

Synergistic Activity of Garlic with Ampicillin against *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus is one of the most frequent causes of skin infections with the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), to multiple antimicrobial agents. Garlic is used as natural spice use as a medicine as well, against many clinical pathogens. Garlic (*Allium sativum*) is claimed to retard development of resistance and produce synergy with conventional antibiotics. The study was conducted to investigate the inhibitory effect of alcoholic extract of Garlic on inhibition of growth of *Staphylococcus aureus* as well as synergistic activity of fresh garlic extract with ampicillin. We screened the clinical isolates of *S. aureus* for susceptibility/resistance against commonly prescribed antibiotics by using agar well diffusion method, disk diffusion method, minimum inhibitory concentration (MIC) Furthermore, we tested garlic solely and in combination with ampicillin to find possible synergistic antimicrobial effects by checkerboard method. *Staphylococcus aureus* became resistant to standard antibiotic ampicillin and gave zone of inhibition 8mm and 10 mm which is considered as resistant whereas, zone of garlic are 21mm and 25mm. The MIC of garlic is obtained at the concentration of 14.4 mg/ml and through checkerboard the result we obtained indicates the highly synergistic activity of garlic and ampicillin. Statistical analysis of garlic synergism with ampicillin had been proved. Garlic can be used as additive in the preparation of different antibiotics to produce their effectiveness against different pathogens.

Keywords: Antibacterial, antibiotic, resistance, *Staphylococcus aureus*, synergism

INTRODUCTION

Currently antimicrobial agents are the most widely utilized and the most generally recommended drugs, in medicinal field, particularly for the treatment of uncontrolled diseases. This is principally on the grounds that when all is said in done they work perfectly against microscopic organisms, simple to convey, and are of few symptoms. In any case, now a large portion of the microorganisms will indicate far reaching resistance against antibiotics (Wilke *et al.*, 2005). In 1961, the primary MRSA was found among *S. aureus* clinical confines. Methicillin resistant *Staphylococcus aureus* (MRSA) is a bacterium in charge of a few hard to-treat diseases in people. It is likewise called oxacillin resistant *Staphylococcus aureus* (ORSA). Dental infection incidence is regularly connected

with a decrease of some oral bacterial strains are connected with dental caries and normally found on the surface of hard dental tissues (Bowden, *et al.*,1975).

Devastating increase of antimicrobial resistance advanced the need of utilizing natural compound. Hence health care providers made an eye towards medicinal plants; numerous medicinal plants are recorded to have antimicrobial potential (Slots and Taubman, 1992). Among which *Allium sativum*, (Garlic) having a place with Alliaceae family, aside from cooking is likewise known for its medicinal qualities and beforehand documented as folk medicine in ancient period. Garlic has the power of killing and inhibiting the growth of pathogens and microorganisms like bacteria and parasites therefore it is said to have antimicrobial activity. Louis Pasteur was

the main portrayed the antibacterial impact of onion and garlic juice (Whitemore *et al.*, 2000). Its typical pungent odor and antibacterial activity rely on upon allicin, which is delivered by enzymatic (allicin lyase) hydrolysis of allicin in the wake of cutting and crushing of the cloves (Ellmore and Feldberg, 1994).

