



Antibacterial activity of dietary spices as adjuvants to ciprofloxacin against resistant uropathogenic *Escherichia coli*

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Abstract: Urinary tract infections (UTIs), particularly those caused by antibiotic-resistant *Escherichia coli*, pose a significant global health challenge. This study investigates the potential of dietary spices garlic (*Allium sativum*) and mint (*Mentha spp.*) as adjuvants to ciprofloxacin in combating resistant uropathogens. Thirty-five clinical *E. coli* isolates were analyzed for susceptibility to ciprofloxacin, garlic, and mint extracts using disk diffusion and broth dilution assays. Results demonstrated notable antibacterial activity, with garlic exhibiting a 25 mm zone of inhibition and mint 21 mm. Synergistic effects were observed when combining these spices with ciprofloxacin, yielding zones of 34 mm (garlic+ciprofloxacin) and 31 mm (mint+ciprofloxacin). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for both spices (10 µg/mL and 15 µg/mL, respectively) matched those of ciprofloxacin, confirming their potency. Optical density (OD 600nm) measurements further validated these findings. The study highlights the promise of garlic and mint as natural enhancers of antibiotic efficacy, offering a potential strategy to mitigate antibiotic resistance in UTIs. These findings advocate for further exploration of plant-based adjuvants in clinical therapeutics.

Keywords: Urinary tract infection, antibiotic resistance, *Escherichia coli*, garlic, mint, ciprofloxacin, synergy, phytotherapy.

1. Introduction

Urinary tract infections (UTIs) have been a health concern that has risen to unsurpassable levels through various factors that have provoked repeated bacteremia and increased antibiotic resistance (Baba, 2022). Due to their high prevalence, UTIs are one of the most common infections in the world, and their occurrence leads to close to 8 million healthcare visits every year (Öztürk, 2020). Urinary tract infection (UTI) is a global health conundrum, which is mainly contributing to the infections by microorganisms like *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis*, and *Staphylococcus saprophyticus* (Mancuso, 2023).

The emerging interest in herbs and herbal preparations is due to their therapeutic actions that include immunomodulation, adaptogenic effects, and anti-mutagenicity (Akram, 2020). The growing misuse of synthetic drugs, which has resulted in more adverse drug reactions, has led many to pursue natural alternatives that are safe (Ekor, 2014). Traditional plant-based medicines have been curing diseases since the dawn of time, and now, most of the conventional treatments available against diseases are failing because of bacterial resistance (AlSheikh, 2020). Therefore, scientists are developing new anti-bacterial agents from plants and semi-synthetic derivatives in combating the resistance of drugs (Bachheti, 2023). It is common to prescribe ciprofloxacin, a broad-based antibiotic also classified under the fluoroquinolone category, in treating UTIs, but there are numerous *E. coli* strains that have become resistant to it (Wagenlehner, 2010).

Garlic (*Allium sativum*) has been used both as a diet and as a treatment agent against infection, since time immemorial (Sasi, 2021). Antibacterial qualities and unique aroma of garlic are largely due to the formation of allicin, which is formed when the alliin is broken with alliinase enzymes that are formed when the cloves are punctured or smashed (Borlinghaus, 2021). While Mint (*Mentha spp.*) is a good source of vitamin A, it is an extremely important nutrient to ensure appropriate vision, especially vision in poor light conditions (Arshad,

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2023). There is no doubt that mint is also an extremely heavy source of antioxidants compared to other herbs and spices (Choudhury, 2006). The conventional use of the herb for gastrointestinal discomfort is best (Spirling, 2001). The use of peppermint oil in alleviating abdominal pain and worsening the symptoms of irritable bowel syndrome has been proven to have minimal side effects (Chumpitazi, 2018).

The current study was designed to determine the frequency of microorganisms commonly involved in UTI and the importance of herbal medicine. This study will be helpful in designing an appropriate antibiogram profile of the isolated pathogenic microorganism to develop a pharmacotherapeutic plan for clinicians to treat UTI.

2. Methodology

The study was conducted at the Department of Microbiology, Abdul Wali Khan University, Mardan, Pakistan, from December 2024 to May 2025.

2.1. Collection and Isolates processing

To preserve the bacteria, the isolates were placed in stock in 25% solution of glycerol. Clinical samples of (35) *E. coli* isolates were obtained at Mardan Medical Complex, Mardan. All the isolates after collection were taken to the Microbiology Lab at the Abdul Wali Khan University, Mardan, for further analysis.

2.2. Reconfirmation of bacterial isolates

The strains were inoculated on CLED agar plates, and the streaked plates were incubated at 37°C for 24 hours. Tests and procedures were carried out for the detection and identification of microorganisms from the growth media. The procedure contains Gram staining, Biochemical tests such as Catalase Test, Coagulase Test, Oxidase test, Urease Test, Citrate Test, Indole Test, and Triple Sugar Iron (TSI) Test. For Antibiotic Susceptibility Testing (AST), we used Mueller-Hinton agar (MHA), which is more commonly used for the routine susceptibility testing of non-fastidious microorganisms by the Kirby-Bauer disk diffusion technique (Igbinsosa, 2022).

