Antibacterial and Phytochemical Studies of *Allium Sativum*

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Abstract: Aqueous, methanolic and ethanolic extracts of *Allium sativum* (Lin), the popular bulbous spice plant was processed by different methods employing a combination of plant/solvent concentration, plant/solvent contact time and evaluated for antibacterial and phytochemicalpropeties and tested against standard sensitive strains of *Staphylococcus aureus* and *Escherichia coli.* The results obtained showed that pre-processed dried garlic samples were not active while freshly crushed samples showed better antibacterial activity on bacteria and that ethanol actually enhanced garlic's antibacterial activity. Antibacterial activity of garlic decreased over time in ethanolic solvent but increased in methanol. The bulb was also seen to minimal quantities of saponins and flavonoids and a high level of hydrocyanides. The study suggests that garlic when used in its raw form has better antibacterial activity, either directly or as an adjuvant in a solvent. The significance of the stability of garlic as an antibiotic is discussed.
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1. Introduction

Garlic (*Allium sativum* Linn.), an Alliaceae, is a perennial plant belonging to the genus *Allium.* It is an erect bulbous herb, 30–60 cm tall, strong smelling when crushed. The underground portion consists of a compound bulb with numerous fibrous rootlets. The bulb gives rise above ground to a number of narrow, keeled, grass like leaves. Garlic has been known to possess dietary and medicinal properties (Ross et al, 2001) and proven to have antimicrobial effects (Reuter et al, 1996; Lawson, 1998). The plant has also been shown to possess phytochemical constituents (Cavallito and Bailey, 1994; Ankri and Mirelman, 1999; Prados-Rosales et al., 2003). The antimicrobial properties of garlic were first described by Louis Pasteur in 1958, and since then, research had demonstrated its effectiveness against bacteria, protozoa, fungi and some viruses (Jaber and Al-Mossawi, 2007).

In its pure form it has been found to exhibit antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant enterotoxicogenic strains of *Escherichia coli* and also possesses antifungal activity, antiparasitic, and antiviral activity (Pai and Platt, 1992; Ross et al, 2001). The antimicrobial activity of garlic has been attributed to the presence of allicin (a thiosulfinate) whose removal completely renders garlic ineffective against microorganisms (Hughes and Lawson, 1991).

Allicin, the main active principle related to *Allium sativum* chemistry, is obtained by crushing or cutting garlic cloves. The odourless 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 (Block, 1985). Allicin is considered to be responsible for the bacteriostatic properties of garlic. *Allium sativum* extracts obtained with ethanol (ethanolic garlic extract, EGE) and acetone (acetonic garlic extract, AGE) extracted by drying at 60^{o}C (Eja *et al*, 2007) and by sohxlet apparatus (El Mahmood, 2007) was claimed to have direct implication in the inhibition of the in-vitro growth of gram positive, gram negative and diarrhoeagenic bacteria responsible for serious gastric diseases such as ulcers and even gastric cancer (Cañizares et al, 2004). The present study was to examine the antibacterial activity of freshly crushed, sun dried and shade dried pre-treated garlic extracted with different solvents on a Gram negative and Gram positive bacteria.

2. Materials and Methods

2.1 Collection of Materials

Garlic was bought from commercial spice sellers in the central Watt market in Calabar, Nigeria in April, 2010. Healthy garlic bulbs with no signs of external damage were used for ethanolic, methanolic and aqueous extractions and phytochemical analysis

(Kučinskaitė *et al*, 2007). Fresh garlic bulbs were washed under running tap water, air dried and chopped to fine pieces that could be weighed, after peeling off the scaly external epidermis. The bulbs were chopped and quickly macerated in a blender to a fine paste and also squeezed in a juice extractor to obtain fresh juice extracts. The mashed paste was immediately suspended in solvents. Up to 15mls of extracts were obtained from squeezed garlic and also immediately measured out in appropriate proportions into solvents.

Garlic bulbs were finely ground by pounding and dried under pressure in a metal plate on the dashboard of a closed automobile left in the sun during the heat of the day. The garlic dried completely in less than three hours and was beaten to a dry fine powdery substance and stored in an air tight container and used the same way as the fresh samples as described vide infra.