Allicin is the precursor of sulfur-containing substances, which are in charge of the flavor, smell and pharmacological properties. When presented to air, allicin is further changed over into diallyldisulphide, which possess antibacterial impacts, and the diminishment by cysteine will disturb the disulphide bond in microbial proteins (Cutler and Wilson, 2004). Allicin, the dynamic element of *A. sativum* has been accounted for to have a scope of potential targets. Garlic likewise used for the safeguarding of sustenance items because of its germicide and disinfectant properties (De *et al.*, 1999). Garlic has been proposed to display synergistic or added substance impact with customary antimicrobial operators against *E. coli* and *S. aureus* (Karuppiyah and Rajaram, 2012). Traditionally garlic has been utilized to treat intestinal diseases, particularly related with digestive issues (Abubakar, 2009). Garlic treats infections of the skin, digestive tract and respiratory pathways. Crude garlic concentrates and garlic supplements in the treatment of stomach ulcers. Some individuals use garlic to counteract colon growth, rectal malignancy, stomach disease, bosom tumor, prostate disease, and lung malignancy. It is likewise used to treat prostate growth and bladder cancer. This study concentrated on seven of the most perilous superbugs called multidrug resistant microbes. Henceforth, the present study meant to assess the adequacy of antimicrobial activity of fluid garlic separate against different microorganisms like *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Preparation of drugs: Stock solution can be made at a standard concentration, e.g. 100mg/mL for Ampicillin and filter sterilizes to achieve. 20 μ L of solution was loaded on each disk, so we need a solution with a final concentration of 0.5mg/mL (0.5 μ g/ μ L).

Preparation of fresh garlic extract: Take 100gm of garlic bulb from market and peeled off. Then crushed in mortar and pestle and allowed to stand for 10 min. Squeezed out using sterile cheesecloth. This filtrate was considered 100% FGE and was stored at -20°C . The extract was prepared freshly every week.

Sample collection: *Staphylococcus aureus* were isolated in the laboratory from the samples of wound, pus and blood collected from the different clinical wards.

Isolation of pure culture: Mannitol Salt agar was used for the isolation of *Staphylococcus aureus*. Different biochemical tests were performed for the confirmed identification of *Staphylooccus aureus*.

Antibiotic susceptibility test: For the antibiotic susceptibility test. Wells of 5 mm were cut on the surface of the agar. A volume of 100 μ l of FGE was added to 4 different wells. In one well normal saline was added. The plates were incubated at 37°C for 24 h. Zone of inhibition was measured by a scale to the nearest mm including disc diameter.

Broth dilution method for determination of minimum inhibitory concentration: 1 ml of standard inoculum of the microorganism was added to each tube containing different concentrations of the garlic extract (15-360 mg/ ml), the final volume of 2 ml of broth. After 24 h of incubation, the tubes were visually examined for turbidity. MIC was determined as the highest concentration of the drug which prevented the visible growth of test isolates.

Checkerboard method for the combination:

Antimicrobial effect of combinations consisting of FGE with ampicillin was assessed by checkerboard method test. Serial dilutions of ampicillin (0.6-32 µg/ml) were mixed in a test tube containing varying concentration of FGE (15-180 mg/ml) in 2 ml of MH broth. A volume of 1 ml of standard inoculum of the microorganism was added to each tube after 24 h of incubation at 37°C.

RESULTS

In the study, we obtained gram +ve, purple colour cocci in grapes like cluster. Gas formation (O₂) in the form bubbles shows that *S.aureus* is catalase +ve. *S.aureus* produces the enzyme catalase this enzyme detoxify hydrogen peroxide (H₂O₂), which is formed from the superoxide radical by superoxide dismutase. No color change or color change after more than 30 sec indicates *S. aureus* is oxidase -ve. The plasma is converted into stiff gel that remains in place when the tube is tilted or inverted this indicates that *S. aureus* is coagulase +ve. In well diffusion method, *S. aureus* showed statistically significant dose dependent increase in the zone

of inhibition at FGE and higher compared with Increasing concentration of FGE increases zone of inhibition. As per well diffusion method, this concentration of FGE produces mean zone of inhibition of 20mm-25mm. Minimum inhibitor concentration of fresh garlic extract (FGE) against *S. aureus* was determined using broth dilution method. MIC value of FGE ranged from 1:5, 1:10 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, :1:50, 1:60. According to our results MIC of garlic is 1:25.

