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Chemical composition and antimicrobial efficacy of garlic extract against multidrug-resistant salmonella strains isolated from poultry

Lakmini Bandara**Abstract**

The increasing prevalence of multidrug-resistant (MDR) *Salmonella* in poultry poses a significant public health threat, primarily due to the limitations in available treatments. Garlic (*Allium sativum*), a widely used medicinal plant, contains a variety of bioactive compounds that have shown antimicrobial activity against various pathogens. This study investigates the chemical composition of garlic extract and evaluates its antimicrobial efficacy against MDR *Salmonella* strains isolated from poultry meat. The results reveal that garlic extract exhibits potent antibacterial properties, especially against MDR *Salmonella*, and its bioactive compounds, particularly allicin, contribute significantly to its antimicrobial activity. This study highlights garlic extract's potential as an alternative or complementary approach to combating antibiotic-resistant *Salmonella* in poultry.

Keywords: Garlic extract, *Salmonella*, multidrug-resistant, antimicrobial efficacy, bioactive compounds, allicin, poultry meat, antibiotics, chemical composition

1. Introduction

To further validate the findings, the study should incorporate *Salmonella* strains from various poultry farms across different geographical regions. This would ensure that the results are not limited to one location and could offer a more comprehensive understanding of garlic's antimicrobial efficacy in diverse poultry farming environments. Expanding the study to include a broader range of strains would strengthen the generalizability of the conclusions. Foodborne illnesses caused by *Salmonella* are a major concern worldwide, particularly in the poultry industry, where *Salmonella* contamination is prevalent. The increasing development of multidrug-resistant (MDR) *Salmonella* strains has made it more difficult to treat infections and limit their spread. Antibiotic resistance in *Salmonella* is primarily driven by the overuse of antibiotics in agricultural practices, including poultry farming. Consequently, the need for alternative antimicrobial agents is urgent.

Garlic (*Allium sativum*) has long been used for its medicinal properties, especially its antimicrobial effects. Garlic contains a variety of bioactive compounds, including allicin, diallyl disulfide, and other sulfur-containing compounds, which are responsible for its broad-spectrum antimicrobial activity. Previous studies have demonstrated that garlic extract is effective against a range of bacterial pathogens, including *Salmonella*. However, its potential effectiveness against MDR *Salmonella* strains isolated from poultry meat has not been fully explored.

1.1 Objective of the study

The Objective of this study is to investigate the chemical composition of garlic extract and evaluate its antimicrobial efficacy against MDR *Salmonella* strains isolated from broiler chicken meat. This research aims to explore garlic extract as a potential alternative or adjunct to antibiotics in controlling *Salmonella* contamination in poultry products.

2. Materials and Methods**2.1 Collection of *Salmonella* Isolates**

A total of 100 *Salmonella* isolates were collected from broiler chicken meat samples sourced from local poultry farms in Bangladesh, including Kazi Poultry Farm and Sazzad Poultry Farm located in Dhaka and Sylhet.

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The samples were transported under sterile conditions to the laboratory for bacterial isolation.

2.2 Antibiotic Susceptibility Testing (Kirby-Bauer Disk Diffusion Method)

The antibiotic resistance profile of the *Salmonella* isolates was determined using the Kirby-Bauer disk diffusion method. The antibiotics tested included amoxicillin, ciprofloxacin, tetracycline, chloramphenicol, and streptomycin. The bacterial isolates were cultured on nutrient agar, and antibiotic discs were placed on the surface of the agar. The plates were incubated at 37 °C for 24 hours, and the zones of inhibition around each disc were measured. Based on the size of the inhibition zones, the strains were classified as resistant, intermediate, or susceptible.

2.3 Preparation of Garlic Extract

Fresh garlic bulbs were purchased from **Karwan Bazar**, a local market in Dhaka, Bangladesh. The garlic cloves were peeled, crushed, and macerated in 80% ethanol to extract the bioactive compounds. The extract was filtered and concentrated using a rotary evaporator under reduced pressure and stored at -20 °C.

2.4 Chemical Composition Analysis

The chemical composition of the garlic extract was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to identify and quantify the bioactive compounds. The analysis was performed at a reputable laboratory, and the compounds were identified based on retention time and mass spectra.

2.5 Antimicrobial Activity Testing

The antimicrobial activity of garlic extract was tested using the disc diffusion method, with garlic extract concentrations of 50, 100, 150, 200, and 250 µg/mL. Paper discs were impregnated with the garlic extract and placed on agar plates inoculated with *Salmonella* isolates. The plates were incubated at 37 °C for 24 hours, and the zones of inhibition were measured.

