

**ANTIBACTERIAL ACTIVITY OF GARLIC EXTRACTS (*ALLIUM SATIVUM*)
AGAINST *STAPHYLOCOCCUS AUREUS***

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ABSTRACT

In the present study *Staphylococcus aureus* was isolated and indentified by morphological, cultural and biochemical characterization. Different concentration of aqueous garlic extracts (100%, 75%, 50%, and 25%) was tested with seven different antibiotics against *S.aureus* showed strong synergistic antibacterial effects with methicillin, teicoplanin, tobramycin and vancomycin as compared to clindamycin and linezolid while ampicillin showed negligible synergistic effects.

KEY WORDS: *S.AUREUS*, *ALLIUM SATIVUM*, ANTIBACTERIAL ACTIVITY.

INTRODUCTION

The popular use of plants as remedies for many infectious diseases, search for substances with antimicrobial activity are frequent. Garlic has been scientifically proven to be powerful natural antibiotics, antiviral and antifungal agent. Allicin is the main biologically active component of freshly crushed garlic and its antimicrobial affect is majorly due to its oxidative interaction with important thiol containing enzymes e.g., alcohol dehydrogenase, thio-redixinreductase, RNA polymerase (Fry et al.2005). A striking aspect of the activity of garlic is the apparent inability of most bacteria develop resistance to because its mode of action is completely different from that of other antibiotics (Cavallito et al., 1944). It has been proposed that the development of resistance to allicin is 1000 fold difficult than the development of resistance to betalactam antibiotics (Ankri, 1999) making garlic a prime candidate for therapeutic use. In addition to its potent infection fighting abilities, garlic also helps lower blood pressure, promotes balanced intestinal health and is a powerful immune system booster. The first report of

S.aureus with resistance to vancomycin was from Japan in 1977 by Hiramastu *et. al.*, raising the threat of incurable Staph infections. As powerful antibiotics lose their punch against “super bugs” such as MRSA, scientists are in search for new antimicrobial agents from natural sources and they all are potent against MRSA (Srinivasan, 2009)

MATERIAL AND METHODS

1. Isolation and identification of *Staphylococcus aureus*

Sample was collected from surgical patient from hospital. The pus sample collected on transport media SWAB and brought to the Microbiology laboratory within 24 hours.

2. Isolation

Media and Culture Condition

On reception the swab was inoculated on manitol salt broth and incubated at 37°C for 1 hour. The broth culture was then subcultured on Manitol salt Agar (Hi-Media). The plates were incubated at 37°C for 24-48 hrs. Manitol fermentation was observed and recorded from Manitol Salt Agar Plate for *S.aureus*, Gram Staining was performed from colonies obtained on manitol salt Agar and *S.aureus* was confirmed. *Staphylococcus* agar No 110 Azide (Hi-media, India) was also used for confirmation of *S.aureus*.

3. Identifications test

Identification of Bacteria was performed by Tube Coagulase test method.

4. Antibacterial activity

Fresh garlic was washed with sterile distilled water 3-4 times. It was allowed to air dry and crushed in mortar & pestle in sterile condition. The extract was taken out the help of sterile muslin cloth to get 100% garlic extract. Different concentrations (75%, 50% and 25%) of aqueous garlic extract were further prepared and used in the study (Bauer, 1966).

Study on synergistic action of garlic extract with seven different antibiotics against *S.aureus*.

Synergistic action was studied using Kirby Bauer disc diffusion method (Daka, 2011). Study was performed on Muller Hinton Agar (MHA) plates according to Clinical and Laboratory standard Institute (CLSI) guidelines. Inoculum was prepared by picking four to five colonies from overnight cultures and inoculated in five ml Tryptone Soya Broth and incubated at 35°C until turbid to 0.5 McFarland standard. Muller –Hinton agar plates were overlaid with the inoculums of the *S.aureus* clinical strain. Five same antibiotic discs were placed on the plate at equal distances. 10µml of aqueous garlic extract of different concentration was pipette out over the four antibiotics disc using micropipette leaving one antibiotic disc as it is. This was followed for all the antibiotics used in the study. Zone diameters were measured at 24 and 48 hrs following CLSI criteria. Methicillin resistance was evaluated by Kirby-Bauer disc diffusion technique with 4% NaCl in MHA and using Methicillin (5mcg) disc.

RESULT AND DISCUSSION

S.aureus was isolated and identified from pus sample on the basis of cultural characteristics, tube coagulase test. Garlic extract showed concentration dependent antibacterial activity against *S.aureus* that is with decrease in concentration of garlic extract, antibacterial activity also decreased. (Table 1). These observations were similar to the observations made by Deresse Daka coworkers (2011).

Table -1 Effect of different concentrations of garlic extract against isolated *S. aureus*.

Sr. no.	Garlic extract	Zone of inhibition in mm diameter
1	100%	27
2	75%	24
3	50%	22
4	25%	No zone

Garlic exhibited strong synergistic activity with methicillin and tobramycin giving an enhanced zone of 14 and 13 mm respectively. Jabar and Mossawi also found aqueous extract of

garlic to be potent against *S.aureus*. It was observed that garlic showed strong synergistic antibacterial Effect with Methicillin, teicoplanin, tobramycin and vancomycin. On the contrary, no synergistic activity activity was observed with vancomycin against *S.aureus* in the work of Betoni et al., (2006). Synergistic activity with clindamycin and linezolid was fair with the least activity shown with ampicillin showed negligible synergistic effect with garlic. These observations were similar to observation made by Betoni et al., (2006) (Table 2)

In our work important observation was made that *S.aureus* was resistant to tobramycin with zone of inhibition of 11mm which was enhanced to 25mm in combination with 100% concentration of garlic extract. Finding of the related work showed that allicin alone did not give good antibacterial activity but it facilitated antibacterial activity of cefazolin and oxacillin when tested against *Staphylococcus* spp. Allicin showed synergistic effect with vancomycin and clarithromycin against *S.aureus* in the work by Shahnaz et al, (2009)

Table 2 Zones of inhibition of Synergistic effect of *Allium sativum* with a panel of seven different antibiotics against *S.aureus*

Antibiotics	Antibiotics without garlic extract (GE)	Antibiotics with 25% (GE)	Antibiotics with 50% (GE)	Antibiotics with 75% (GE)	Antibiotics with 100% (GE)
Ampicillin (2µg)	29 mm(S)	30mm	30mm	30mm	30 mm
Clindamycin (2µg)	32mm (s)	35mm	35mm	35mm	35 mm
Linezolid (30µg)	32 mm(S)	32mm	33mm	33mm	35mm
Methicillin (5µg)	30 mm(S)	18mm	29mm	30mm	29mm
Teicoplanin 30µg)	15 mm(S)	20mm	21mm	25mm	25mm
Tobramycin (10µg)	11 mm(R)	13mm	15mm	23mm	25mm
Vancomycin (10µg)	20 mm (s)	21 mm	23mm	26mm	28mm

In 2008, Ron Cutler and the University of East London found that allicin was far more effective as compared to multiple antibiotics against MRSA. Also they have documented that the combination of allicin with antibacterial could inhibit both antibiotic susceptible and antibiotic-resistant pathogenic bacteria and most combination are synergistic.

Conclusion: It is concluded that overall synergistic activity of garlic with different antibiotics used against *S.aureus* was effective. So it is stress that more and more natural product should be used in clinical treatment and general public awareness programmes should be carried out to encourage the layman in avoid misuse of life saving drugs.

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