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Full Length Research Paper

Antibacterial activity of fresh juice of *Allium sativum* (garlic) against multi – drug resistant isolates of *Staphylococcus aureus*

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***Staphylococcus aureus* causes a variety of infections in humans and animals. The growing emergence of drug - resistant pathogens led to the development of natural antimicrobials. In this study the antimicrobial effect of fresh garlic juice against multi – drug resistant *S. aureus* isolates has been studied. Antibacterial activity was measured by agar well diffusion method. All the test bacteria were found sensitive to the garlic juice. The minimum inhibitory concentration (MIC) of the fresh garlic juice against the test bacteria were ranging from 6.25 to 12.5% v/v and the minimum bactericidal concentration (MBC) ranging from 25 to 50% v/v. Based on this finding garlic juice possess significant antibacterial potency against multi-drug resistant isolates of *S. aureus* may be used as a natural therapeutic agent and antimicrobial preservative in food industry.**

Key words: Antibacterial activity, MDR *Staphylococcus aureus* and garlic juice.

INTRODUCTION

Staphylococcus aureus causes a variety of infections ranging from mild to severe in humans as well as economically important infections in animals (Dinges et al., 2000). *S. aureus* grows on fresh (raw), cooked or improperly stored foods and produces heat – resistant *Staphylococcus* enterotoxins (Nkanga and Uriah, 1981; Adeslyun, 1984) which are responsible for one of the most important food – borne disease in industrial countries. Because of great genetic variability of *S. aureus* and having the ability to develop changes in sensitivity to antimicrobials, most clinical isolates of *S.aureus* are resistant to a number of antibiotics (Sibanda et al., 2010).

The growing concern of microbial resistance towards chemical antimicrobial agents and growing sensitivity of consumers to the presence of chemical preservatives in food, have recently led to the development of natural antimicrobials. Spices are one of the most commonly used natural antimicrobial agents to control food – borne pathogens (Souza et al., 2005). Garlic (*Allium sativum*) is a well – known spice which is used in foods all over the world and its antibacterial effect against food borne pathogens like *Salmonella*, *Shigella* and *S. aureus* has been recognized (Teferi and Hahn, 2002).

In the present study there is a renewed interest in investigating the antimicrobial potential of fresh garlic juice on multiple drug resistance isolates of *S. aureus*.

MATERIALS AND METHODS

Test microorganism

A total of 30 clinical isolates of *S. aureus* were collected from different hospitals in Rasht. The antimicrobial susceptibility test for each isolate was performed on Muller Hinton agar by Kirby – Bauer disk diffusion method (Bauer et al., 1966).

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The susceptibility pattern of the isolates to the selected antimicrobial agents including Vancomycine (30 µg), Methicilline (5 µg), Amoxicillin (25 µg), Amoxi-Clav (30 µg), Cefazolin (30 µg), Cefalexin (30 µg) and Gentamycin (30 µg) provided from Padtan Teb company (Iran), was determined and 19 isolates which were resistant to three or more antimicrobial classes were defined as multi-drug resistant. Standard strain of *S. aureus* ATCC 6538 was used as control.

Fresh garlic juice preparation

Garlic bulbs were peeled and washed with sterile distilled water. Then the bulbs were blended to form juice. Fresh garlic juice was sterilized by filtration and used for testing antibacterial activity against multi-drug resistant *S. aureus* isolates.

Antimicrobial activity

Antimicrobial activity of fresh garlic juice was evaluated by using agar well diffusion method. Muller – Hinton agar plates were inoculated with 100 µl of standardized inoculum (1.5×10^8 CFU/ m1) of each bacterium and spread with sterile swabs. Wells of 6 mm in diameter were made with sterile borer in to each agar plate containing the bacterial inoculum. A little molten agar medium was used to seal the bottom of the wells. All plates were carried out in triplicates and the wells filled with 50 µl of garlic juice. One well in each plate was set up as control by adding 50 µl of freshly prepared sterile distilled water. The plates were left at room temperature for 15 min to allow the diffusion of plant juice in to the agar (Rios et al., 1988). After incubation at 37°C for 24 h the inhibiti on zone around each well was measured in millimeter. If the diameter of zone of inhibition was less than 9 mm was considered as inactive, 9 to 12 mm as partially active, 13 to 18 mm as active and more than 18 mm as very active (Junior and Zanil, 2000).

Minimum inhibitory concentration (MIC) of the fresh garlic juice against the test bacteria was determined using the microdilution method (Eloff, 1998). The fresh garlic juice was serially diluted two fold in Muller – Hinton Broth medium. Then 100 µl of standardized test organism suspension (1.5×10^8 CFU/ml) was inoculated in to each dilution. Tubes containing growth medium and different dilution of garlic juice without inoculum were used as controls. All tubes were incubated at 37°C for 24 h. Then the tube with highest dilution (lowest concentration) with no visible growth when compared with control was considered as the MIC. Minimum bactericidal concentration (MBC) was determined by culturing 100 µl of the contents of all tubes with no visible growth on Muller – Hinton agar and incubating at 37°C for 24 h. The low est concentration which inhibited colony formation on agar was determined as MBC for the garlic juice.