2.3. Preparation of Extract

Locally sourced fresh mints (*Mentha spp*) and garlic (*Allium sativum*) were obtained from a local market. The plant materials were washed properly, shade-dried for 10 days under dark conditions, and transferred into a fine powder using an electric spice grinder. To do this, the airtight containers were filled with powdered samples and placed in storage. In the extraction in aqueous medium, the powder was immersed in distilled water at room temperature and left to stand overnight, after which it was then filtered using Whatman filter paper, and the filtrate was dried in an oven at a temperature of 40°C during a period of 5 days to get the crude extract (Shaikh, 2014).

Table 1. Distribution of spices items

Spices	Weight	Distilled Water
Mint	50gm	500ml
Garlic	100gm	500ml

2.4. Antibacterial Susceptibility Testing

The microbiological assessment of the *E. coli* isolated strains was performed in terms of susceptibility to antimicrobials using common microbiological methods. Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion technique, whereas the effectiveness of the plant extracts was determined using the well diffusion technique. Mueller-Hinton agar plates were loaded with bacterial suspension to produce a confluent lawn by applying sterile swabs thoroughly. Aseptic placement of antibiotic-impregnated disks (*Ciprofloxacin* 5µg) into the surface of the agar was done with the standardized interval of the disk using sterile forceps. After incubation of 37°C at an interval of 24 hours, the bacterial resistance profiles were determined by the diameter of the zones of inhibition around each disk. To test whether conventional antibiotics can show synergistic action when used with dietary spices (*Mentha and Allium sativum*), a comparative disk diffusion test was carried out. A total of five sterile markers were placed on each agar plate to divide them into two equal parts. Untreated antibiotic disks were laid on one part, whereas the subsequent antibiotic disks that had been treated in advance with the spice extracts (concentration of 100g/mL prepared in DMSO) were laid on the opposite part. An evaluation of the independent antimicrobial activity of the spice extract was also done by the



addition of a central disk with the addition of spice extract alone. Under this design, individual and combined antimicrobial effects could be simultaneously evaluated ([Chumpitazi, 2018](#)).

2.5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The broth dilution test was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant extracts as well as *ciprofloxacin*. A sterile syringe was used to add 3mL of nutrient broth to sterile test tubes. 5µl of a standardized nutrient broth culture containing bacterial suspension was taken into each of the tubes. Serial dilution of plant extract (5, 10, 15, and 20µl) was placed in different test tubes. After 24 hours of incubation at 37 C0, the tubes were checked in terms of turbidity. Clear or hazy tubes after growth represented an indication of bacterial growth inhibition (MIC) or the lack of such an effect, respectively. Confirmation of MBC was done by re-culture of clear broth on solid media and determination of the viability of bacteria. The same methodology was used, but in the case of *ciprofloxacin*, serial dilutions of the pure antibiotic (active form) were employed in place of the plant extract. The MIC was documented as the lowest concentration of the antibiotic that eliminated the occurrence of visible growth, whereas the MBC was the lowest concentration needed to kill the bacteria, confirmed by subculturing ([Chikezie, 2017](#); [Zhang, 2016](#)).

2.6. Optical density (OD 600nm)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of a spectrophotometer were confirmed by observation of the optical density (OD 600nm). During this procedure, the cuvette containing the bacterial culture was introduced into the spectrophotometer, in which UV light was allowed to percolate through the sample cuvette. The sensor would measure the optical density of the bacterial suspension then the results were shown in digital form ([Parvekar, 2020](#)).

3. Results and discussion

Urinary tract infection (UTI) is a highly prevalent disease because of numerous causes that result in chronic bacteremia, resistance to drugs, and is the second most prevalent organ infection in human beings, causing nearly 8 million cases among health practitioners. In our study, a total of 35 *E. coli* isolates were retrieved from MMC Mardan. These isolates were grown on CLED Agar and showed yellow opaque colonies on CLED agar. The Gram staining results showed that the bacterial isolates are Gram-negative rods.

3.1. Cultural and morphological characteristics of bacterial species

Our study recorded that, following the assessment of cultural characteristics, the bacterial isolates were fully identified through biochemical testing. All specimens tested positive for the Triple Sugar Iron (TSI) test, catalase, and indole, while yielding negative results for oxidase, urease, citrate utilization, and coagulase tests. The antibiotic susceptibility standard values were applied, provided by CLSI (Clinical and Laboratory Standards Institute).

Table 2. Cultural and morphological characteristics of bacterial species

Isolates	Colonies color	Shape	Gram Staining
<i>E. coli</i>	Yellow Opaque	Rod	Negative

According to CLSI M100 (2024), the disk should be 5µg, and the zone of inhibition of *Ciprofloxacin* for *E. coli* is Susceptible (≥ 31 mm), Intermediate (21–30 mm), and Resistance (≤ 20 mm). The results showed that *Ciprofloxacin* produced a (28mm) zone of inhibition against *E. coli*. The zone produced by Garlic extract (25mm), followed by mint extract, showed a 21mm zone of inhibition against *E. coli*. *Ciprofloxacin*+Garlic showed (34mm) zone of inhibition against *E. coli*, while *Ciprofloxacin*+Mint showed (31mm) zone of inhibition against *E. coli*.

