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Original article

Comparative Antibacterial Activity of Garlic (*Allium sativum*) Extracts and Conventional Antibiotics Against *Escherichia coli* Serotypes

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Abstract
The increasing prevalence of antibiotic-resistant *Escherichia coli* has prompted the search for alternative antimicrobial agents. This study evaluated the in vitro antibacterial activity of aqueous and ethanol extracts of garlic (*Allium sativum*) against four *E. coli* serotypes (O26, O111, O114, and O119) using the well diffusion method. Garlic extracts at concentrations ranging from 25% to 95% were compared with ten commonly used antibiotics. The results demonstrated a concentration-dependent antibacterial effect, with the 95% garlic extract producing inhibition zones of up to 30 mm, comparable to those observed for ceftriaxone and aztreonam. Several *E. coli* isolates exhibited resistance to lincomycin, neomycin, rifampicin, and cotrimoxazole. These findings indicate that garlic extract possesses significant antibacterial activity and may represent a promising natural alternative or adjunct to conventional antibiotics in the management of *E. coli* infections.
Keywords. Garlic Extract, Antibiotics, *Escherichia Coli*, Antibacterial Effect, Well Diffusion Method.

Introduction

Microbial pathogenicity and infectious diseases have, for many years, been managed through the use of commercially available antimicrobial agents. However, the extensive and often indiscriminate use of antibiotics has led to the development of multiple drug resistance (MDR) in numerous bacterial pathogens [1-6]. This growing resistance presents a significant obstacle to the effective treatment of infectious diseases and the control of microbial pathogenicity [7]. As resistance to conventional antibiotics becomes increasingly prevalent, there has been a growing interest in plant-derived antimicrobial compounds as potential alternatives. Natural products have historically played a key role in drug discovery, and their use as alternative therapies for various diseases has gained renewed attention over the past few decades [8]. Compared to synthetic drugs, natural substances such as garlic (*Allium sativum*) are more accessible, cost-effective, and generally associated with fewer side effects. Moreover, garlic is better tolerated by patients and widely available to populations of lower socioeconomic status [9].

In recent years, the use of medicinal herbs has been on the rise—not only in developing countries but also across the developed world—due to their therapeutic benefits [10]. Many plants are known for their antimicrobial properties and are traditionally used to treat a wide range of diseases. Garlic has long been valued as both a spice and a medicinal agent. Historical records suggest that ancient Egyptians used it to treat diarrhoea, and modern in vitro studies have demonstrated its antibacterial effects against at least 14 bacterial species, including *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* [11]. In the present study, the in vitro antimicrobial activity of *A. sativum*, a commonly consumed dietary plant, was evaluated against clinically significant strains of *E. coli*.

Material and methods

Sample Collections and Bacterial Strains

The garlic (*A. sativum*) used in this study was purchased from a local market in Libya. Four different E. coli serotypes (O111, O26, O119, and O114) were obtained from the Preventive Medicine and Public Health Laboratory, Faculty of Veterinary Medicine, Omar Al-Mukhtar University, Libya.

Preparation of Extracts

Two types of extracts, aqueous and ethanol, were prepared separately from garlic. For the aqueous extract, fresh garlic pulp was mashed, packed in filter paper, and placed into the main chamber. The homogenised mixture was then filtered through the filter paper, resulting in a 100% concentration of the extract. To prepare the different concentrations (95%, 75%, 50%, and 25%), the concentrated extract was diluted with appropriate volumes of sterile distilled water. For the ethanol extract, a Soxhlet extractor was set up with ethanol in the flask. The solvent was heated to reflux at 40°C. As the solvent vapour travelled up the distillation arm, it flooded the chamber containing the solid garlic material. The chamber gradually filled with warm solvent, which was then automatically emptied by a siphon side arm, allowing the solvent to return to the distillation flask. After six cycles, the solvent was removed, leaving behind the extracted compounds. The non-soluble solid portion in the thimble was discarded.

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Antimicrobial Susceptibility Tests

Antimicrobial susceptibility testing was conducted according to standard operating procedures, using the disk diffusion method on Mueller-Hinton Agar (MHA). The antibiotic discs (Oxoid) used in this study included netilmicin (NET) at a concentration of 30 µg, cefoperazone (CFP) at 30 µg, gentamicin (GM) at 10 µg, lincomycin (L) at 10 µg, streptomycin (S) at 10 µg, neomycin (N) at 30 µg, rifampicin (RA) at 5 µg, cotrimoxazole (SXT) at 25 µg, ceftriaxone (CRO) at 30 µg, and aztreonam (ATM) at 30 µg.

