

EXPLORING THE THERAPEUTIC EFFICACY OF CUMIN EXTRACT AGAINST UTI-CAUSING PATHOGENS

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Abstract

In recent years, treatment of urinary tract infection has become increasingly challenging due to the prevalence of antimicrobial resistance patterns in the UTI causing pathogens. Plant extracts could be used to increase the efficiency of the conventional antibiotics without increasing antibiotics doses due to the presence of bioactive compounds. The current study has explored the potential of cumin seed extract to inhibit the growth of UTI pathogens. The potential was assessed using solvent including methanol, ethanol, acetone and water at different duration including 24, 48 and 72 hours for each extract. The pathogens included *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. It was observed that the cumin seed extract exhibited antimicrobial activity against all tested pathogens. Results demonstrates that methanol extracts exhibited stronger antimicrobial potential with the highest zones of inhibition observed for *P. vulgaris* and *E. coli* (19mm), *K. pneumoniae* (18mm) and *S. aureus* (15mm). Ethanol extracts also exhibited significant antimicrobial potential particularly against *K. pneumoniae* (18mm) and *E. coli* (17mm). Acetone and distilled water showed comparatively lower activity. The results proved that the cumin seed extract could be used as a potential source of antimicrobial agent, suggesting a promising compound for healthcare applications, especially antimicrobial resistance.

Keywords: *Cuminum cyminum*, Natural extract, Antimicrobial, Synergistic Effect, Therapeutic.

Introduction

The World Health Organization has identified the antibiotic resistance as one of the major healthcare threats to human health. The bacterial infections that were once easily treatable are becoming extremely challenging to cure because of the development of resistance against the antibiotics prescribed (Sher et al., 2024). It has been estimated that antibiotic resistance alone is responsible for over 700,000 deaths each year which is expected to reach nearly 10 million deaths by the year 2050 (Huemer et al., 2020). The bacteria have shown to develop many resistance patterns against the antibiotics simply because there has been immense misuse of the drugs including taking it unnecessarily for extensive periods of time, taking it when you don't actually need them or poor identification and infection control in healthcare settings. Antibiotics can kill the bacteria but resistant strains survive and multiply increasing the risk of more threatening infection in the near future. In case of contagious organisms, this also leads to increase of active bacteria that can easily spread to other patients and the environment.

UTIs remain one of the most commonly diagnosed infections, afflicting over 150 million individuals annually, with a substantial burden on healthcare systems globally. UTIs are mainly bacterial infections as discussed by Allaq et al. (2020), and in case of lack of treatment or ineffective therapy, they may develop severe consequences, including pyelonephritis and urosepsis. The current issues concerning the frequency of antibiotic resistance among uropathogens have complicated the modern treatment process thus reducing the effectiveness of conventional therapeutics. *E. coli* continues to be the main pathogen in nosocomial UTIs; other pathogens, such as *Klebsiella pneumoniae*, *Proteus mirabilis* and *Staphylococcus aureus*, also play significant roles in the burden caused by the infections. The current standard of antibiotic treatment is progressively becoming ineffective, as the resistant mechanism of the bacteria has been evolving rapidly on the enzymatic level, the drug targets, the altered permeability and the efflux pumps. This way, the elaboration of novel measures to enhance the effectiveness of antibiotics and reinstate microbial susceptibility is of acute necessity (Bullens et al., 2022; Butler, 2020). Key resistance determinants include extended spectrum β lactamases (ESBLs), carbapenemases (e.g., KPC, NDM 1) and plasmid borne qnr genes conferring fluoroquinolone tolerance. Biofilm formation on catheters further aggravates antibiotic penetration barriers. Thus, combating UTIs demands agents that (i) permeate or dismantle biofilms, (ii) bypass or inhibit enzymatic inactivation, and (iii) are deployable on abiotic surfaces to pre-empt colonization (Almatroudi 2025; Firouzjaei et al., 2023; González, 2020).

Due to this rapid evolution, it is necessary to explore new therapeutic ways to enhance antimicrobial effectiveness (Ortega-Lozano et al., 2023). In many experiments, it was noted that traditional medicines and natural extracts are promising sources of new antimicrobial agents and can possess the potential for synergistic antimicrobial effect with traditional antibiotics (Akinsiku et al., 2023; Eloutify et al., 2023).

Cuminum cyminum, commonly known as cumin, is a household staple and traditional medicinal plant with various therapeutic properties including anti-inflammatory, antioxidant and antibacterial as well as carminative, diuretic and antispasmodic properties (Mohammed et al., 2024; Mughal, 2022; Allaq et al., 2020). It is frequently used in medicine to treat several types of digestive, respiratory and inflammatory issues including indigestion, diarrhea, jaundice and other pulmonary disorders. These properties are due to the presence of bioactive compounds like cumin aldehyde, cumene and terpenoids (Srinivasan, 2018; Kedia et al., 2015).

These compounds contribute to a wide array of biological activities, including antioxidant, antimicrobial, anti-

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inflammatory, anticarcinogenic, anti-diabetic, and hepato-protective effects (Bai et al., 2025; Shaheen et al., 2023, Sulieman et al., 2023). In this regard, the current study was designed towards the green synthesis follows the principles of green chemistry. *Cuminum cyminum* plant extracts was prepared using different solvents for various time interval to identify the potential of different phytochemicals against the UTI causing pathogens.

