

Full Length Research Paper

Antibacterial effect of garlic (*Allium sativum*) on *Staphylococcus aureus*: An *in vitro* study

Deresse Daka

Hawassa University College of Health Sciences, Department of Medical Laboratory Technology P. O. Box 1560, Awassa Ethiopia. E-mail: drsdk2000@yahoo.com. Tel: +251911968912. Fax: +0462205421.

Accepted 6 July, 2009

Garlic (*Allium sativum*) has had an important dietary and medicinal role for centuries. It is a large annual plant of the Liliaceae family, which grows in most of Africa and in Ethiopia. Ethiopian garlic is used in traditional medicine for infectious disease and some other cases. The present study tested the aqueous extract of garlic *in vitro* for its antibacterial activity. The extract showed concentration dependent antibacterial activity against *Staphylococcus aureus*. The traditional use of Ethiopian garlic for infectious diseases and for controlling fever appears to be justified.

Key words: Garlic (*A. sativum*), *Staphylococcus aureus*, antibacterial activity.

INTRODUCTION

It is charming to observe how different cultures that have never come into contact with one another came to the same conclusion about the role of garlic in health and disease. Some of the earliest references to this medicinal and culinary plant are found on Sumerian clay tablets dating from 2600-2100 BC. Garlic was an important medicine to the ancient Egyptians listed in the medical text Codex Ebers (ca. 1550 BC) especially for the working class involved in heavy labor (Lawson et al., 1998; Moyers 1996). There is evidence that during the earliest Olympics in Greece, garlic was fed to the athletes for increasing stamina (Lawson et al., 1998).

The great herbalists and physicians of the ancient world record garlic historical use. "Garlic has powerful properties and is of great benefit against changes of water and of residence," wrote Pliny the elder, the first century Roman naturalist (23 - 79 AD) (Foster, 1996; Koch and Lawson, 1995). Garlic has been in used since ancient times in India and China for a valuable effect on the heart and circulation, cardiovascular disease (Kris-Etherton, 2002; Yeh. and Liu, 2001; Gardner et al., 2003), and regular use of garlic may help to prevent cancer, to treat malaria, and to raise immunity. Garlic has also proposed to treat asthma, candidiasis, colds, diabetes, and antibacterial effect against food borne pathogens like *Salmonella*, *Shigella* and *Staphylococcus aureus* (Teferi and Hahn, 2002).

Therapeutic use of garlic has been recognized as a potential medicinal value for thousands of years to different micro-organisms. For example, antifungal, antiviral,

antibacterial antihelmantic, antiseptic and anti-inflammatory properties of garlic are well documented. Moreover, garlic extracts exhibited activity against both gram negative (*E. coli*, *Salmonella* species and *Citrobacter* *Enterobacter*, *Pseudomon*, *Kilabsella*) and gram positive (*S. aureus*, *S. pneumonia* Group A streptococcus and *Bacillus anthrax*) all of which are causes of morbidity world wide. This study will focus on recent research on protective effects of garlic against *S. aureus*.

There is extensive literature on the antibacterial effects of fresh garlic juice, aqueous and alcoholic extracts, lyophilized powders, steam distilled oil and other commercial preparations of garlic. Fenwick and Hanely (1985) undertook a thorough review of the antibacterial effects of garlic and other allium vegetables up to mid 1984. More recently, the antibacterial effects of garlic have been studied by Reuter et al. (1996). The present study tested an aqueous extract of dried garlic *in vitro* for its antibacterial activity against *S. aureus*.

METHODS

The influence of garlic bacterial growth was studied with 30 clinical isolates of *S. aureus*. Fresh Ethiopian garlic was peeled and meshed in a blender. After filtration the substance was freeze dried and stored at -10°C until use.

Antibacterial effect of garlic on *Staphylococcus aureus*

Susceptibility of *S. aureus* was determined by the agar dilution

method using Muller Hinton agar (The NCCLS Modified Kirby-Bauer susceptibility testing technique). Clearly prepared garlic powder was thoroughly mixed with distilled water and the concentration was determined with varying amounts of crude preparation of garlic to give the final concentration of 7.50, 15.00, 22.50, 30.00, 37.50, 45.00, 52.50 and 60.00 mg/ml of media and the final volume of 20 ml.

