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Antibacterial Effect of Garlic (*Allium sativum*) on *Staphylococcu aureus*: An *in vitro* Study

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Abstract: Garlic (*Allium sativum*) has 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: Antibacterial activity, garlic, *A. sativum*, *Staphylococcus aureus*

INTRODUCTION

It is charming to observe how different cultures 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 inheavy labor Lawson *et al.*, 1998; Moyers *et al.*, 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 used from the time when ancient times in India and China for a valuable effect on the heart and circulation, cardiovascular disease (Kris-Etherton *et al.*, 2002; Koscielny *et al.*, 1999; Yu-Yan 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 *S. aureus* (Teferi and Hahn, 2002).

Therapeutic use of garlic has been recognized as a potential medicinal value for thousands of years to different microorganisms. 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* sp. and *Citrobacter enterobacter*, *Pseudomona kilabsella*) and gram positive (*S. aureus*, *S. pneumonia* Group A streptococcus and *Bacillus anthrax*) all of which are cause of morbidity worldwide. This study will focus on recent research on protective effects of garlic against *Staphylococcus 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) understood 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 *Staphylococcus aureus*.

MATERIALS AND METHODS

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

Antibacterial effect of garlic on *Staphylococcus aureus*: Susceptibility of *Staphylococcus 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 mg/ml and 60.00mg/ml of media and the final volume of 20 ml.

Table 1: The antibacterial activity of crude preparation of garlic, Minimum Inhibitory Concentration (MIC) mg/ml of media at room temperature

S. aureus in different samples	N^{o}	%	Concentration of garlic (mg/ml) 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

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 0.01ml of *Staphylococcus aureus* suspension (which was clearly grown on MSA) (McFarland 3) and incubated for 18-24 hours at 35-37ºC 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 August 31st 2008 – January 1st 2009 in at Hawassa University.

RESULTS AND DISCUSSION

The pH of each of the garlic solution was 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 has no pH effect on the garlic activitywas observed. This was similar to the observation of Shokrzadeh and Ebadi (2006). The MIC of extract on *S. aureus* was determined to be greater than 7.50mg/ml, which is almost similar with the work of Shokrzadeh and Ebadi (2006). The lower concentration of garlic had no antibacterial effect in this work, however; it may effective as Shokrzadeh and Ebadi (2006). This is may be due 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 121ºC for 15 min. There was no antibacterial effect of garlic in contrast to the study of Shokrzadeh and Ebadi (2006) (Table 2). The antibacterial effect of crude preparation of garlic at room temperature (fresh garlic) and refrigerated at -10ºC has the same antibacterial effect however; the fresh garlic shows greater effectiveness.

Several studies Stewart and Holt (1962), Cuviello (1999) and Koneman *et al.* (1986) 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) and 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) and 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 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 10^4 CFU/ml, garlic in concentration of (15.00-60.00 mg/ml) was capable of causing the inhibition of growth of bacteria (Table 1 and 3).

At the same protocol garlic has a bactericidal effect at the lower concentration of 30.00mg/ml for clinical isolate of *S. aureus*. However, this concentration level may vary as different authors for instance 160mg/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

Table 2: The antibacterial activity of crude preparation of garlic, Minimum Inhibitory Concentration (MIC) mg/ml of Media autoclaved at 121ºC for 15 min

S. aureus in different samples	Nº	%	Concentration of garlic (mg/ml) 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 -10ºC

S. aureus in different samples	Nº	%	Concentration of garlic (mg/ml) 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

might be due to the structural characteristics of organisms which play a role in the bacterial susceptibility to garlic constituents Tyneka and Gos (1975), particularly *Staphylococcus 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 *Staphylococcus aureus* lastingly.

CONCLUSION AND RECOMMENDATION

This study indemnifies that garlic (*Allium sativum*) has antimicrobial properties against *Staphylococcus aureus*. It has both a bacteristatic and bactericidal activity when tested *in vitro* using crude preparation of garlic.Therefore, garlic may be used successfully for treating food poisoning causative agent like *Staphylococcus aureus*. It may be effective on other microbes on gram positive and gram-negative bacteria, so that further *in vivo* and *in vitro* studies are necessary.

In our situation people's uses garlic after cooking very well with other food, so this may affect antimicrobial effect of garlic *in vivo*. Further study is very important on the temperature and PH effect on garlic activity. More importantly there is 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.

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