2.2 Extraction

Aqueous, methanolic and ethanolic extraction

Prepared ground fresh garlic extracts, raw fresh garlic juice, ground dried garlic materials were extracted in distilled water using 1:2 – 1:10 (logarithmic) garlic/solvent incremental ratios (to obtain 500, 200, 125 and 100mg/ml of crushed garlic) for 2 and 72 hours respectively. Sohxlet extracted ground dried garlic materials extracted with ethanol and methanol was also used. The materials were filtered through layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 2 and 72 hours the supernatant was collected and concentrated by evaporation to make the final volume one-fifth of the original volume. The procedure was repeated using fresh garlic samples without the sterilization. The procedure was also performed using 70% and 98% concentrations of methanol and ethanol respectively.

2.3 Antibacterial Testing

Testing for the antibacterial effects of garlic extracts was done using the technique described by Kirby- Bauer (Prescott et al, 2005). Wells were cut with a cork borer in solid Mueller Hinton agar which had previously been prepared and inoculated with *Staphylococcus aureus* and *Escherichia coli* in McFarland's concentration (NCCLS, 1996).. The wells (6mm in diameter) were seeded with approximately 3 drops (about 0.06ml) of garlic extracts and incubated at 37ºC for 24 hours. The antibiotics, ampicillin (AMP) and ciprofloxacin (CPX), were used as controls for comparison with the raw extract. The plates were observed for zones of inhibition after incubation followed by calculation of the mean zones of inhibition (mm).

2.4 MIC and MBC determination

Minimum inhibitory concentration and Minimum bactericidal concentration of the garlic were determined by serial dilution to various concentrations according to the macro broth dilution technique described by Baron and Finegold (1990).

2.5 Phytochemical Screening

The phytochemical composition of garlic was evaluated quantitatively by direct chemical estimation adopted by Krishnaiah et al, (2009).

3. Results

3.1 Antibacterial Activity

The antibacterial activity of garlic against test organisms are presented in tables 1 to 3. Table 1 shows antibacterial activities of freshly crushed and macerated *A. sativum* paste suspended in solvent by direct decoction on *S. aureus* and *E. coli*. The highest activity was seen in the 500mg/ml concentration of the 98% ethanolic extract at 2 hours (20mm) against E. coli and at 200mg/ml and 125mg/ml concentrations against the same organism (20mm) after 72 hours. Against *S. aureus* activity was observed to be as high as 27mm at 200mg/ml concentration after 2 hours of decoction. Aqueous extract had activity against *E. coli* (16mm) at 2 hours in both the 500mg/ml and 200mg.ml concentrations.

Minimal activity was observed in 70% methanolic and ethanolic extracts while aqueous extracts had activity against *E. coli* as low as 100mg/ml concentration and at 200mg/ml against *S. aureus*.

Table 1: Antibacterial activity of freshly crushed *A. sativum by* direct decoction on *S. aureus* and *E. coli*

Organism/Solvent	Aqueous				Methanol(70%)				Methanol(98%)				Ethanol(70%)				Ethanol(98%)					
Conc. (mg/ml)	500	200	125	100	500	200	125	100	500	200	125	100	500	200	125	100	500	200	125	100		
	Zones of inhibition (mm)																					
Staph aureus					8	8	8	8	15	16			7	8			18	27	12	7	14	16
2hrs			16														10					
72hrs																						
Escherichia coli	16	16			7			8	17	16	8		8	8	8	8	8	20	19	14	20	20
2hrs					10	10																
72hrs																						

Each value is mean of 3 replicates

<10 = Resistant, 10 – 15 = moderately sensitive, >16 = Sensitive (Cheesebrough (1991)

Sohxlet 70 % and 98% methanolic extracts of garlic had activity only on *Staph aureus* with 20mm and 18mm zone diameters. Activity was only observed against *E. coli* when absolute ethanolic extracts was used recording diameters of up to 22mm and 18mm agaist *S. aureus*, as shown in table 2.

The antibiotic controls used were very active against the test organism at zones of inhibition above 20mm in culture. No antibacterial activity was detected with sohxlet extracted dried *A. sativum* against *S. aureus* and *E. coli.* Sohxlet extracted freshly crushed *A. sativum* showed activity in methanol and absolute ethanol against *S. aureus* but showed activity against *E. coli* only in the 98% ethanolic extract.