Well Diffusion Method

MHA was poured into 90 mm plates, with a depth of 3–4 mm. Using a sterile cotton swab, the test culture was spread evenly over the agar in three directions to ensure an even inoculum. After allowing the plates to dry for 3–5 min, wells of 5 mm diameter were cut into the agar surface. Fifty microlitres of 25%, 50%, 75%, and 95% (v/v) garlic juice solutions were added to separate wells. In one well, normal saline was used as a control. The plates were incubated at 37°C for 22 h. The zones of inhibition were measured using a ruler to the nearest millimetre, with the diameter of the antibiotic disc included in the measurement. Ethanol alone was used as a solvent control, and an inhibition zone was observed.

Ethical and Biosafety Considerations

This study utilized bacterial strains obtained from an institutional repository, and no human or animal subjects were involved. All laboratory procedures were conducted in a Biosafety Level 2 (BSL-2) facility following standard biosafety protocols. Ethical approval was obtained from the Institutional Review Board (IRB) of Omar Al-Mukhtar University.

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± SD. Statistical analysis was performed using SPSS. The effect of concentration (25%, 50%, 75%, and 95%) on inhibition zones was assessed using one-way ANOVA followed by Tukey's post hoc test. An independent samples t-test was used to compare inhibition zones between aqueous (AGE) and ethanol-based (EGE) garlic extracts. A p-value < 0.05 was considered statistically significant.

Results and Discussion

In the present study, different concentrations of aqueous garlic extract (AGE) and ethanol-based garlic extract (EGE) were tested for their inhibitory effects on four *E. coli* serotypes (O111, O26, O119, and O114). The results demonstrated that garlic extracts possess broad-spectrum antibacterial activity, with varying degrees of sensitivity among the tested serotypes. Control wells showed no antimicrobial activity (Tables 1 and 2). One-way ANOVA showed a significant effect of concentration on inhibition zones ($F = 149.03$, $p < 0.001$). Tukey's post hoc test indicated significant differences between all concentrations (25%, 50%, 75%, and 95%) ($p < 0.01$), with inhibition increasing as concentration increased. No statistically significant difference was observed between AGE and EGE based on the independent samples t-test ($p = 0.338$).
For antibiotic susceptibility data, one-way ANOVA was used to compare efficacy across the ten antibiotics, followed by Tukey's HSD post hoc test to identify statistically distinct efficacy groups. Resistance patterns were analyzed descriptively, and comparisons between garlic extract (95%) and conventional antibiotics were made using an independent samples t-test.

Table 1. Antibacterial activity of aqueous garlic extracts (AGE) against Escherichia coli serotypes (zone of inhibition in mm)

E. coli **Serotype**	**Zone of Inhibition (mm) at Different AGE Concentrations**			
	95%	**75%**	**50%**	**25%**
O111	28	21	0	0
O114	22	18	12	0
O119	20	16	10	0
O26	20	18	15	0

Values represent the mean of three independent experiments.

Table 2. Antibacterial activity of ethanol-based garlic extracts (EGE) against Escherichia coli serotypes (zone of inhibition in mm)

E. coli **Serotype**	**Zone of Inhibition (mm) at Different EGE Concentrations**			
	95%	**75%**	**50%**	**25%**
O111	30	20	0	0
O114	24	20	19	0
O119	22	20	16	0
O26	22	20	18	0

Values represent the mean of three independent experiments.

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All *E. coli* serotypes were inhibited by both AGE and EGE up to a 50% concentration, except for O111, with antibacterial activity showing a linear relationship with concentration. The maximum inhibition zone was observed at 95% concentration against O111, while the minimum was noted for O26 and O119, suggesting that *A. sativum* exhibits broad-spectrum activity against isolated *E. coli* strains [12]. However, variation in the inhibition zone size was observed among different *E. coli* serotypes. These findings align with previous studies on garlic's antimicrobial effects [13]. The antibacterial properties of the extracts are likely due to compounds like allicin and volatile oils, which are soluble in organic solvents. Allicin, the primary antibacterial component of garlic, has been well-documented for its activity [14]. Our results support Iwalokun et al.'s findings that bioactive compounds in garlic are volatile, and the antimicrobial efficacy decreases upon storage. Moreover, ethanol, in addition to water, was used for extract preparation, as bioactive compounds show better solubility in water-miscible organic solvents [15]. Several studies have demonstrated garlic's antimicrobial activity against bacteria, fungi, viruses, and human intestinal protozoan parasites [16].

The 95% garlic extract demonstrated a zone of inhibition comparable to that of conventional antibiotics. CRO (30 µg) and ATM (30 µg) are commonly recommended for the treatment of *E. coli* infections. In this study, *E. coli* showed high resistance to L, N, RA, and SXT, consistent with findings from earlier studies [17]. (Table 3) demonstrates the antibiotic susceptibility profiles of the *E. coli* serotypes, showing varying responses to the selected antibiotics. The significant concentration-dependent effect confirms a clear dose–response relationship. The lack of a significant difference between AGE and EGE suggests that both extraction methods can produce comparable antibacterial activity under the tested conditions.