3.2. Antibacterial activity of Ciprofloxacin and dietary spices

[Okunye \(2020\)](#) has carried out a study of antibacterial performance of ethanolic extract of *Allium sativum* (garlic) against urinary tract bacteria. After bacterial identification, antibiotic susceptibility testing was carried out via the disc diffusion method, using antibiotics, i.e., Gentamicin, Ciprofloxacin, Ceftazidime, Cotrimoxazole, and Imipenem. The findings indicated that the highest resistance rate was against Ceftazidime (67 %). Later, the

antibacterial activity of different concentrations (100%, 50%, 25% and 12.5% w/v) of *Allium sativum* ethanolic extract against *A. phagocytophilum* was then determined through cup plate agar diffusion assay. The extract had shown activity against clinical isolates and reference strains. The most susceptible of the tested bacteria was *Enterococcus faecalis*, which showed a mean of 20.8 mm inhibition zone at 100 percent concentration, whereas *Pseudomonas aeruginosa* was ranked as the least susceptible bacterium and had a 17.2 mm inhibition zone. The garlic extract could inhibit all the tested bacterial strains at the same concentration of 12.5% (w/v).

Table 3. Antibacterial activity of Ciprofloxacin and Dietary Spices

Zone of Inhibition				
Ciprofloxacin	Garlic	Mint	Garlic+Ciprofloxacin	Mint+Ciprofloxacin
28mm	25mm	21mm	34mm	31mm

According to our study findings, the MIC and MBC values of dietary spices are shown in Table 4. The MIC value of (10 µg/ml) and MBC (15 µg/ml) were observed for garlic against *E. coli*. MIC (10 µg/ml) and MBC (15 µg/ml) were noticed by mint against *E. coli*. Similarly, MIC (10 µg/ml) and MBC (15 µg/ml) were noticed by Ciprofloxacin against *E. coli*.

3.3. MIC and MBC of Dietary Spices and Antibiotics

According to [Mohammed \(2019\)](#), the phytochemical components of the dried powdered ginger plant portions were extracted by application of organic and aqueous solvents (ethanol and methanol). *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella spp.* were the bacterial isolates that have been characterized and identified. The ginger extracts had inhibitory (active) effects on all the test isolates. It was revealed that the minimum inhibitory concentration (MIC) of the extracts on the test isolates was lowest against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella spp.*, and against *Escherichia coli*, the highest MIC was observed to be 50mg/ml. Medicinal efficacy against the test isolates was observed in ginger (*Zingiber officianale*).

[Saeed \(2016\)](#), tested the activity of *C. cyminum* extract and essential oil against bacterial isolates that cause urinary tract infection. The result indicated that the antibacterial activity of essential oil and extract of *C. cyminum* against uropathogen isolates was superior to amoxicillin and the difference was significant, but this activity is not better than the other antibiotics.

Table 4. MIC and MBC of Dietary Spices and Antibiotics

MIC and MBC (µg/ml)					
Garlic		Mint		Ciprofloxacin	
MIC	MBC	MIC	MBC	MIC	MBC
10	15	10	15	10	15

The optical density (OD 600nm) of garlic against *E. coli* was noticed as MIC (0.502) and MBC (0.391). Similarly, the optical density of other dietary spices is shown in Table 5.

Table 5. OD 600nm (MIC and MBC) of dietary spices

(OD) MIC and MBC				
Garlic		Mint		
MIC	MBC	MIC	MBC	MBC
0.502	0.391	0.685		0.302

The existing research endeavors of different workers have revealed that spices in the diet have remarkable medicinal powers and could be employed effectively in medical terms. In this study, an attempt has been made to observe the in vitro antibacterial properties, and based on the findings, it can be said that there is the possibility of giving dietary spices orally combined with antibiotics in the case of UTI infections, i.e., *E. coli*.

4. Conclusions

The research focuses on increasing the problem of antibiotic-resistant urinary tract infection (UTI), especially of *E. coli* origin, and investigates the possibilities of using spices in food, namely garlic (*Allium sativum*) and mint (*Mentha spp.*), as an adjuvant therapeutic option. This shows that both garlic and mint extracts have a great



antibacterial effect on *E. coli*, though in the case of garlic, there is a high zone of inhibition (25mm) compared to that of the mint (21mm). It is noteworthy that the synergetic blend of such spices with *ciprofloxacin* increased the efficacy of the antibacterial, which was confirmed by the extension of the zones of inhibition (34 mm of garlic+ciprofloxacin and 31 mm of mint+ciprofloxacin). These findings are consistent with other studies that highlighted the therapeutic effects of phytochemicals as an alternative to countering the resistance to antibiotics.

CRedit authorship contribution statement

Hamad Ali, Experiment and analyze data, Writing-original draft; Muhammad Usama, Hammad Khan, Muhammad, Data analysis; Abdullah Jan, Sadeeq Bacha, Subhan Ullah, Syed Taimur Shah. Writing-original draft, editing, proofreading.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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