg/ml to 100 mg/ml. 0.5 ml of each concentration of the extracts were dispensed to 9 ml of nutrient broth containing 0.5 ml of standardized test organisms (bacteria and fungi) cells. The tubes were incubated aerobically at 37 ^{0}C for 24 hr for bacterial controls were equally set up by using solvents and test organisms without the extract. The tube with the least concentration of extract that does not show growth after incubation was picked as the minimum inhibitory concentration (MIC) [16].

2.9 Determination of Minimum Bactericidal Concentration (MBC)

The culture tubes used in MIC (Minimum Inhibitory Concentration) determination that did not show turbidity or any visible growth after the period of incubation were drawn with a syringe (0.5 ml) and dispense onto the surface of Nutrient agar. The inocula were seeded on the surface of the media. The plates were incubated at 37 ^{0}C. The lowest concentration of the extract that inhibits the growth of the organisms on the plates after incubation was regarded as Minimum Bactericidal Concentration (MBC) [14].

2.10 Phytochemical screening

The phytochemical screening of the garlic bulb extract was carried out according to the procedures described by Trease and Evans [17] and Fadiji *et al*. [14].

2.11 Statistical analysis

The data obtained in this study were expressed as mean ± SEM and subjected to analysis by one-way ANOVA. *p* values of 0.05 or less were considered significant using SPSS software version 17.0.

3. Results

3.1 Antimicrobial effects of aqueous and ethanolic extract of plant samples

The screening for antimicrobial activity of the plant samples used in this study revealed that the plant extracts had varying effects on the growth of the clinical isolates. All the plant extracts have inhibitory strength on the test organisms ranging from 2-32 mm. The methanolic extract of *Alium sativum* had the highest zone of inhibition approximately on all pathogens tested with extracts having 29 mm and 32 mm against *Pseudomonas aeruginosa* and *Escherichia coli* at 250 mg/mL (Table 1). The lowest inhibition was recorded in ethanolic extracts with 2 mm against *S. aureus* at 100 mg/mL.

Table 1. Antimicrobial effects of ethanolic and methanolic extract of *Alium sativum* (garlic) on tested organisms

Name of Organisms	Zone of inhibition (mm)									
	Ethanolic extract					**Methanolic extract**				
	250 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	Control	250 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	control
Pseudomonas Aeruginosa	25 ± 0.01	13 ± 0.17	10 ± 0.03	-	-	29 ± 0.04	17 ± 0.03	13 ± 0.13	-	-
Escherichia coli	26 ± 0.03	17 ± 0.02	-	-	-	32 ± 0.03	22 ± 0.06	13 ± 0.03	9 ± 0.13	-
Salmonella Typhi	19 ± 0.08	12 ± 0.01	9 ± 0.01	3 ± 0.13	-	24 ± 0.05	19 ± 0.11	11 ± 0.19	5 ± 0.01	-
Klebsiella sp	11 ± 0.01	7 ± 0.05	-	-	-	16 ± 0.01	11 ± 0.02	5 ± 0.12	-	-
S. aureus	6 ± 0.03	2 ± 0.02	-	-		13 ± 0.17	8 ± 0.04	3 ± 0.11	-	-
P level (0.05)	***	***	***	***	***	***	***	***	***	***

KEY: - = No Zone of inhibition. Values expressed as mean ± SEM; *** = Means square significant at $p < 0.001$

3.2 Minimum inhibitory concentration and minimum bactericidal / fungicidal concentration of aqueous and ethanolic extracts of plant samples on the tested organism

The MIC and MBC values obtained for the plant extracts on *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella sp*, *S. aureus*, and *Salmonella typhi* varied from one extract to another. The methanolic extracts had the best MIC and MBC on all the clinical isolates tested compared to the aqueous extracts. Both extracts had considerable MIC and MBC values for all the pathogenic bacteria tested. The values ranged from 20 - 76 mg/mL for ethanolic extract, while methanolic extract has ranged from 13 - 62 mg/mL. These concentrations gave bactericidal and fungicidal effects after 24 hr of incubation. They are therefore regarded as the lowest concentrations of the extract sufficient to kill a defined proportion of viable organisms at a specified period (Table 2). All values from the treatments were significantly ($p < 0.05$) different from each other.