RESULTS

Antimicrobial susceptibility test in *S. aureus* isolates

The results of antimicrobial susceptibility test of a total of 19 multi-drug resistant *S. aureus* isolates are shown in Table 1.

Antimicrobial activity of garlic juice

The results of the antimicrobial activity of fresh garlic juice against multi – drug resistant *S. aureus* isolates are shown in Table 2. The zone of inhibition of the growth of the isolates is a function of antimicrobial activity of the garlic juice.

Minimum inhibitory concentration and minimum bactericidal concentration of garlic juice

Minimum inhibitory concentration of the garlic juice varied between 6.25 to 12.5% v/v and the minimum bactericidal concentration were between 25 to 50% v/v.

DISCUSSION

In the present study the effect of garlic juice on the growth and viability of multi – drug resistant *S. aureus* isolates was investigated *in vitro*. The results revealed the antimicrobial potential of fresh garlic juice and the entire test organisms were susceptible to it with inhibi-tion zone diameter between 39 to 55 mm. MIC of the fresh garlic juice against the test bacteria were ranging from 6.25 to 12.5% v/v and the minimum bactericidal concentration ranging from 25 to 50% v/v. This low value of MIC indicates that the garlic possess promising potential to combat multi – drug resistant *S. aureus* also the MIC and MBC values revealed that this plant extract was bacteriostatic at low concentrations and bactericidal at higher concentrations.

The antimicrobial activity of garlic juice can be attributed to the presence of allicin and other diallyl sulfide compound (Lawson, 1996). Also the structural characteristics of microorganism play a role in bacterial susceptibility to the garlic components (Tynecka and Gos, 1975).

The susceptibility of test multi-drug resistant bacteria to the garlic juice is encouraging because strains of *S. aureus* resistant to multiple antibiotics including methicillin were increasingly responsible for many outbreaks all over the world. The results obtained in this study confirm the earlier reports. Iwalokun et al. (2004) have found the antimicrobial properties of aqueous garlic extract against multi – drug resistant bacteria including *S. aureus*.

A recent study focused on the garlic extract anti-microbial 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/m^1 (Daka, 2011). Kivank (1997) studied the antimicrobial effect of fresh juice of 17 plants *in vitro*. Fresh garlic juice showed the highest inhibitory effect on the bacteria.

These results are contrary to those of Onyeagaba et al. (2004) who reported that the aqueous and ethanolic extracts of garlic did not inhibit the growth of test organism including *S. aureus*. From the results obtained in this study, garlic juice may be used as a natural

Table 1. Antimicrobial sensitivity testing of multi – drug resistant *S. aureus* isolates (Diameter of zone of inhibition in millimeter) Standard antibiotics

Test bacteria	M	V	Amx	Amc	CZ	G	CN
MDR - 1	0	16	0	0	0	0	0
MDR - 2	10	17	13	12	20	0	23
MDR - 3	0	13	9	0	0	11	0
MDR - 4	9	17	12	10	0	14	8
MDR - 5	0	17	0	0	0	0	0
MDR - 6	11	10	12	12	24	0	16
MDR - 7	9	16	10	12	25	10	21
MDR - 8	8	17	10	15	19	0	28
MDR - 9	10	17	13	13	20	0	23
MDR - 10	9	16	12	10	21	0	20
MDR - 11	0	15	0	0	0	0	0
MDR - 12	11	17	12	13	24	0	26
MDR - 13	9	10	13	11	22	8	24
MDR - 14	22	10	10	10	20	0	20
MDR - 15	0	11	9	0	0	0	0
MDR - 16	0	16	0	0	0	0	0
MDR - 17	11	16	16	11	14	0	24
MDR - 18	10	15	14	15	25	8	10
MDR - 19	0	15	10	10	16	0	11

MDR= Multi drug resistant, M = Methcillin, V = Vancomycin, Amx = Amoxicillin, Amc = Amoxi-Clav, CZ = Cefazolin, CN = Cefalexin, G = Gentamycin.

Table 2. Susceptibility of the multi – drug resistant *S. aureus* isolates to the garlic juice (Diameter of zone of inhibition in millimeter).

Test bacteria	Garlic juice (50 µl) zone of inhibition (mm)
MDR - 1	45
MDR - 2	43
MDR - 3	43
MDR - 4	45
MDR - 5	41
MDR - 6	55
MDR - 7	43
MDR - 8	41
MDR - 9	41
MDR - 10	45
MDR - 11	55
MDR - 12	47
MDR - 13	40
MDR - 14	53
MDR - 15	55
MDR - 16	54
MDR - 17	47
MDR - 18	44
MDR - 19	39

therapeutic agent and natural antimicrobial preservative in food industry because of its very active antimicrobial properties.

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