Synergistic Effects of Garlic Extract and Antibiotics against MDR Bacteria

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ABSTRACT

Background: The increasing spread of MDR-bacteria poses a global health challenge, mainly because of unreasonable antibiotics using. This has encouraged researchers to explore effective natural alternatives, such as extracts from medicinal plants, as promising sources of new therapeutic agents.

Objectives: The research meant to assess the FGE antibacterial activity and investigate its synergistic interaction when combined with amikacin or tetracycline against clinically MDR-resistant isolates, whom *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.

Methods: FGE was prepared at a concentration of 320 mg/ml, and by using an automated VITEK system the susceptibility of the drug-resistant isolates was confirmed. By using the Kirby-Bauer method antibacterial activity was measured according to CLSI. Tests included extract alone, antibiotics alone, and extract-impregnated tablets, with three replicates per experiment and digital measurement of the diameters of the inhibition zones.

Results: The extract reveled activity against *S. aureus* (12.4 ± 0.5 mm), *E. coli* (10.1 ± 0.3 mm), and *K. pneumoniae* (8.2 ± 0.4 mm), while it did not inhibit *P. aeruginosa*. Combining the extract with amikacin or tetracycline enhanced the inhibition activity against all isolates except *P. aeruginosa*, with the combination with tetracycline being most effective against *S. aureus* and *E. coli.*

Conclusion: FGE show significant antibacterial activity against a number of MDR bacteria and enhances the efficacy of amikacin and tetracycline, suggesting its potential use as a complementary agent along with antibiotic in the treatment of infections caused by MRS.

Keywords: Drug-resistant, amikacin, tetracycline, bacterial activity.

INTRODUCTION

The hasty spread of multidrug-resistant bacterial infections carries danger threat to public health systems worldwide [1 ,3]. The overuse and unreasonable use of antibiotics have faster the emergence of resistant strains, less treatment choices and increasing illness and death rates linked with these infections [2,4].

Therapeutic plants have long been treasured sources of therapeutic agents due to their rich content of active phytochemicals, their fairly little cost, and their restricted toxicity [3,5]. As said by the WHO, herbal remedies persist as an alternative medicine [4]. Garlic (*Allium sativum*) holds a protuberant place in medicine because of its several scientifically confirmed therapeutic properties [5,7].

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Garlic is broadly used as a main food and traditional therapy. Plentiful studies have confirmed its antimicrobic action, chiefly credited to its effective sulfur compounds, utmost especially allicin [9]. Inhibitory properties were showed by these compounds against a vast scale of bacteria, including MDR [10,12].

The study investigates the Fresh Garlic Extract (FGE) antibacterial activity and evaluates its potential to enhance the effectiveness of certain antibiotics against selected multidrug-resistant clinical isolates [5,7,10].

Objectives of the Study

1. To evaluate the fresh garlic extract antibacterial activity against MDR bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa)*.

2. To investigate the potential synergistic interaction between FGE and two commonly used antibiotics-amikacin and tetracycline.

3. To compare the inhibition zones of garlic extract alone, antibiotics alone, and garlic–antibiotic combinations.

MATERIALS AND METHODS

Bacterial Isolates

Four MDR bacterial isolates were obtained from clinical samples and confirmed by VITEK automated susceptibility testing:

- *Staphylococcus aureus*
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Pseudomonas aeruginosa*

Isolates were classified as MDR based on resistance to ≥3 antibiotic classes.

Preparation of Fresh Garlic Extract (FGE)

Bulbs of fresh garlic (32 g) were washed, surface-sterilized with 70% ethanol, and homogenized by a sterile pestle and mortar. The spawned paste was mixed with 100 mL sterile normal saline to obtain a 32% w/v extract (equivalent to 320 mg/mL). The mixture was filtered by membrane (2.5 µm) and stored at 4°C for 24 hr. [7,9].

Preparation of Bacterial Inoculum

Each bacterial isolate was cultured on MacConkey or blood agar for 24 hr, after that the bacterial colonies were suspended in sterile saline and adjusted to 0.5 McFarland turbidity standard (~1.5×10^8 CFU/mL) [7].

