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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 the increased antibiotic resistance (Baba, 2022). Due to their high-rate prevalence, UTIs are one of the most common infections in the whole world, and their occurrence leads to close to 8 million healthcare visits every year (Öztürk & Murt, 2020). Urinary tract infection (UTI) is a global health conundrum, which is mainly contributing to the infections by microorganisms like *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Enterococcus faecalis and Staphylococcus saprophyticus* (Mancuso et al., 2023).

The emerging interest in herbs and herbal preparations is due to their therapeutic actions that include immunomodulation, adaptogenic effects, and the anti-mutagenicity (Akram et al., 2020). The growing misuse of synthetic drugs which has resulted in more adverse drug reactions has made many to pursue natural alternatives that are safe (Ekor, 2014). Traditional plant-based medicines are curing diseases since the dawn of time and now, most of the conventional treatments available against diseases are failing because of bacterial resistance (AlSheikh et al., 2020). Therefore, scientists are developing new anti-bacterial agents from plants and sem-synthetic derivatives in combating the resistance of drugs (Bachheti & 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, which have become resistant to it (Wagenlehner et al., 2010).
Garlic (*Allium sativum*) has been used both as a diet and as a treatment agent against infection, since time immemorial (Sasi et al., 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 et al., 2021). While Mint *(Mentha spp.)* is a good source of vitamin A, it is

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an extremely important nutrient to ensure appropriate vision and especially visions in poor light conditions (Arshad et al., 2023). There is no doubt that mint is also an extremely heavy source of antioxidants compared to other herbs and spices (PAUL CHOUDHURY et al., 2006). The conventional use of the herb in gastrointestinal relieving discomfort is best (Spirling & Daniels, 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 et al., 2018).

The current study was designed to determine the frequency of microorganisms commonly involved in UTI and importance of herbal medicine. This study will be helpful to design 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, 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), it is more commonly used for the routine susceptibility testing of non-fastidious microorganism by the Kirby-Bauer disk diffusion technique (Igbinosa et al., 2022).

2.3. Preparation of Extract

Local 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 the dark conditions and transferred into a fine powder using an electric spice grinder. To do this the airtight containers were filled with the powdered samples and placed in storage. In extraction in aqueous medium, the powder was immersed in distilled water at room temperature and left to stand overnight out of which it was then filtered using Whatman filter paper and the filtrate dried over an oven at a temperature of 40C0 during a period of 5 days to get the crude extract (Shaikh et al., 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 antimicrobial using common microbiological methods. Antibiotic susceptibility was determined using Kirby-Bauer disk diffusion technique whereas the effectiveness of the plant extracts was determined using well diffusion technique. Mueller-Hinton agar plates were loaded with bacteria 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 C0 at an interval of 24 hours the bacterial resistance profiles were determined by the diameter of the zones of inhibition each disk. To test whether the conventional antibiotics can show synergistic action when used with dietary spices (*Mentha and Allium sativum*), 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 on 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 combine antimicrobial effects could be simultaneously evaluated (Chumpitazi et al., 2018).

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

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 were 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 in 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 which eliminated the occurrence of visible growth whereas the MBC was the lowest concentration needed to kill the bacteria, confirmed by subculturing (Owuama, 2017; Zhang et al., 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 bacterial culture was introduced in 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 et al., 2020).

3. Results and discussion

Urinary tract infection (UTI) is a highly anticipating disease because of numerous causes that result into chronic bacteremia, resistance to drugs, and is the second prevalent organ infection in human being causing nearly 8 million cases of the health practitioners. In our study total (35) *E. coli* isolates were retrieved from MMC Mardan. These isolates were grown on CLED Agar and showed yellow opaque color colonies on CLED agar. The gram staining results showed that bacteria 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 Standard Institute).

Table 2. Cultural and morphological characteristics of bacterial species

Isolates	Colonies color	Shape	Gram Staining
E. coli	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* produce (28mm) zone of inhibition against *E. coli*. The zone produced by Garlic extract (25mm) followed by mint extract showed (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 et al. (2020) have carried out a study of antibacterial performance of ethanolic extract of Allium sativum (garlic) of urinary tract bacteria. After bacterial identification, antibiotic susceptibility testing was carried on via the mode of disc diffusion, using antibiotics, i.e. Gentamicin, Ciprofloxacin, Ceftazidime, Cotrimoxazole, and Imipenem. The findings indicated that the most 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 showed activity against clinical isolates, and references strains. The most susceptible of the tested bacteria was *Enterococcus faecalis* that showed a mean of 20.8 mm inhibition zone at 100 percent concentration, whereas *Pseudomonas aeruginosa* was ranked as the least susceptible bacteria and had a 17.2 mm inhibition zone. The garlic extract could inhibit at 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) was noticed by garlic against *E. coli*. MIC (10 µg/ml) and MBC (15 µg/ml) was noticed by mint against *E. coli*. Similarly, MIC (10 µg/ml) and MBC (15 µg/ml) was noticed by *Ciprofloxacin* against *E. coli.*

3.3. MIC and MBC of Dietary spices and Antibiotics

According to Mohammed et al. (2019), the phytochemical components of the dried powdered ginger plant portions were taken by application of organic and aquatic solvents (ethanol and methanol). *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella spp*. were the bacteria 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)*.

Saee et al. (2016) tested the activity of *C. cyminum* extract and essential oil against bacteria 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
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+ciprofloxaci*n*). These findings are consistent with other studies that highlighted the therapeutic effects of phytochemicals as an alternative to countering the resistance to antibiotics.

Author's contribution

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.

