



PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF GARLIC EXTRACTS

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ABSTRACT

The antibacterial potency of aqueous and methanol extracts of garlic was determined invitro against three bacterial isolates (Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa) by agar well diffusion method. Preliminary phytochemical screening revealed the presence of tannin, flavonoid, carbohydrate, protein, saponin, alkaloid and glycoside in the plants while anthraquinone was absent. Both the aqueous and methanol extract of garlic were observed to be more potent against E. coli with maximum zone of growth inhibition of 21.5mm at 200mg/ml and 24.0mm at 200mg/ml respectively. The minimum inhibitory concentration (MIC) of the aqueous and methanol extracts of garlic against E. coli was 100mg/ml and 50mg/ml respectively, and for S. aureus it was 200mg/ml and 100mg/ml respectively. Similarly, The MIC against Pseudomonas aeruginosa for the aqueous and methanol extracts was 200mg/ml and 200mg/ml respectively. Higher minimum bactericidal concentration (MBC) of 300mg/ml was observed against P. aeruginosa with aqueous extract and 250mg/ml with the methanol extract. The MBC for both the aqueous and methanol extract was 200mg/ml respectively against E. coli while MBC of 300mg/ml was observed against S. aureus for the aqueous extract and 200mg/ml for the methanol extract. These findings therefore justify the traditional medicinal use of garlic.

Keywords; Phytochemical, Antibacterial, Efficacy, Garlic, Isolates.

INTRODUCTION

In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants to produce cost-effective remedies that are affordable to the population. The rising incidence of multidrug resistance among pathogenic microbes has further necessitated the need to search for newer antibiotics. (Doughari, 2006). Approximately 20% of the plants in the world have been subjected to pharmacological and/or biological evaluation and a substantial number of new antibiotics introduced into the market are obtained from natural or semi synthetic sources (Mothana and Lindequist 2005).

Initial reports of antimicrobial activity of garlic showed that allicin (allyl 2-propene thiosulfinate); a notable flavonoid in garlic is formed when garlic cloves are crushed (Ross *et al.*, 2000). Allicin formation follows the action of an enzyme, allinase of the bundle sheet cells upon the alliin of the mesophyll cells. When crushed, *Allium sativum* yields allicin, a powerful antibiotic and antifungal compound (phytoncide). However, due to poor bioavailability, it is of limited use for oral consumption. Garlic also contains some sulphur-containing compounds such as alliin, ajoene, diallylsulphide, dithin, S-allylcysteine, enzymes and other non sulphur-containing compounds including vitamin B, proteins, minerals, saponins and flavonoids (Johnson *et al.*, 2008).

Garlic has been used to treat many conditions. The root bulb of garlic has been used

traditionally for thousands of years to treat many disease conditions. The root bulb of garlic has a high concentration of sulfur containing compounds among which allicin appears to be among the most active compounds (Tattelman, 2005). The elucidation of the chemical structures of some of these compounds has led to the synthesis and production of more potent and safer drugs (Bhattacharjee *et al.*, 2005). Therefore, the need for new therapeutic agents is pertinent and garlic is considered as one of the most promising agents.

MATERIALS AND METHODS

Plants procurement

Cloves of garlic were purchased from Sokoto central market located at Sokoto central senatorial district in the state capital in July, 2011. They were skinned, washed and blot dried at room temperature. They were chopped to pieces and further dried under shade for three (3) days to remove moisture after which they were meshed into smaller granules.

Plant extraction

The extraction methods described by Harbone (1973) and Aliyu *et al.* (2009) were adopted. These methods are adequate for both initial and bulk extraction. The plant powder is placed in a cellulose thimble in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser.

A suitable solvent is added to the flask, and the set up is heated under reflux. When a certain level of condensed solvent has accumulated in the thimble, it is siphoned into the flask beneath. Eighty (80g) of the dried powdered plant material was soaked in water and methanol respectively. The mixture of each solvent was agitated in a mechanical shaker overnight, filtered and concentrated using water bath at a temperature of 56°C and transferred to a Soxhlet apparatus, the filtrate was evaporated and the residues were used for phytochemical analysis and bioassay.

Phytochemical test

The methods described by Cannel (2000) and Hassan *et al.* (2004) were used for the Phytochemical test.