Table 2: Antibacterial activity of sohxlet extracted freshly crushed *A. sativum* on *S. aureus* and *E. coli*

Organism/Solvent Conc. (mg/ml)	Aqueous	Methanol(70%) Zones of inhibition(mm)	Methanol(98%)	Ethanol(70%)	Ethanol(98%)
Staph aureus	ND[+]	20	18	NAD[*]	18
Escherichia coli	ND	NAD	NAD	NAD	22

* = No activity detected, + = not done

Table 3: Antibacterial activity of freshly extracted *A. sativum* juice on *S. aureus* and *E. coli*

Organism/Solvent conc. (mg/ml)	Aqueous 500	200	125	100	Methanol(70%) 500	200	125	100	Methanol(98%) 500	200	125	100	Ethanol(70%) 500	200	125	100	Ethanol(98%) 500	200	125	100
					Zones of inhibition(mm)															
Staph aureus																7				
2hrs		18					8						12	12			22	29	16	10	18
72hrs					8	8	8		18	20							20	12			
Escherichia coli				20				7								12					
2hrs	22				8			10					10	12			24	22		18	22
72hrs					10				17	17	8						20				

In table 3, garlic antibacterial activities peaked at 29mm against *S. aureus* when freshly squeezed garlic juice was suspended in 98% ethanolic solvent at 200mg/ml concentration. Aqueous extract also showed a considerable level of activity against *E. coli* with 20mm and 22mm zone diameter at 500mg/ml and 200mg/ml concentrations respectively after 2 hours decoction. Even when fresh garlic juice was suspended in solvent, 70% methanol and ethanol proved to be relatively inactive against test bacteria. Activity of garlic juice in absolute ethanol was more pronounced against test bacteria than in methanol. In methanol, activity was observed only after 72 hours of contact between garlic juice and solvent showing 17mm each zone diameters at 500mg/ml and 200mg/ml concentrations respectively.

Table 4 presents the MIC and MBC of garlic while table 5 represents the quantitative phytochemical values for garlic. Alkaloids are not found in garlic as shown in table 5.

MIC and MBC remained the same against *S. aureus* and *E. coli* at 156µg/ml and 312µg/ml respectively. Garlic showed a high occurrence of hydrocyanides and very little amounts of flavonoids, tannins and saponines.

Table 4: MIC and MBC of *A. sativum* extracts at 200mg/ml concentration on *E. coli* and *S. aureus* (µg/ml)

Test organism/Plant	MIC	MBC
Escherichia coli	312	312
Staphylococcus aureus	156	156

Table 5: Quantitative phytochemical values for *A. sativum* (mg/g)

Phytochemical(mg/gm)/ Plant sample	*Allium sativum*
Saponins	0.023
Flavonoids	0.137
Alkaloids	NAD[±]
Tannins	0.382
Hydrocyanides	3.298

± = no activity detected

4. Discussion

Activity of *Allium sativum* was significant only when fresh crushed garlic cloves were used as shown in tables 1 and 3. In table 1, the fresh crushed cloves were decocted directly in solvent. Antibacterial activity tended to reduce with time in the ethanolic (98%) extract at all the concentrations used while activity petered out completely in the aqueous extract after 3 days of decoction. Garlic was also found to be more active against *E. coli* as shown by the results obtained, as has been shown in a previous study by Eja et al, 2007. The fresh sohxlet extracts were considerably more active than the direct decoction extracts. The antibacterial activity of garlic tended to also reduce with concentration. The 200mg/ml (1:5 w/v dilution) concentration of 98% ethanolic and methanolic extracts expressed more activity than the 500mg/ml (1:2 w/v dilution) on *S. aureus* activity of garlic in table 3 were more significant and better at all concentrations when extracted garlic juice was used. This may be because the freshly extracted juice was more concentrated in allicin than an equivalent weight of whole garlic marsh and allicin was still very potent considering the time it took to macerate garlic and weigh out.

Shade dried crushed garlic samples extracted with sohxlet and direct decoction showed absolutely no activity against test bacteria as also has been reported by Onyeagba et al (2004) and Eja et al (2006).

Maceration of dried garlic was next to impossible and very difficult by both mortar and pestle and by blending. The hygroscopic and fibrous nature of garlic could not allow proper processing of shade dried garlic. Measurement (weighing) of the dried material was difficult due to stickiness. The dried powdered material retained the solvent when introduced into the Soxhlet apparatus. Dried garlic is not very miscible in alcoholic solvents. This did not agree however with work done by El-Mahmood (2009) who reported using soxhlet extraction for shade dried garlic cloves made into powder. Eja, et al, (2011) however reported activity of fresh garlic juice extract that was pre-weighed and dried in an oven to a constant weight.