DISCUSSION

Methicillin resistant *Staphylococcus aureus* (MRSA) is very widespread in healthcare settings world wide. Current surfacing of community-associated (CA) MRSA worldwide has further alarmed to the epidemiology of *S. aureus* infections. Scientists from divergent fields are investigating plants to their antimicrobial usefulness. Here are 10 plants which were found to be useful for antibacterial aid. They are local as well (Calendula, Cannabis, Cayenne, Garlic, Peppermint, Rose, St. John's wort, Tea Tree & Cedar).

Researchers have indicated *S. aureus* as a

Table I. Biochemical characterization of isolates

Organism	Biochemical test	Results
<i>S. aureus</i>	Catalase	Bubble appears indicates positive result.
<i>S. aureus</i>	Oxidase	No colour indicates negative result.

Table II. Standard zone diameter Interpretation of Ampicillin

Standard zone diameter Interpretation of Ampicillin			
Ampicillin	Susceptible	Intermediate	Resistant
<i>S. aureus</i>	>26	23-25	<22

Table III: Antimicrobial Activity of Garlic and Ampicillin against *S. aureus*

Inhibition zone diameter		
Organism	Garlic	Ampicillin
<i>S. aureus</i>	21mm	8mm
<i>S. aureus</i>	25mm	10mm