Screening for Antibacterial Activity

Clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* were obtained from the Microbiology Laboratory, Usmanu Danfodiyo University Teaching Hospital Sokoto. The isolates were identified by standard microbiological procedure as described by Cheesbrough (2002). The susceptibilities of the test organisms to the plant extract were assayed as described by Aliyu, *et al.* (2009). Briefly, the test organisms from growth on nutrient agar incubated at 37 °C for 18hr were suspended in saline solution (0.85% NaCl) and adjusted to match a turbidity of 0.5 (10⁸ cells/ml) McFarland standard. The standardized suspension was used to inoculate the surfaces of Mueller Hinton agar plates (90mm in diameter) using sterile cotton swab. Six millimeter diameter wells were punched using cork borer in agar and filled with the desired concentrations (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) of the aqueous and methanol extracts. Commercial antibiotic (Ciprofloxacin 30µg) was used as reference standard to determine the sensitivity of the isolates. Disc was directly placed onto the bacterial culture. The plates were allowed to stand for 5 hours at room temperature for extract to diffuse into the agar and then incubated at 37°C over night. The entire test was conducted in duplicate. Antibacterial activities were evaluated by measuring inhibition zone diameters as recommended by National Committee for Clinical Standard (1999). The minimum inhibitory concentration was also determined. Each extract from aqueous and methanol

was separately dissolved in sterile distilled water and 2ml of sterile Mueller Hinton broth was transferred into a set 5 tubes and 2ml of each concentration (400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml) of the extracts was added to obtain final concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml respectively. Each test organism was inoculated into the labeled tube by taking a loopful of the standardized bacterial suspension using a flame sterilized wire loop except the control; the tubes were incubated at 37°C for 18 hours. The MIC was taken as the lowest concentration that prevented visible growth. The minimum bactericidal concentration was determined according to the National Committee for Clinical Standard (1999). From the test tubes used in the determination of MIC, the tubes that showed no visible growth were sub cultured onto freshly prepared Mueller Hinton agar and incubated at 37°C for 48. The least concentration at which the organisms did not recover and grow was taken as the MBC.

RESULTS

Table 1 shows the results of preliminary phytochemical screening of garlic (*Allium sativum*). Tannins, flavonoids, terpenoids, Alkaloids and saponin, carbohydrates, protein, and cardiac glycosides were found in the extracts while anthraquinones was absent.

Table 2 and 3 showed the diameter of zone of inhibition of the extracts at different concentrations against the test organism, the aqueous and methanol extract of garlic were observed to be more potent against *E. coli* with maximum zone of growth inhibition of 21.5mm at 200mg/ml and 24.0mm at 200mg/ml respectively.

Results of the MIC and MBC for garlic extract are presented in Table 4. The MICs of the aqueous and methanol extracts of garlic against *E. coli* were 100mg/ml and 50mg/ml respectively while *S. aureus* were 200mg/ml and 100mg/ml. *Pseudomonas aeruginosa* had 200mg/ml and 200mg/ml respectively. Higher MBC of 300mg/ml was observed against *P. aeruginosa* with aqueous extract and 250mg/ml with the methanol extract. The MBC for both the aqueous and methanol extract was 200mg/ml against *E. coli* while MBC of 300mg/ml was observed against *S. aureus* for the aqueous extract and 200mg/ml for the methanol extract.

Table 1: Phytochemical profile of aqueous and methanol extracts of *Allium sativum*

Phytochemical Constituent	Aqueous Extract	Methanol Extract
Carbohydrate	–	–
Anthraquinones	+	+
Saponins	+	+
Steroids	–	–
Triterpenes	+	–
Flavonoids	+	–
Tannins	+	+
Alkaloids	–	+
Glycosides	+	–

+ = Present - =absent`

Table 2: Diameter of zones of inhibition (mm) of garlic aqueous extract against the bacteria

Test organism	zone of inhibition (mm)/concentration (mg/ml)			
	200	100	50	25
<i>Escherichia coli</i>	21.5	18.0	16.0	6.0
<i>Staphylococcus aureus</i>	20.0	17.0	14.0	6.0
<i>Pseudomonas aeruginosa</i>	21.0	17.0	6.0	6.0

Table 3: Diameter of zones of inhibition (mm) of garlic methanol extract against the test Organism

Test organism	zones of inhibition (mm)/concentration (mg/ml)			
	200	100	50	25
<i>Escherichia coli</i>	24.0	21.0	19.0	6.0
<i>Staphylococcus aureus</i>	23.0	19.0	18.0	16.0
<i>Pseudomonas aeruginosa</i>	20.0	18.0	17.0	6.0