Isolation of *Staphylococcus aureus*

Clinical samples (sputum, nasal swab, stool and ear discharge) were collected and cultured on Nutrient and Manitol Salt agars (MSA) as well as other biochemical tests (coagulase and catalase test) were done. Agar plates were inoculated with 0.01 ml of *S. aureus* suspension (which was clearly grown on MSA) (McFarland 3) and incubated for 18 - 24 h at 35 - 37 °C under aerobic condition. The minimum inhibition concentrations (MIC) and minimum bactericidal concentrations (MBC) of the garlic were determined using clinical isolates and control strains (*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 which get hold of Ethiopian Nutrition and Health research institute ENHRI Addis Ababa, Ethiopia, Pasture institute). The study was conducted from 1st July 2008 - 1st January, 2009 at Hawassa University.

RESULTS AND DISCUSSION

The aqueous preparation of garlic was prepared to determine the antibacterial effect. The pH of each of the garlic solution was measured and obtained as 6.90, 7.00, 7.08, 7.14, 7.18, 7.28, 7.29 and 7.40. The activity of the garlic was tested in these different pH levels and no pH effect on the garlic activity was observed. This was similar to the observation of (Shokradeh and Ebadi 2006). The MIC of extract on *S. aureus* was determined to be greater than 7.50 mg/ml which is almost similar with the work of (Shokradeh and Ebadi 2006). The lower concentration (7.5 mg/ml) of garlic had no antibacterial effect in this work, however; it may be effective (Shokradeh and Ebadi 2006). This is may be due to the species difference or the garlic difference in different biologic condition.

This study revealed that antibacterial activity of the garlic extract was heat sensitive. All clinical isolates (30) of *S. aureus* were tested on garlic extract which was autoclaved at 121 °C for 15 min. There was no antibacterial effect of garlic in contrast to the work of (Shokradeh and Ebadi 2006) (Table 2). The antibacterial effect of crude preparation of garlic at room temperature (fresh garlic) and refrigerated at -10 °C has the same antibacterial effect however; the fresh garlic shows greater effectiveness.

Several studies (Stewart and Holt, 1962; Cuvillo, 1999; Koneman et al., 1988) have confirmed that *S. aureus* is an important cause for both nosocomial and community acquired infections, which result in substantial morbidity and mortality. Although scientific antimicrobials were of help in the initial phase of their development, their fast emergence of drug resistant *S. aureus* strains have created a problem in the control and treatment of various infections (Barber, 1961; Monzone, 1971;

Pearson, 2000). As medicinal chemists advance in their search for new bacterial targets to attack, bacteria relentlessly evolve; as a result, a large number of bacterial species have become resistant to antibacterial drugs (Garau, 1994; Gould, 1994; Sanders and Sanders, 1992). Thus there is a need to develop alternate strategies. Because garlic is known to act synergistically with antibiotics, and resistance has not been reported for garlic, more dose-response preclinical studies and eventually clinical studies should be done to assess the use of an antibiotic/garlic combination for bacteria that are difficult to eradicate.

On consideration of the problems, the study focused on the garlic antibacterial activity on *S. aureus* has shown that dilute solutions of garlic can completely inhibit the growth of *S. aureus* at the concentration of more than 7.50 mg/ml. This could be due to the action of biological active ingredient of allicin which exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action (Feldberg et al., 1988).

According to Onyeagba and his colleague the crude extracts of garlic and ginger applied singly and in combination did not exhibit any *in vitro* inhibition on the growth of test organisms including *Staphylococcus* spp. (Onyeagba et al., 2004). In contrast the study has clearly shown that for *S. aureus* with inoculums density of 10⁴ CFU/ml, garlic in concentration of (15.00 - 60.00 mg/ml) was capable of causing the inhibition of bacteria growth (Tables 1 and 3).