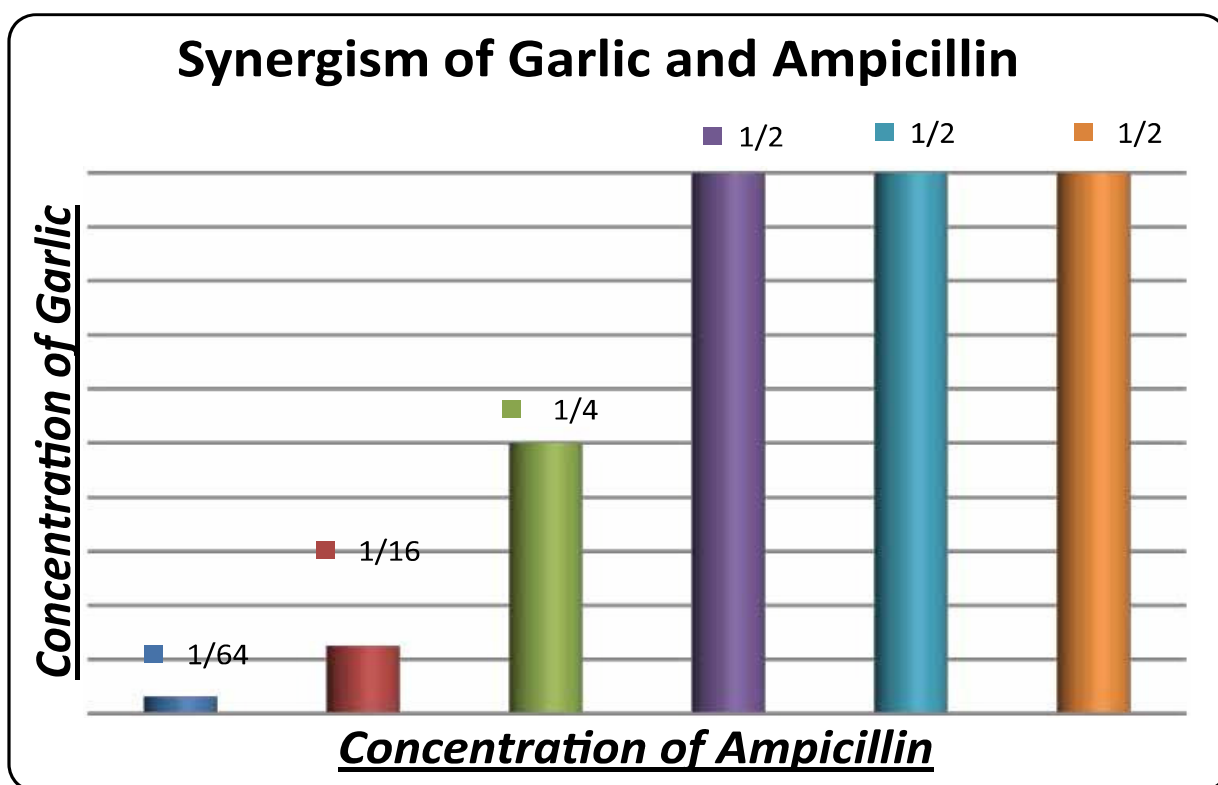


Figure 1. Synergism activity of Ampicillin and Garlic

extremely sensitive bacterium to aqueous and ethanol extracts. As we performed experiment on antibacterial activity of garlic against ampicillin resistant *Staphylococcus aureus* and observed positive results of garlic on ampicillin resistant *Staphylococcus aureus* as well as synergism activity of garlic with ampicillin against MDR's *Staphylococcus aureus* which is corresponding to previous studies (Bakri and Douglas, 2005; Hall *et al.*, 1983).

This has been proved in research that garlic gave the zone of range 21mm-25 mm. which indicates highly resistivity from garlic. As well as synergism activity of garlic and Ampicillin check through checkerboard method indicates that we can use garlic as an additive in medicines to increase their susceptibility against different bacterial species. Foremost part of garlic determined to be accountable for a great deal of the therapeutic and antimicrobial properties of garlic is Allicin. Allicin found in compressed garlic has an inhibitory effect against broad

spectrum of microorganisms including gram positive as well as gram negative bacteria.

CONCLUSION

The results suggest that fresh garlic extract (FGE) can improve the antibiotic sensitivity of *S. aureus* to some antibiotics. Thus, combinations of FGE with ampicillin can improve sensitivity of *S. aureus* to ampicillin thereby can help conserving the usage of vancomycin. This will not only significantly reduce the cost of the therapy but also significantly lower the adverse drug reactions due to potentially toxic drugs. However, further detailed pharmacokinetic and pharmacodynamic studies are needed to study the effect of combination of FGE with various antimicrobials *in vivo*.

REFERENCES

Abubakar EMM. 2009. Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *Escherichia coli*,

- Staphylococcus aureus, Streptococcus pneumoniae and *Pseudomonas aeruginosa*. *J. Med. Plants Res.*, 3(4): 179-185.
- Bakri IM and Douglas CWI. 2005. Inhibitory effect of garlic extract on oral bacteria. *Arch. of Oral Biol.*, 50(7): 645-651.
- Bowden GH, Hardie JM and Slack GL. 1975. Microbial variations in approximal dental plaque. *Caries research*, 9(4): 253-277.
- Cutler RR and Wilson P. 2004. Antibacterial activity of a new, stable, aqueous extract of allicin against methicillin-resistant *Staphylococcus aureus*. *BJMS*, 61(2): 71-74.
- De M, De K and Banerjee AB. 1999. Antimicrobial screening of some Indian spices. *Phytotherapy Res.*, 13(7): 616-618.
- Ellmore GS and Feldberg RS. 1994. Allin lyase localization in bundle sheaths of the garlic clove (*Allium sativum*). *Amer. J. Bot.*, 81(1): 89-94.
- Hall MJ, Middleton RF and Westmacott D. 1983. The fractional inhibitory concentration (FIC) index as a measure of synergy. *J. Antimicro. Chemother.*, 11(5): 427-433.
- Karuppiah, P. and Rajaram, S., 2012. Antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. *Asian Pacific J. Trop. Biomed.*, 2(8): 597-601.
- Slots J and Taubman MA. 1992. *Contemporary oral microbiology and immunology*. Mosby Year Book.
- Whitemore BB, Naida AS. 2000. Thiosulfinate In: Naida A.S (Ed). *Natural food antimicrobial systems* Boca Roton, FL: CRC press. pp. 265-380.
- Wilke MS, Lovering AL and Strynadka NCJ. 2005. β -Lactam antibiotic resistance: a current structural perspective. *Curr. Opin. Microbiol.*, 8(5): 525-533.