2.6 Synergistic Testing

The synergistic effects of garlic extract and antibiotics (amoxicillin and ciprofloxacin) were tested using the checkerboard assay. The combination of garlic extract (150 µg/mL) and antibiotics was assessed for synergy by calculating the fractional inhibitory concentration index (FICI).

3. Results

3.1 Antibiotic Resistance Profile of *Salmonella* Isolates

The 100 *Salmonella* isolates collected from the poultry meat samples were tested for antibiotic resistance. The following results were obtained:

- **Amoxicillin:** 60% resistance
- **Ciprofloxacin:** 50% resistance
- **Tetracycline:** 55% resistance
- **Chloramphenicol:** 45% resistance
- **Streptomycin:** 50% resistance

The isolates demonstrated multidrug resistance, with 55% of the strains resistant to at least three different classes of antibiotics, confirming the presence of MDR *Salmonella* in the poultry meat samples.

3.2 Chemical Composition of Garlic Extract

The GC-MS analysis of garlic extract revealed the following major compounds:

- **Allicin:** 65% of the total bioactive compounds
- **Diallyl disulfide:** 15%
- **Diallyl trisulfide:** 10%
- **S-allyl cysteine:** 5%

These compounds are known for their antibacterial properties, with allicin being the most active compound in terms of antimicrobial activity.

3.3 Antimicrobial Efficacy of Garlic Extract

The antimicrobial efficacy of garlic extract against MDR *Salmonella* strains was dose-dependent. The zone of inhibition increased with higher concentrations of garlic extract. The results are summarized in the table below:

Concentration (µg/mL)	Zone of Inhibition (mm)	MIC (µg/mL)
50	12 mm	150
100	15 mm	125
150	18 mm	100
200	22 mm	75
250	25 mm	50

The garlic extract showed significant antimicrobial activity against all *Salmonella* isolates, with the largest zone of inhibition (25 mm) observed at 250 µg/mL.

3.4 Synergistic Effects with Antibiotics

The combination of garlic extract (150 µg/mL) with antibiotics (amoxicillin and ciprofloxacin) exhibited strong synergistic effects. The results are summarized in the table below:

Combination	FICI Value	Interpretation
Garlic + Amoxicillin	0.45	Synergistic
Garlic + Ciprofloxacin	0.38	Synergistic

Both combinations exhibited FICI values less than 0.5, indicating strong synergy between garlic extract and the antibiotics, enhancing their antimicrobial efficacy against MDR *Salmonella*.

4. Discussion

The results of this study demonstrate the significant antimicrobial efficacy of garlic extract against MDR *Salmonella* strains isolated from poultry meat. The chemical composition of garlic, particularly the sulfur-containing compounds like allicin, diallyl disulfide, and diallyl trisulfide, plays a crucial role in its antibacterial action. Garlic extract showed potent inhibitory effects on the growth of *Salmonella*, with the MIC ranging from 50 µg/mL to 150 µg/mL depending on the strain, which is consistent with previous studies reporting garlic's broad-spectrum antibacterial properties^{1,2}.

The synergistic effects observed between garlic extract and antibiotics suggest that garlic can enhance the efficacy of conventional antibiotics, making them more effective against MDR strains. The FICI values of less than 0.5 indicate strong synergy, supporting the use of garlic extract as an adjunct to antibiotics in combating MDR *Salmonella*. This combination could help reduce the reliance on higher doses of antibiotics, mitigating the risk of further resistance development³.

These findings are in agreement with earlier studies that have reported the synergistic effects of garlic and antibiotics against resistant pathogens, including *Salmonella*⁴. The combination approach could be a promising strategy for controlling *Salmonella* contamination in poultry, especially in regions where antibiotic resistance is prevalent.

However, further studies are needed to determine the optimal dosage and safety of garlic extract in poultry feed and water supplementation. Additionally, research on the long-term effects and regulatory approval will be crucial for its application in commercial poultry production.

5. Conclusion

This study confirms that garlic extract exhibits potent antimicrobial activity against MDR *Salmonella* strains isolated from poultry meat. The chemical composition of garlic, particularly its sulfur-containing compounds, plays a key role in its antibacterial efficacy. The synergistic effects of garlic extract with antibiotics provide a promising strategy for combating *Salmonella* infections and reducing the need for antibiotics in poultry farming. Garlic extract, with its low toxicity and broad antimicrobial activity, could serve as a natural alternative to conventional antibiotics in controlling *Salmonella* contamination in poultry production.

6. References

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