Using the same protocol garlic has a bactericidal effect at the lower concentration of 30.00 mg/ml for clinical isolate of *S. aureus*. However, this concentration level may vary as different authors have stated; for instance 160 mg/ml was observed by Sivam et al., (1997). This might be due to the garlic species variation in different country, the processing difference on the garlic species and the inoculums' densities. The bactericidal effect of garlic might be due to the structural characteristics of organisms which play a role in the bacterial susceptibility to garlic constituents (Tynecka and Gos, 1975), particularly *S. aureus* contains only 2% lipid (Salton, 1964) so that the lipid content of the membranes will have an effect on the permeability of allicin and other garlic constituents. Hence these phenomenons may favor the destruction of the cell wall and genetic materials of *S. aureus*.

Conclusion

Garlic (*A. sativum*) has antimicrobial properties against *S. aureus*. It has both a bacteristatic and bactericidal activity when tested *in vitro* using crude preparation of garlic. Therefore, gallic may be used successfully for treating food poisoning causative agent like *S. aureus*. Further *in vivo* studies are necessary. More importantly there is

Table 1. The antibacterial activity of crude preparation of garlic; minimum inhibitory concentration (MIC) mg/ml of media at room temperature.

<i>S. aureus</i> in different samples			Concentration of garlic (mg/ml)							
	No.	(%)	0.75	15.00	22.50	30.00	37.50	45.00	52.50	60.00
Sputum	10	33.33	+	-	-	-	-	-	-	-
Stool	12	40.00	+	-	-	-	-	-	-	-
Nasal swab	14	46.33	+	-	-	-	-	-	-	-
Ear discharge	4	13.33	+	-	-	-	-	-	-	-
Total	30	100.00								

+ Indicates growth of bacteria; - indicates inhibition of bacteria.

Table 2. The antibacterial activity of crude preparation of garlic; minimum inhibitory concentration (MIC) mg/ml of media autoclaved at 121 °C for 15 min.

<i>S. aureus</i> in different samples			Concentration of garlic (mg/ml)							
	No.	(%)	0.75	15.00	22.50	30.00	37.50	45.00	52.50	60.00
Sputum	10	33.33	+	+	+	+	+	+	+	+
Stool	12	40.00	+	+	+	+	+	+	+	+
Nasal swab	14	46.33	+	+	+	+	+	+	+	+
Ear discharge	4	13.33	+	+	+	+	+	+	+	+
Total	30	100.00								

+ Indicates growth of bacteria; - indicates inhibition of bacteria.

Table 3. The antibacterial activity of crude preparation of garlic; minimum inhibitory concentration (MIC) mg/ml of media refrigerated at -10 °C.

<i>S. aureus</i> in different samples			Concentration of garlic (mg/ml)							
	No.	(%)	0.75	15.00	22.50	30.00	37.50	45.00	52.50	60.00
Sputum	10	33.33	+	-	-	-	-	-	-	-
Stool	12	40.00	+	-	-	-	-	-	-	-
Nasal swab	14	46.33	+	-	-	-	-	-	-	-
Ear discharge	4	13.33	+	-	-	-	-	-	-	-
Total	30	100								

+ Indicates growth of bacteria; - indicates inhibition of bacteria.

need for detailed scientific study of traditional medical practices to ensure that valuable therapeutic knowledge of some plants is preserved and also to provide scientific evidence for their efficacies. Also another study will be needed to establish the exact component or pharmacological standardization and clinical evaluation in garlic.

ACKNOWLEDGEMENTS

This work was possible because of encouragement and guidance of Hawassa University and Jimma University, Department of Medical Laboratory Technology, Ethiopia, Staff of Armour Hanson Research institute AHARI, Addis Ababa, Ethiopia, and Staff of Ethiopian Nutrition and Health research institute ENHRI Addis Ababa, Ethiopia.

