Antibacterial activity of garlic extract on streptomycin-resistant *Staphylococcus aureus* and *Escherichia coli* solely and in synergism with streptomycin

M. N. Palaksha, Mansoor Ahmed¹, Sanjoy Das

Aditya Institute of Pharmaceutical Sciences and Research, A.D.B. Road, Aditya Nagar, Surampalem, East Godavari District, Andhra Pradesh, ¹Department of Pharmacology, Sri Siddaganga College of Pharmacy, B. H. Road, Tumkur, Karnataka, India

Address for correspondence:
Mr. M. N. Palaksha, Aditya Institute of Pharmaceutical Sciences and Research, A. D. B. Road, Aditya Nagar, Surampalem, East Godavari District, Andhra Pradesh – 533 437, India.

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Abstract

This study focuses the significant antibacterial activity of Garlic (*Allium sativum* Linn.) extract on streptomycin-resistant strains solely and in synergism with streptomycin. Gram-positive *Staphylococcus aureus* ATCC BAA 1026 and gram-negative *Escherichia coli* ATCC 10536 were made resistant to standard antibiotic streptomycin used as a 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 streptomycin synergism with streptomycin control had proved it significant.

**Key words:** Antibacterial, antibiotic, *Escherichia coli*, resistance, *Staphylococcus aureus*, synergism

INTRODUCTION

Since ancient time, naturally occurring plants have played an important role in the discovery of new therapeutic agents.[1] Almost all antibiotics are subjected to the problem of bacterial resistance. Therefore, newer herbal antibacterial compounds from plants and their semisynthetic derivatives to overcome the resistance are under investigation.

Garlic (*Allium sativum* Linn.) has an important dietary and medicinal role for centuries. Its therapeutic uses include beneficial effects on the cardiovascular system, antibiotic, anticancer, anti-inflammatory, hypoglycemic, and hormone-like effects.[2] Garlic extracts have been used to treat infections for thousands of years.[3] Its typical pungent odor and antibacterial activity depend on allicin, which is produced by enzymatic (alliin lyase) hydrolysis of alliin after cutting and crushing of the cloves.[4]

Streptomycin was widely used for more than four decades, which is still effective against gram-positive and gram-negative bacteria. This study reveals better efficacy of garlic extract and its streptomycin synergism than streptomycin on 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, India.

Development of streptomycin resistance in selected bacterial strains

Each organism was subcultured from a nutrient agar (Qualigens Fine Chemicals, Mumbai, India) slant to standard methods broth (Human Diagnostic and Surgichem, kolkata, India), PH 7.8, and incubated overnight. With stock solutions of standard antibiotic streptomycin (gift sample on request from Karnataka Antibiotics and Pharmaceuticals Limited, Bangalore, India), which were prepared by diluting weighed aliquots of this drug in
sterile 1% phosphate buffer PH 6.0, twofold dilutions were prepared daily. The dilution series were usually consisted of ten 100×13 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°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.[1]

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 cloves were surface-sterilized by immersing them into 70% (v/v) ethanol for 60s.[6] Residual ethanol on surface was evaporated in sterile laminar airflow chamber followed by homogenizing aseptically in sterile mortar and pestle. The homogenized mixture was filtered through sterile cheesecloth. 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°C for 8 h. 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 of each set, 100 μl test samples of following concentrations: (1) standard streptomycin 10 mg/ml in sterile distilled water; (2) 50% sterile garlic extract; (3) streptomycin 10 mg/ml in 50% sterile garlic extract; (4) streptomycin 10 mg/ml in 75% sterile garlic extract; (5) streptomycin 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 of the following concentrations: (1) standard streptomycin 10 mg/ml in sterile distilled water; (2) 50% sterile garlic extract; (3) streptomycin 10 mg/ml in 50% sterile garlic extract; (4) streptomycin 15 mg/ml in 50% sterile garlic extract; (5) streptomycin 20 mg/ml in 50% sterile garlic extract) were added. Every plate used according to the aforementioned procedure was performed in triplicate for statistical average.

RESULTS
Mean zones of inhibition were expressed in mm ± standard error of mean. Mean zones of inhibition of different treatment groups were measured by agar-well-diffusion assay and compared with the control. Statistical comparison of sole garlic extract and streptomycin synergism (same concentration of streptomycin in garlic extract of different strengths and different concentration of streptomycin in the garlic extract of same strength as stated in Tables 1 and 2, respectively) with streptomycin control by one-way ANOVA post-test using the software Graphpad Instat 3 (trial) had proved it significant. Figures 1 and 2 illustrate Table 1 whereas Figures 3 and 4 do the same for Table 2.

DISCUSSION
The findings of this study reveal the distinct antibacterial profile of Allium sativum Linn. solely and in streptomycin synergism against streptomycin-resistant S. aureus ATCC BAA 1026 and E. coli ATCC 10536 as witnessed from prominent zones of inhibition. E. coli is a common pathogenic bacteria 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.

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

| Drug, Dose | Zone of inhibition (mm ± S.E.M.) | S. aureus ATCC BAA 1026 | E. coli ATCC 10536 |
|-----------|---------------------------------|--------------------------|---------------------|
| Streptomycin<sup>a</sup> (control) 10 mg/ml | 7 ± 0.2887 | 8 ± 0.2887 |
| Sterile garlic extract<sup>b</sup> 50% | 14 ± 0.5775<sup>c</sup> | 14.5 ± 0.2887<sup>c</sup> |
| Streptomycin<sup>b</sup> 10 mg/ml | 15 ± 0.2887<sup>c</sup> | 16 ± 0.2887<sup>c</sup> |
| Streptomycin<sup>b</sup> 10 mg/ml | 22 ± 0.2887<sup>c</sup> | 24 ± 0.5774<sup>c</sup> |
| Streptomycin<sup>b</sup> 10 mg/ml | 26 ± 0.2887<sup>c</sup> | 28 ± 0.2887<sup>c</sup> |

*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 post-test.

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

| Drug, Dose | Zone of inhibition (mm ± S.E.M.) | S. aureus ATCC BAA 1026 | E. coli ATCC 10536 |
|-----------|---------------------------------|--------------------------|---------------------|
| Streptomycin<sup>a</sup> (control) 10 mg/ml | 6 ± 0.2887 | 7 ± 0.2887 |
| Garlic extract<sup>b</sup> 50% | 15 ± 0.2887<sup>c</sup> | 14 ± 0.2887<sup>c</sup> |
| Streptomycin<sup>b</sup> 10 mg/ml | 16 ± 0.2887<sup>c</sup> | 15 ± 0.2887<sup>c</sup> |
| Streptomycin<sup>b</sup> 15 mg/ml | 19 ± 0.5774<sup>c</sup> | 17 ± 0.2887<sup>c</sup> |
| Streptomycin<sup>b</sup> 20 mg/ml | 20 ± 0.2887<sup>c</sup> | 19 ± 0.5774<sup>c</sup> |

*Solvent: sterile distilled water. **Solvent: 50% sterile garlic extract. ***P < 0.01 as compared with control according to one-way ANOVA post-test.
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Synergistic use can prevent the pathogenic organism grow their resistance against antibiotic.

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