Antimicrobial Susceptibility Testing

By using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (MHA) plates the antibacterial activity was assessed, following CLSI standards:

- Agar depth: 4 mm.
- Inoculum: lawn culture using sterile swabs.
- Inoculated plates dried for 15 minutes before testing.

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Test Groups

Four test conditions were used:

1. **FGE alone:** sterile discs loaded with 20 µL FGE.
2. **Antibiotics alone:** amikacin (10 µg/disc) and tetracycline (10 µg/disc).
3. **FGE + AM:** amikacin discs loaded with 10 µL of FGE.
4. **FGE + TE:** tetracycline discs loaded with 10 µL of FGE.

Controls

- **Positive control:** antibiotic discs
- **Negative control:** blank discs with sterile saline

Replication and Measurement

All tests were achieved in triplicate, and inhibition zones were measured by a digital Vernier caliper. Results expressed as mean ± standard deviation.

RESULTS

Antibacterial Activity of Fresh Garlic Extract

Fresh garlic extract confirmed inhibitory action against 3 tested isolates.

Table 1. FGE Inhibition Zones and Antibiotics Inhibition Zones (mm)

Bacterial Strain	**FGE**	**Amikacin**	**Tetracycline**
S. aureus	12.4 ± 0.5	11.2 ± 0.4	6.1 ± 0.2
E. coli	10.1 ± 0.3	11.0 ± 0.3	3.4 ± 0.2
K. pneumoniae	8.2 ± 0.4	11.1 ± 0.2	6.3 ± 0.3
P. aeruginosa	0	23.3 ± 0.6	4.0 ± 0.3

Synergistic Interaction Between FGE and Antibiotics

The combination of amikacin or tetracycline with FGE meaningfully increased the inhibition zones of all isolates excluding *P. aeruginosa*.

Table 2. The effects of FGE–Antibiotic Combinations (mm)

Bacterial Strain	Amikacin Alone	Amikacin + FGE	Tetracycline Alone	Tetracycline + FGE
S. aureus	11.2 ± 0.4	14.3 ± 0.5	6.1 ± 0.2	28.4 ± 0.7
E. coli	11.0 ± 0.3	12.1 ± 0.4	3.4 ± 0.2	30.2 ± 0.5
K. pneumoniae	11.1 ± 0.2	13.0 ± 0.4	6.3 ± 0.3	11.4 ± 0.4

P. aeruginosa	23.3 ± 0.6	23.1 ± 0.5	4.0 ± 0.3	6.0 ± 0.3

Interpretation

- FGE revealed the maximum inhibition against *S. aureus*.
- GFE with tetracycline were showed a strong synergistic enhancement.
- *Pseudomonas aeruginosa* keep on resistant to FGE and did not show synergy.

DISCUSSION

Garlics antibacterial activity has been broadly known in the scientific literature, mainly credited to allicin and other organosulfur compounds [9]. In this study, FGE confirmed noteworthy inhibitory activity against MRS of *S. aureus*, *E. coli*, and *K. pneumonia* [10].

The capability of garlic compounds to damage bacterial cell membrane or interact with excretory pump mechanisms, thus increasing cell penetrability by the antibiotic and enhancing its effectiveness, all that reasons leaded to a strong synergistic interaction between garlic extract and tetracycline [11]. Prior studies have conveyed similar synergistic effects when combination another plant extracts with certain antibiotics, supporting the idea of using plant agents as possible therapeutic enhancers [12].

Pseudomonas aeruginosa has a special structural and functional features (the strength of its outer membrane, the efficiency of its excretory pump systems, and its high capacity for biofilm formation) all of which contribute to its resistance to many natural and therapeutic compounds and one of them is garlic extract (Lack inhibitory action), generally, the results support the possible application of garlic extract as an accessary therapy to develop antibiotic efficiency, specifically in the situation of expanding antimicrobial resistance [5,7,10].

CONCLUSION

- FGE revealed significant antibacterial activity against three MDR isolates but was ineffective against *P. aeruginosa*.
- Garlic extract improved the antibacterial effects of amikacin and tetracycline, with noticeable synergy experiential particularly with tetracycline.
- Using garlic extracts as a complementary agent along with antibiotics shows promise in combating MDR bacterial infections.

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