REFERENCES

- Barber M (1961). Methicillin-Resistant Staphylococci. *J. Clin. Pathol.* 14: 385-393
- Fenwick GR, Hanley AB (1985). The genus *Allium*-Part 3. Medicinal effects. *CRC Crit. Rev. Food Sci. Nutr.* 1: 71-74
- Foster S (1996). *Garlic-Allium sativum*. Botanical series, N^o 311, 2nd ed. Austin, Texas: American Botanical council.
- Feldberg RS, Chang SC, Kotik AN, Nadler M, Neuwirth Z, Sundstrom DC, Thompson NH (1988). *In vitro* mechanism of inhibition of bacterial growth by allicin. *Antimicrob. Agents Chemother.* 32: 1763-1768.
- Garau J (1994). β -Lactamases: current situation and clinical importance. *Intensive Care Med.* 20(suppl.): 3: S5-S9.
- Gould IM (1994). Risk factors for acquisition of multi-drug-resistant gram-negative bacteria. *Eur. J. Clin. Microbiol. Infect. Dis.* 13 (suppl.): S30-S38.
- Gardner C, Chatterjee LM, Carlson JJ (2003). Soy garlic and ginkgo biloba: their potential role in cardiovascular disease prevention and treatment. *Curr. Atheroscler Rep.* 5: 468-475.

- Koneman EW, Allen SD, Janda WM (1986). Color Atlas and Textbook of Diagnostic Microbiology, 4th ed; J.B Lippincott Co.
- Koch HP, Lawson LD (1995). Garlic-the science and Therapeutic Application of *Allium sativum* L. Related species, 2nd ed, Baltimor: Williams and Wilkins,
- Kris-Etherton PM (2002). Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. J. Am. Med. 113: 71s-88s.
- Lawson LD (1998). Garlic: a review of its medicinal effects and indicated active compounds. In: Phytomedicines of Europe. Chemistry and Biological Activity. Series 691 (Edited by: Lawson LD & Bauer R) American Chemical Society, Washington, DC, pp. 176-209.
- Moyers S (1996). Garlic in Health, History and World Cuisine. Suncoast Press, St. Petersburg, FL, pp. 1-36.
- Monzone H (1971). Biological Basis of infections and infestations. F.A. Davis Company, Philadelphia, pp. 30-40.
- Onyeagba RA, Ugbogu OC, Okeke CU, Iroakasi O (2004). Studies on the antimicrobial effects of garlic (*Allium sativum* Linn), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). Afr. J. Biotechnol. 3(10): 552-554.
- Cuviello PV (1999). The many Faces of Staphylococcus. J. Am. Med. Technol. 1(1): 21-24.
- Pearson S (2000). MRSA. A Modern Plague. Biomed. Scient. 44(6): 528-530.
- Reuter HD, Koch HP, Lawson DL (1996). Therapeutic effects and Applications of garlic and its Preparation. In: Garlic: the science and Therapeutic Applications of *Allium sativum* L. and related Species, 2nd Edn., Koch HP and Lawson DL (Eds), pp: 135-212
- Shokradeh M, Ebadi AG (2006). Antibacterial effect of Garlic (*Allium sativum*) on staphylococcus. Pak. J. Biol. Sci. 9(8): 1577-1579.
- Sivam GP, Lampe JW, Ulness B, Swanzy SR, Potter JD (1997). Helicobacter pylori-*in vitro* susceptibility to garlic (*Allium sativum*) extract. Nutr. Cancer 27: 118-121.
- Salton MRJ (1964). The Bacterial Cell Wall. Elsevier, Amsterdam, The Netherlands.
- Stewart GP, Holt RJ (1962). Evolution of Natural Resistance to the new Penicillin, Br. Med. J. 1309-311.
- Sanders CC, Sanders Jr WE (1992). b-Lactam resistance in gramnegative bacteria: global trends and clinical impact. Clin. Infect. Dis. 15: 824-839.
- Teferi G, Hahn HJ (2002). Treatment of malaria in Ethiopia folk medicine. Trop. Doc. 32: 206-207.
- Tynecka Z, Gos Z (1975). The fungistatic activity of garlic (*A. sativum*) *in vitro*. Ann. Univ. Mariae Currie skoldowaska sect D. Med. 30: 5-13.
- Yeh YY, Liu L (2001). Cholesterol lowering effect of garlic extracts and organosulfur compounds: Human and Animal studies. J. Nutr. 131: 989s-993s.