Research Article

Studies on *Invitro* Synergistic Antibacterial Activity of Amoxycillin and *Allium sativum* on Streptomycin and Rifampicin Resistant Strains

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ABSTRACT
In the present study focuses the significant antibacterial activity of Garlic (*Allium sativum* Linn.) extract on streptomycin and Rifampicin resistant strains alone or in synergism with Amoxycillin. Gram positive *Staphylococcus aureus* ATCC BAA 1026 and gram negative *Escherichia coli* ATCC 10536 were made resistant to standard antibiotics streptomycin and rifampicin. Amoxycillin is used as control in the experiment. Zones of inhibition of different treatment groups were measured by agar well diffusion assay and compared with control. Statistical comparison of sole extract and Amoxycillin synergism with Amoxycillin control had proved it significantly.

Keywords: Antibacterial, *Escherichia coli*, *Staphylococcus aureus,* Amoxycillin, *Allium sativum*.

INTRODUCTION

Number of antimicrobial agents are release to market every year & number of antimicrobial agents withdrawn from market every year due to several reasons (i.e., safety, bacterial resistance, adverse drug reactions etc). *Allium sativum* been used worldwide as a spice, food, and folk medicine. It contains alliin as a major sulfur containing compound. When the raw garlic clove is damaged, alliin is hydrolyzed to sulfenate, pyruvate, and ammonia by allinase. Condensation of 2 mol of sulfenate gives allicin a major sulfur containing intermediate, which was isolated and identified as an antibacterial substance[1].

Garlic (*Allium sativum* Linn.) has an important dietary and medicinal role for centuries. Its therapeutic uses include beneficial effects on the cardiovascular system, antibiotic, anti cancer, anti-inflammatory, hypoglycemic and hormone-like effects[2]. Garlic extracts have been used to treat infections for thousands of years[3]. There is great concern about the worldwide increase in antibiotic resistance especially of gram positive bacteria. *Allium sativum* especially the thiosulfinate allicin has been known for its antibacterial activity against gram positive and gram negative organisms[4].

Amoxycillin was widely used for more than three decades which is still effective against gram positive and gram negative bacteria. This study reveals better efficacy of garlic extract and Amoxycillin synergism on Streptomycin and Rifampicin resistant strains.

MATERIALS AND METHODS

Source of bacterial strains

Both Gram positive *Staphylococcus aureus* ATCC BAA 1026 and gram negative *Escherichia coli* ATCC 10536 were collected from clinical specimens of Padmashree Diagnostic Centre, Tumkur.

Development of Rifampicin resistance in selected bacterial strains

Each organism was sub cultured from a nutrient agar (Qualigens Fine Chemicals, Mumbai) slant to Standard methods broth (Human Diagnostic and Surgichem, Kolkota), PH 7.8, and incubated overnight. With stock solutions of standard antibiotics Streptomycin and Rifampicin (gift sample on request from Karnataka Antibiotics and Pharmaceuticals Limited, Bangalore) which were prepared by diluting weighed aliquots of this drug in sterile 1 per cent phosphate buffer PH 6.0, two fold dilutions were prepared daily. The dilution series were usually consisted of ten 100 X 13 mm test tubes each containing 0.5 ml of the antibiotic dilution. To each tube was added 1.5 ml of a 1:100 dilution in broth of the 18-24 h broth culture prepared above and all the tubes were incubated at 37 ^{o}C for 24 h. The last tube showing inhibition of the organism in the dilution series indicated the initial sensitivity of the strain in micrograms of the antibiotic. The second tube showing growth in dilution series was selected for preparing 1:100 broth dilutions for the second exposure to streptomycin dilution series. To increase the resistance of the strain to the particular antibiotic, the procedure described above was repeated[5]. Same procedure is used to produce Rifampicin resistance strains.

Preparation of aqueous garlic extract

Fresh garlic (*Allium sativum* L.) bulbs were purchased from local market. The bulbs were peeled, weighed (100 gm) and cleaned. Cleaned bulbs were surface-sterilized by immersing them into 70 % v/v ethanol for 60 seconds[6]. Residual ethanol on surface was evaporated in sterile laminar air flow chamber followed by homogenizing aseptically in sterile mortar and pestle. The homogenized mixture was filtered through sterile cheese cloth. This extract was considered as the 100 % concentration of the extract. The concentrated mother extract was further diluted to 75 % and 50 % by mixing with appropriate sterile distilled water[7].