Allicin is volatile at a temperature of 50^{o}C and may have been evaporated from the garlic powder. When the garlic bulb is crushed, minced, or otherwise processed, alliin is released from compartments and interacts with the enzyme alliinase in adjacent vacuoles. Hydrolysis and immediate condensation of the reactive intermediate (allylsulfenic acid) forms allicin (*European pharmacopoeia*, 1997). Allicin itself is an unstable product and will undergo additional reactions to form other derivatives e.g. products, depending on environmental and processing conditions (Reuter and Sendl, 1994). This accounts for the lack or absence of antibacterial activity of garlic when exposed for unnecessarily long periods and pretreatments of any kind before bacteria is challenged with it.

Fujisawa et al (2008) demonstrated the instability of garlic (*Allium sativum* L.)-derived allyl 2-propenylthiosulfinate (allicin) in various aqueous and ethanolic solutions as well as in vegetable oil through chemical and biological analyses performed simultaneously. Their study reported that crushed fresh garlic cloves generated antibacterial activity and chemically detectable allicin, a major antibacterial principle, and both declined on a daily basis in aqueous and ethanolic solutions at room temperature, showing biological and chemical half-lives of about 6 and 11 days, respectively. Allicin was more stable in 20% alcohol than in water. Allicin is considered to be the most potent antibacterial agent in crushed garlic extracts, but it can be unstable, breaking down within 16 h at 23°C (Hahn, 1996). However, the use of a water-based extract of allicin stabilizes the allicin molecule. This may be due to two factors: the hydrogen bonding of water to the reactive oxygen atom in allicin can reduce its instability; and/or there may be water-soluble components in crushed garlic that destabilize the molecule (Lawson, 1996). The disadvantage to this approach is that allicin can react with water to form diallyl disulphide, (Lawson and Wan, 1995) which does not exhibit the same level of antibacterial activity as allicin. The aim of this work was to study the efficacy of garlic given different approaches of pre treatment and solvent types viz a viz solvent concentration and contact time between solvent and garlic samples on bacteria.

Allicin is one of the most active principles of freshly crushed garlic homogenates first discovered in 1944 by Cavallito and Bailey who noted its potent antimicrobial activity. It has a variety of antimicrobial and antifungal activities. However, no clinical trials have been performed with allicin and it was never developed into a drug or commercial product due to its instability, inability to be absorbed, and offensive odor. Allicin is considered to be of limited value inside the body and is presently regarded by the scientific community as just a transient compound which rapidly decomposes to other compounds.

5. Conclusion

Garlic is a well know spice consumed as food in many parts of the world either in the raw form or added to cooked food. It is acclaimed to have antihypertensive and other beneficial health properties. This study shows that the plant has ample antibacterial activity against Gram negative and Gram positive bacteria when extracted with alcoholic and aqueous solvent only as a fresh preparation. Garlic is not miscible with alcoholic solvents when dried and as

such would not allow extraction of any active materials from it. Despite the low presence of phytochemical compounds in garlic, allicin remains the most prominent active antibacterial compound in the plant.