Conflicts of Interest

The authors declare no conflict of interest.

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References

Akram, M., Riaz, M., Wadood, A. W. C., Hazrat, A., Mukhtiar, M., Ahmad Zakki, S., Daniyal, M., Shariati, M. A., Said Khan, F., & Zainab, R. (2020). Medicinal plants with anti-mutagenic potential. *Biotechnology & Biotechnological Equipment*, *34*(1), 309-318.

AlSheikh, H. M. A., Sultan, I., Kumar, V., Rather, I. A., Al-Sheikh, H., Tasleem Jan, A., & Haq, Q. M. R. (2020). Plant-based phytochemicals as possible alternative to antibiotics in combating bacterial drug resistance. *Antibiotics*, *9*(8), 480.

Arshad, M. K., Fatima, I., Ahmad, W., Ellahi, S., Mumtaz, M., Akhtar, M. U., Aslam, M. S., & Siddique, W. A. (2023). Mint (Mentha): A herb and used as a functional ingredient. *Scholars International Journal of Traditional and Complementary Medicine*, *6*(03), 38-52.

Baba, R. T. (2022). *Urinary Tract Infection Bacterial Profile and Antibiotic Susceptibility Pattern among Pregnant Women Attending Antenantal Clinic at Selected Hospitals in Ilorin* Kwara State University (Nigeria)].

Bachheti, R. K., & Bachheti, A. (2023). *Secondary metabolites from medicinal plants: Nanoparticles synthesis and their applications*. CRC Press.

Borlinghaus, J., Foerster, J., Kappler, U., Antelmann, H., Noll, U., Gruhlke, M. C., & Slusarenko, A. J. (2021). Allicin, the odor of freshly crushed garlic: A review of recent progress in understanding allicin's effects on cells. *Molecules*, *26*(6), 1505.

Chumpitazi, B. P., Kearns, G., & Shulman, R. J. (2018). The physiological effects and safety of peppermint oil and its efficacy in irritable bowel syndrome and other functional disorders. *Alimentary pharmacology & therapeutics*, *47*(6), 738-752.

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in pharmacology*, *4*, 177.

Igbinosa, E., Ogofure, A., & Beshiru, A. (2022). Evaluation of different agar media for the antibiotic susceptibility testing of some selected bacterial pathogens. *University of Lagos Journal of Basic Medical Sciences*, *8*(1-2).

Mancuso, G., Midiri, A., Gerace, E., Marra, M., Zummo, S., & Biondo, C. (2023). Urinary tract infections: the current scenario and future prospects. *Pathogens*, *12*(4), 623.

Mohammed, S., Bashir, A., David, A., Abdul-Rahman, A., & Ninyio, N. (2019). Phytochemical Constituents and Antibacterial activity of Ginger (Zingiber officianale) Extract on Selected Clinical Isolates Associated with Urinary Tract Infections (UTIS). *Ife Journal of Science and Technology*, *3*(1), 74-85.

Okunye, O. L., Idowu, P. A., Adeleke, E. O., & Babalola, C. O. (2020). Antimicrobial activity of garlic (Allium sativum) on selected uropathogens from cases of urinary tract infection. *Annals of Tropical Pathology*, *11*(2), 133-138.

Owuama, C. I. (2017). Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method. *African journal of microbiology research*, *11*(23), 977-980.

Öztürk, R., & Murt, A. (2020). Epidemiology of urological infections: a global burden. *World journal of urology*, *38*(11), 2669-2679.

Parvekar, P., Palaskar, J., Metgud, S., Maria, R., & Dutta, S. (2020). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against Staphylococcus aureus. *Biomaterial investigations in dentistry*, *7*(1), 105-109.

Paul, C., R., Kumar, A., & Garg, A. (2006). Analysis of Indian mint (Mentha spicata) for essential, trace and toxic elements and its antioxidant behaviour. *Journal of pharmaceutical and biomedical analysis*, *41*(3), 825-832.

Saee, Y., Dadashi, M., Eslami, G., Goudarzi, H., Taheri, S., & Fallah, F. (2016). Evaluation of antimicrobial activity of Cuminum cyminum essential oil and extract against bacterial strains isolated from patients with symptomatic urinary tract infection. *Novelty in Biomedicine*, *4*(4), 147-152.

Sasi, M., Kumar, S., Kumar, M., Thapa, S., Prajapati, U., Tak, Y., Changan, S., Saurabh, V., Kumari, S., & Kumar, A. (2021). Garlic (Allium sativum L.) bioactives and its role in alleviating oral pathologies. *Antioxidants*, *10*(11), 1847.

Shaikh, S., Yaacob, H. B., & Rahim, Z. H. A. (2014). Prospective role in treatment of major illnesses and potential benefits as a safe insecticide and natural food preservative of mint (Mentha spp.): a Review. *Asian Journal of Biomedical and Pharmaceutical Sciences*, *4*(35), 1.

Spirling, L. I., & Daniels, I. R. (2001). Botanical perspectives on health peppermint: more than just an after-dinner mint. *The journal of the Royal Society for the Promotion of Health*, *121*(1), 62-63.

Wagenlehner, F. M., Weidner, W., Perletti, G., & Naber, K. G. (2010). Emerging drugs for bacterial urinary tract infections. *Expert opinion on emerging drugs*, *15*(3), 375-397.

Zhang, Y., Liu, X., Wang, Y., Jiang, P., & Quek, S. (2016). Antibacterial activity and mechanism of cinnamon essential oil against Escherichia coli and Staphylococcus aureus. *Food control*, *59*, 282-289.