Testing of antibacterial activity using agar well diffusion method

Resistant bacterial strains were inoculated into 10 ml of sterile nutrient broth, and incubated at 37^{0}C for eight hours. Each culture was swabbed on the surface of sterile nutrient agar plate in duplicate. In each agar plate of both sets, five wells were prepared with the help of sterilized cork borer of 10 mm diameter. In the wells of first plate, 100 µl test samples (1. Standard amoxycillin 10 mg/ml in sterile distilled water, 2. 50 % sterile garlic extract, 3. Amoxycillin 10 mg/ ml in 50 % sterile garlic extract, 4. Amoxycillin 10 mg/ ml in 75 % sterile garlic extract, 5. Amoxycillin 10 mg/ ml in 100 % sterile garlic extract) were added by using micropipette. In the wells of second plate of each set, 100 µl test samples (1. Standard Amoxycillin 10 mg/ml in sterile distilled water, 2. 50 % sterile garlic extract, 3. Amoxycillin 10 mg/ ml in 50 % sterile garlic extract, 4. Amoxycillin 15 mg/ ml in 50 % sterile garlic extract, 5. Amoxycillin 20 mg/ ml in 50 % sterile garlic extract) were added. Every plate used according to above mentioned procedure was performed in triplicate for statistical average[8].

Fig. 1: G1- 50 % garlic extract, G2- 75 % garlic extract, G3- 100 % garlic extract. The concentration of Amoxycillin alone and in G1, G2, G3 etc. is 10 mg/ mL. Concentration of garlic used alone is 50%

Fig. 2: G1- 50 % garlic extract, G2- 75 % garlic extract, G3- 100 % Garlic extract. The concentration of Amoxycillin alone and in G1, G2, G3 etc. is 10 mg/ml. Concentration of garlic used alone is 50 %

RESULTS AND DISCUSSION

Mean zones of inhibition were expressed in mm ± SEM. Mean zones of inhibition of different treatment groups were measured by agar well diffusion assay and compared with control. Statistical comparison of sole garlic extract and amoxycillin synergism (same concentration of amoxycillin in garlic extract of different strengths and different concentration of amoxycillin in garlic extract of same strength as stated in table 1 and table 2 respectively) with amoxycillin control by one-way ANOVA post-test using the software graphpad Instat 3 (trial) had proved it significant. Figure 1 and 2 say illustrate table 1where figure 3 and 4 do the same for table 2.

The findings of the present work reveal the distinct antibacterial profile of *Allium sativum* Linn. solely and in amoxycillin synergism against streptomycin and Rifampicin-resistant *Staphylococcus aureus* ATCC BAA 1026 and *Escherichia coli* ATCC 10536 as witnessed from prominent zones of inhibition. *E. coli* is a common pathogenic bacterium for urinary tract infection and *S aureus* is the cause of pneumonia and several infections in gut, urinary tract etc. Use of garlic extract solely is fruitful. Synergistic use can prevent the pathogenic organism grow their resistance against antibiotic.

Fig. 3: A1- 10 mg/ml, A2- 15 mg/ ml, A3- 20 mg/ml of Amoxycillin, the concentration of garlic extract used alone and with A1, A2, A3 etc. is 50 % garlic extract. Concentration of Amoxycillin used alone is 10 mg Per ml

Fig. 4: A1- 10 mg/ ml, A2- 15 mg/ ml, A3- 20 mg/ml of Amoxycillin the concentration of garlic extract used alone and with S1, S2, S3 etc. is 50% garlic extract. Concentration of Amoxycillin used alone is 10 mg/ml

Table 1: Inhibition of resistant bacteria due to sole garlic extract and synergism of same concentration of rifampicin in garlic extract of different strengths in presence of rifampicin control

S. No.	Drug	Dose	Zone of inhibition (mm ± SEM)	
			***Staphylococcus aureus* ATCC BAA 1026**	**Escherichia coli ATCC 10536**
1	Amoxycillin # (control)	10 mg/ml	14±0.5774	13.5±0.5774
2	Sterile garlic extract#	50 %	19±0.5775**	19.5±0.2887**
3	Amoxycillin ##	10 mg/ml	21±0.2887**	22±0.2887**
4	Amoxycillin ###	10 mg/ml	22.5±0.2887**	22.5±0.2887**
5	Amoxycillin ####	10 mg/ml	23.5±0.2887**	24±0.5774**

solvent- sterile distilled water, ## solvent- 50 % sterile garlic extract, ### solvent- 75 % sterile garlic extract, #### solvent- 100 % sterile garlic extract, ** P< 0.01 as compared with control according to one-way ANOVA.

Table 2: Inhibition of resistant bacteria due to sole garlic extract and synergism of different concentration of amoxycillin in garlic extract of same strength in presence of amoxycillin control

S. No.	Drug	Dose	Zone of inhibition (mm ± SEM)	
			***Staphylococcus aureus* ATCC BAA 1026**	**Escherichia coli ATCC 10536**
1	Amoxycillin # (control)	10 mg/ml	13±0.2887	13±0.2887
2	Garlic extract#	50 %	20±0.2887**	19±0.2887**
3	Amoxycillin ##	10 mg/ml	21.5±0.2887**	22±0.2887**
4	Amoxycillin ##	15 mg/ml	24±0.2887**	23.5 0.5774**
5	Amoxycillin ##	20 mg/ml	26±0.5774**	25±0.2887**

solvent-sterile distilled water, ## solvent- 50 % sterile garlic extract, ** P< 0.01 as compared with control according to one-way ANOVA.

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