Table 2. Minimum inhibitory concentration and minimum bactericidal concentration of both ethanolic and methanolic of extracts of *Allium sativum*

Microorganisms	**Ethanolic Extract**		**Methanolic extract**	
	MIC (mg/mL)	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**
Pseudomonas Aeruginosa	30 ± 0.02	59 ± 0.19	21 ± 0.13	46 ± 0.11
Escherichia coli	45 ± 0.014	68 ± 0.15	33 ± 0.08	52 ± 0.13
Salmonella Typhi	25 ± 0.08	52 ± 0.17	19 ± 0.09	37 ± 0.03
Klebsiella sp	20 ± 0.02	34 ± 0.07	13 ± 0.05	26 ± 0.18
S. aureus	48 ± 0.14	76 ± 0.09	39 ± 0.01	62 ± 0.06
p level (0.05)	***	***	***	***

KEY: - = Not determined, MIC= Minimum Inhibitory concentration, MBC= Minimum bactericidal concentration, MFC= Minimum fungicidal concentration.
Values expressed as mean ± SEM; *** = Means square significant at $p < 0.001$

3.3 Phytochemical analysis of the plant samples

The phytochemical analysis of the plant extracts had shown that the tested plant parts contain some active components. The ethanolic and methanolic extracts of *Alium sativum* showed that it contains glycosides, steroids, phlobatanins, flavonoid, alkaloids, terpenoids and carbohydrate in a tangible amount with more presence in the methanolic extract was observed when compared to the ethanolic extract, while saponin, phenolics and tannins were absent (Table 3).

Table 3. Phytochemical analysis of aqueous and ethanolic extract of *Alium sativum* (Garlic)

S. No.	***Allium sativum* (Garlic)**		
	Chemical constituents	**Ethanolic**	**Methanolic**
1	Alkaloids	++	+++
2	Tannins	-	-
3	Saponins	-	-

4	Phlobatanins	+	++
5	Flavonoids	+	++
6	Terpenoids	+	+++
7	Glycosides	+	+++
8	Steroids	-	+
9	Phenolics	-	-

KEY +++ = More Strongly Present, ++ = Strongly Present, + = Present, - = Absent

4. Discussion

In this study, the five clinical bacterial pathogens were studied to assess the antimicrobial properties of garlic. The findings showed that they were sensitive to the plant extracts. The plant extracts showed varying degrees of antimicrobial activity on the microorganisms. This is in agreement with the work of Rojas et al. [18] on ten medicinal plants.

Phytochemical screening of the garlic bulb extracts indicated the presence of glycosides, steroids, phlobatanins, flavonoid, alkaloids, terpenoids and carbohydrate while saponin, phenolics and tannins were absent. These bioactive compounds found in most plants are believed to be responsible for the observed antibacterial activities of the plant extract. A previous study attributed the antimicrobial effect of plant extract to the presence of these secondary plant metabolites [19]. Similar phytochemical analysis of plant extracts of *Ocimum gratissmum* revealed the presence of alkaloids, flavonoids, cardiac glycosides, steroidal terpenes and tannins [14]. The presence of active compounds in the bulb extract of *A. sativum* could be used to establish its usage in traditional medicine.

The inhibitory activities of the plant extract agree with the report of Leven et al. [20]. Nwadiaro and Nwachukwu [5] linked the antimicrobial activities of plants to the presence of tannins, alkaloids, flavonoids and saponins. It has been reported that various plants that are rich in alkaloids, tannins and glycosides possess antimicrobial activity against a number of microorganisms [21]. This suggests that the plant extract used in this study has a general antimicrobial activity. The antibacterial potential of *A. sativum* bulb extract is more pronounced in the methanolic extract than its ethanolic extracts. This is in agreement with an earlier report of Gomaa and Hashish [22] in which the inhibitory property of garlic extracts on the growth of some microorganisms including *Salmonella typhi* was reported.

The varying inhibition sizes recorded in this study is an indication of the bioactive strength of phytochemicals contained in the garlic bulb. The varying MICs and MBCs of the plant extracts on the test organisms are in agreement with the report indicating that microorganism exhibits a different level of susceptibility to plant extracts [23]. Lower MIC and MBC values were recorded in methanolic extracts as compared with ethanolic extract is an indication that methanolic extract has more antibacterial strength than the ethanolic extracts.

Conclusion

The present study, therefore, shows that *A. sativum* bulb extracts have useful antimicrobial properties. The strong antibacterial activities showcased by both the ethanolic and methanolic extracts of *A.* sativum bulb against the test organisms brought about its likely recommendation for frequent input of garlic in pharmaceutical and foods and products. Structural elucidation of the bioactive compounds is recommended in order to be able to ascertain the actual antimicrobial activity of the various phytochemicals. Also, efforts should be made by pharmaceutical companies towards the development of novel drugs which are of natural origin.

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