Table 4: MIC and MBC of garlic extract against the test organisms (mg/ml)

Test organism	MIC (mg/ml)		MBC (mg/ml)	
	Aqueous	methanol	aqueous	methanol
<i>E. coli</i>	100	50	200	200
<i>S. aureus</i>	200	100	300	200
<i>P. aeruginosa</i>	200	200	300	250

DISCUSSION

Antibacterial evaluation of aqueous and methanol extracts of garlic revealed a significant antibacterial potency against the test organisms. The phyto-constituents of garlic have long been known and its antibacterial properties have been widely reported (Roy *et al.*, 2006). The antimicrobial activity of extracts of garlic have long been linked to the presence of some bioactive compounds. These secondary metabolites also serve to protect the plants themselves against bacterial, fungal and viral infections (El-Mahmood and Amey, 2007). These bioactive compounds are known to work synergistically to produce various effects on the human and animal subjects (Amagase, 2006). However, most reports on the activity of garlic have focused mainly on the commensal microflora and community acquired infections, while information on their activity against hospital based pathogens is scanty. The large sizes of zones of growth inhibition produced by the garlic extracts against the test organisms indicated the potency of the active principle in them. Drugs present in plants are known as active principle and these active principles are divided chemically into a number of chemical classes including glycosides, alkaloids, volatile oils, steroids flavonoids, resins and sterols. Most of these active principles have measurable antibacterial activities against microorganisms. In this study, garlic extract showed higher activity against the test organisms. Gram-negative *E. coli* was most susceptible to the active principle present in garlic with a maximum zone of growth inhibition of 24mm, at 200mg/ml for methanolic extract and 21.5mm, at 200mg/ml for aqueous extract. Although, gram-negative bacteria tend to have higher intrinsic resistance to most antimicrobial agent (Donlan, 2001). Impressive activity against this gram-negative bacterium was observed. *Escherichia coli* are incriminated in

gastrointestinal and urinary tract infections: the susceptibility of *E. coli* to the extracts is an indication of the therapeutic potentials of these extracts against such infections. The garlic aqueous and methanol extracts have an appreciable potency against *S. aureus* with inhibition zones of 20.0mm, at 200mg/ml and 23.0mm, at 200mg/ml respectively. *Staphylococcus aureus* is a cause of skin and soft tissue infections, thus, the potency of the extract on the organism justifies the folkloric use of the plant in the treatment of wounds and guinea worm sores. The aqueous and methanol extracts of garlic shows an appreciable potency against *P. aeruginosa* with an inhibition zones of 21.0mm, at 200mg/ml and 20.0mm, at 200mg/ml respectively, a bacterium that has been reported to have possibly developed resistance to most antibiotics even before their discovery (Mukhtar and Tukur 2001).

MIC values of garlic aqueous and methanolic extract were respectively, 100mg/ml and 50mg/ml for *E. coli*, 200mg/ml and 100mg/ml for *S. aureus* and 200mg/ml and 200mg/ml for *P. aeruginosa*. While the MBC values were respectively, 300mg/ml and 250mg/ml for *P. aeruginosa*, for *S. aureus* 300mg/ml and 200mg/ml and 200mg/ml for both aqueous and methanol extract for *E. coli*. The antibacterial activity of these extracts could be attributed to their various phytochemical constituents. In general the growth of all test bacteria was inhibited though varying degrees, which agrees with the findings of Ankri and Mirelman (1999). The work of Jaber and Al-Mossawi (2007) also shows that *S. aureus* was more susceptible to garlic extract than *E. coli*, which is in contrast to what was observed in this study. The methanolic extracts have been observed to be more potent than the aqueous extracts which is in conformity to the findings by Debnath (2005). This account for the influence of the solvent system, which also affects the antibacterial activity of the crude extract.

CONCLUSION

This study has demonstrated the effectiveness of garlic against clinical isolates of *E. coli*, *S. aureus* and *P. aeruginosa* that are associated with various infectious diseases. This has provided justification that if well processed, garlic can be used to develop bioactive substances that may have promising effect on the treatment of some diseases.

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Recommendation

Further work on the phyto-constituents isolation and purification of the bioactive component of this plant is recommended as it could lead to the development of more effective substances that can be used to treat infections.

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