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References

6. Ankri, S. and Mirelman, A. Antimicrobial properties of allicin from garlic. *Microbes Infect.* 1999; 2, 125-129
7. Bauer, AW, Kirby, WM, Sherris, JC and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 1996; 45, 413-416.
8. Block E. The chemistry of garlic and onions. Science Am. 1985; 252, 114-19.
9. Cañizares, P., Gracia, I., Gómez, LA, García, A., Martín De Argila, C., Boixeda D, de Rafael, L. Thermal degradation of allicin in garlic extracts and its implication on the inhibition of the in-vitro growth of *Helicobacter pylori.* Biotechnology Prog. 2004; 20(1), 32-7
10. Cavallito C., Bailey J.H. Allicin, the antibacterial principal of *Allium sativum*. Isolation, physical properties and antibacterial action. Journal of American Chemical Society 1944; 66, 1944–52.
11. Cheesbrough, M. (1991). Medical Laboratory Manual for Tropical countries. English Language Book Society Publishers, London. 479.
12. Eja, M.E., Udo, S.M., Andy, I.E., Arikpo, G.E., Ikpeme, E.M and K.H. Enyi-Idoh. Comparative study of antibacterial activities of extracts of garlic (*Allium sativum*) obtanined by two methods. Nigerian Journal of Microbiology 2006; 20(3), 1238 – 1343
13. Eja, M.E., Asikong, B.E., Abriba, C., Arikpo, G.E., Anwan, E.E. and K.H. Enyi-Idoh. A comparative Assessment of the Antimicrobial Effects of Garlic (Allium sativum) and antibiotics on Diarrheagenic Organisms. Southeast Asian Journal of Tropical Medicine and Public Health 2007; 38 (2), 343 - 348
14. Eja, M. E., Arikpo, G. E., Enyi-Idoh K. H. and Ikpeme, E. M. An evaluation of the antimicrobial synergy of Garlic (*Allium sativum)* and Utazi (*Gongronema latifolium*) on *Escherichia coli* and *Staphylococcus aureus.* Malaysian Journal of Microbiology 2011; 7(1), 45-49
15. EL-mahmood Muhammad Abubakar . Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniea and Pseudomonas aeruginosa.* Journal of Medicinal Plants Research 2009; 3(4), 179-185
16. Fujisawa, H,, Suma, K,, Origuchi, K,, Kumagai, H,, Seki, T, and Ariga T. Biological and chemical stability of garlic-derived allicin. *Journal of Agric Food and Chemistry* 2008; 56(11), 4229-35.
17. Hahn, G. In: Koch HP, Lawson LD, eds. *Garlic: the science and therapeutic application of* Allium sativum *L and related species* (2nd edn). Baltimore Williams and Wilkins, 1996; 1–24.
18. Hughes B.G., Lawson L.D. Antimicrobial effects of *Allium sativum* L. (garlic), *Allium ampeloprasum* (elephant garlic) and *Allium cepa* L. (onion), garlic compounds and commercial garlic supplement products. Phytother Reseatrch 1991; 5, 154-8.
19. Jaber, M.A., Al-Mossawi, A. Susceptibility of some multiple resistant bacteria to garlic extract. African Journal of Biotechnology 2007; 6(6), 771-776
20. Krishnaiah D., Devi T., Bono A. and Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. Journal of Medicinal Plants Research 2009; 3(2), 067-072
21. Kučinskaitė, A., Pobłocka-Olech, L., Krauze-Baranowska, M., Sznitowska, M., Savickas, A. and Briedis, V. Evaluation of biologically active compounds in roots and rhizomes of *Rhodiola rosea* L. cultivated in Lithuania. Eksperimetinis Tyrimas. Medicina (Kaunas), 2007*;* 43(6), 487
22. Lawson, L.D. The composition and chemistry of garlic cloves and processed garlic. In: Koch HP, Lawson LD, eds. *Garlic: the science and therapeutic application of Allium sativum L and related species* (2nd ed). Baltimore: Williams and Wilkins, 1996; 37–107.
23. Lawson, L.D., Wang, Z.Y.J. Changes in the organosulphur compounds released from garlic during aging in water, dilute ethanol or dilute acetic acid. Journal of Toxicology 1995; **14,** 214.
24. Lawson L.D. Garlic: a review of its medicinal effects and indicated active compounds. In: Lawson LD, Bauer R, eds. Phytomedicines of Europe: their chemistry and biological activity. Washington DC: American Chemical Society, 2007; 69.
25. National Committee for Clinical Laboratory Standards 1996. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved Standard M31-A. NCCLS, Wayne Pa.

26. Onyeagba, RA, Ugbogu, OC, Okeke, CU, Iroakasi, O. Studies on the antimicrobial effects of garlic (*Allium sativum* Linn), ginger (*Zingiber officinale Roscoe)* and lime (*Citrus aurantifolia* Linn). African Journal of Biotechnology 2004; 3, 552-554.
27. Pai, S.T.., Platt, M.V. Antifungal effects of *Allium sativum* (garlic) extract against the *Aspergillus* species involved in otomycosis. Clinical Microbiology 1992; 30, 2881-6.
28. Prados-Rosales, R.C., Luque-GARCIA, jl. and Luque de Castro, M.D. Rapid analytical method for the determination of p4sticides residues in sunflower seeds based on focused microwave-assisted soxhlet extraction, prior to GC-MS-MS. Journal of Chromatography 2003; A 993, 121-129
29. Prescott, L. M., Harley, J. P. and Klein, D. A. Microbiology. 6th edn. McGraw-Hill, Boston. 2005; pp. 992.
30. Reuter, H.D., Koch, H.P. and Lawson, L.D. Therapeutic effects and applications of garlic and its preparations. In: Koch HP, Lawson LD, eds. Garlic. The science and therapeutic application of *Allium sativum* L. and related species. Baltimore: Williams and Wilkins. 1996; 135-212.
31. Ross, Z.M., O'Gara, E.A., Hill, D.J., Sleightholme, H.V., Maslin, I.J. Antimicrobial properties of garlic oil against human enteric bacteria: Evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Applied Environmental Microbiology 2001; 67, 475-80.

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