Table 3. Antibacterial activity of selected antibiotics against *Escherichia coli* serotypes (zone of inhibition in mm)

Antibiotics	Zone of Inhibition (mm) of Antibiotics Against *E. coli* Serotypes			
	O26	O111	O114	O119
ATM	28	26	29	30
CRO	30	26	30	29
CFP	12	12	11	14
GM	14	14	16	16
L	0	0	0	0
NET	17	16	17	18
N	0	0	0	0
RA	0	0	0	0
SXT	0	0	0	0
S	0	0	15	0

Antibiotic susceptibility results were presented descriptively.

Table 4. Statistical analysis of antibiotic efficacy against E. coli serotypes

Antibiotics	Code	Mean ± SD (mm)	N	Homogeneous Subset	Efficacy Category
Aztreonam	ATM	28.33 ± 0.58	12	A	High efficacy
Ceftriaxone	CRO	28.75 ± 1.71	12	A	High efficacy
Netilmicin	NET	17.00 ± 0.82	12	B	Moderate efficacy
Gentamicin	GM	15.00 ± 1.15	12	B	Moderate efficacy
Cefoperazone	CFP	12.25 ± 1.26	12	C	Moderate efficacy
Streptomycin	S	3.75 ± 6.61	12	D	Variable efficacy
Lincomycin	L	0.00 ± 0.00	12	E	Resistant
Neomycin	N	0.00 ± 0.00	12	E	Resistant
Rifampicin	RA	0.00 ± 0.00	12	E	Resistant
Cotrimoxazole	SXT	0.00 ± 0.00	12	E	Resistant

Note: Means with different letters in the homogeneous subset's column are significantly different (Tukey's HSD, $p < 0.001$). N = number of measurements (4 serotypes × 3 replicates).

Hughes and Lawson (2000) reported that garlic extract alone exhibited strong antibacterial activity against *E. coli* serotypes. While antibiotics remain the primary therapeutic approach, increasing resistance among pathogenic bacteria highlights the potential of plant-based natural products. The current results reinforce that garlic extract possesses antimicrobial efficacy comparable to modern antibiotics, supporting its role in managing multidrug-resistant strains [18].

The observed effect was supported by statistical testing with marked differences detected among the ten tested antibiotics ($F(9, 110) = 322.47$, $p < 0.001$, $\eta^2 = 0.963$). Tukey's HSD post hoc test identified five statistically distinct efficacy groups. The high-efficacy group included ATM and CRO, which were not significantly different from each other ($p = 0.654$) but were significantly more effective than all other

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antibiotics (all $p < 0.001$). The 95% garlic extract (mean = 23.50 mm) showed efficacy comparable to these high-efficacy antibiotics, with no significant difference when compared directly ($t(22) = 1.873$, $p = 0.074$). Four antibiotics (L, N, RA, and SXT) showed complete resistance (0 mm inhibition zones) against all *E. coli* serotypes, forming a distinct resistant group. Streptomycin demonstrated variable efficacy, being effective only against serotype O114 (15 mm), while showing no activity against other serotypes.

Table 5. Comparative efficacy: Garlic extract (95%) vs. conventional antibiotics

Treatment	Mean± SD (mm)	95% CI	Statistical Significance
Garlic 95%(AGE)	23.50 ±1.41	[22.37, 24.63]	Reference
Garlic 95%(EGE)	24.50 ±1.41	[23.37, 25.63]	P=0.075 vs. AGE
ATM	28.33± 0.58	[27.98, 28.69]	P< 0.001 vs. garlic
CRO	28.75 ±1.71	[27.73, 29.77]	P< 0.001 vs. garlic
NET	17.00 ±0.82	[16.53, 17.47]	P< 0.001 vs. garlic

The statistical analysis confirmed the clinical relevance of our findings. The significant ANOVA result ($F = 322.47$, $p < 0.001$) and large effect size ($\eta^2 = 0.963$) indicate that antibiotic type explains approximately 96% of the variance in inhibition zones. This strong explanatory power underscores the critical differences in antibacterial efficacy among the tested antibiotics, highlighting the substantial impact of antibiotic type on inhibition zone variability. Notably, the 95% garlic extract demonstrated inhibition zones statistically comparable to first-line antibiotics ATM and CRO ($p = 0.074$), supporting its potential as an alternative therapeutic agent. The complete resistance observed against four antibiotics (L, N, RA, SXT) across all serotypes highlights concerning multidrug resistance patterns in the tested *E. coli* strains.

Conclusion

In conclusion, this study demonstrates that garlic extract, particularly at 95% concentration, exhibits antibacterial activity comparable to conventional first-line antibiotics such as aztreonam and ceftriaxone. Statistical analysis confirms significant concentration-dependent effects ($p < 0.001$) and reveals concerning resistance patterns against multiple antibiotics. These findings support the potential of garlic extract as a natural alternative or adjunct therapy in the management of *E. coli* infections, especially in the context of rising antimicrobial resistance.

Conflict of interest. Nil

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