Methodology

Cumin seeds were obtained from a local market in Karachi, Pakistan. The seeds were washed multiple times with distilled water to remove any dust particles and air-dried. The dried seeds were grounded into a fine powder using a sterile grinder and stored in airtight containers.

The microbial strains used in this study were obtained from the Ziauddin Hospital. The specific strains selected for their relevance as common urinary tract infection (UTI) pathogens were *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumonia*, and *Staphylococcus aureus*. They were then streaked on nutrient agar plates to isolate single colonies. The plates were incubated at 37°C for 24 hours. After incubation, the isolated colonies were stored at 4°C for further use.

DMSO were products of Sigma-Aldrich. Deionized water was used during preparation of all solutions. Every glassware's was washed using distilled water and air-dried before use. All instruments and media were sterilized using autoclave before each experiment.

The research work conducted has been approved by the Ethical Approval Committee of the Institute (ERC/ZUFESTM/0016). The experiment has been performed as per the guideline.

Seed Cleaning and Powdering

Cumin seeds were thoroughly washed 3–5 times with deionized water to remove dust or contaminants. 100 g of whole cumin seeds were washed with sterile distilled water (three cycles) to eliminate surface impurities. Seeds were air-dried in a sterile environment for 48 hours. Dried seeds were ground into a fine powder using a sterile electric grinder. The powder was sieved (mesh size ~0.5 mm) and stored in airtight amber glass bottles at 4°C until further use.

Extract Preparation

In this study, four different solvents were used to extract active compounds from cumin seeds: methanol, ethanol, acetone, and water. The extracts were prepared at 1:10 ratio of cumin powder to solvent. 10 grams of finely ground cumin powder was added in 100 mL of each solvent. The extracts were stirred with the help of magnetic stirrer at approximately 25°C for 24 hours. This was done to extract the bioactive compounds from the cumin powder into the solvents. After 24-hour, the liquid extract and solid residue were separated from each flask using Whatman filter paper. Same procedure was followed for 48-hour and 72-hour extracts. All the extracts were stored at 4°C until further use. The representation of extract preparation is illustrated in Figure 1.



Figure 1: Schematic representation of *Cumin Cyminum* extract preparation

Antimicrobial Activity of Cumin Extracts

Four bacterial cultures; *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* were inoculated in 5 mL of nutrient broth at 37°C for 2 hours. This was done to achieve the log phase of bacterial growth. Then 50 µL of each bacterial broth culture was spread on Mueller-Hinton Agar (MHA) plates using a sterile glass spreader. To assess the antimicrobial activity of the plant extracts, wells were made with a borer of 6 mm diameter. For each UTI pathogen, four separate MHA plates were prepared. On each plate of the pathogen, four wells were created to add 20 µL solvents with the concentration of 24, 48, 72-hour extraction period cumin seed extract was added. The fourth well was used as the positive control where DMSO was added. The plates were incubated at 37°C for 24 hours. Following incubation, the plates were examined for zones of inhibition. The clear zones around the wells indicated the antimicrobial activity of the extracts.

Results and Discussion

All extracts of cumin demonstrated considerable zones of inhibition (ZOI) against the four selected UTI pathogens *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Different antimicrobial activity was recorded based on each solvent used and the extraction duration (24 h, 48 h, and 72 h). Increased antimicrobial activity was observed with longer extraction times, with methanolic extracts showing the highest zones of inhibition across four pathogens tested. This increased antimicrobial activity in longer extraction periods is linked with the increased extraction and diffusion of phytochemicals responsible for antimicrobial action like terpenoids, flavonoids and aldehydes into the solvents. *E. coli* showed a ZOI of 19 mm for methanolic extract after 72 h. *P. vulgaris* showed a ZOI of 19mm at 72 h methanolic extract. The antimicrobial activity of distilled water was comparatively lower at 24 and 48 h but demonstrated some activity at 72 h. These results align with literature from Vanlalveni et al. (2021), who reported that longer extraction times significantly elevate the phytochemical extraction which increases the antimicrobial potential (Table 1).

Table 1: Zones of inhibition (ZOI) for cumin seed extracts across methanol against selected UTI pathogens

Microorganism	Zone of Inhibition (mm)			
	24 hours	48 hours	72 hours	+ve control
<i>E. coli</i>	15	16	19	7

<i>P. vulgaris</i>	15	18	19	11
<i>K. pneumoniae</i>	16	16	18	12
<i>S. aureus</i>	10	10	15	11

Table 2: Zones of inhibition (ZOI) for cumin seed extracts across ethanol and durations against selected UTI pathogens.

Microorganism	Zone of Inhibition (mm)			
	24 hours	48 hours	72 hours	+ve control
<i>E. coli</i>	14	16	17	10
<i>P. vulgaris</i>	10	12	16	12
<i>K. pneumoniae</i>	15	17	18	13
<i>S. aureus</i>	9	12	15	10

Ethanol and acetone extracts exhibited slightly variable patterns depending on the microbial strain (Table 2 and Table 3). For example, *K. pneumoniae* showed higher activity for all the solvents across all concentrations, suggesting that these strains are particularly susceptible to the extracted bioactive compounds present in solvents. The antimicrobial activity of distilled water is summarized in Table 4.

Table 3: Zones of inhibition (ZOI) for cumin seed extracts across acetone and durations against pathogens.

Microorganism	Zone of Inhibition (mm)			
	24 hours	48 hours	72 hours	+ve control
<i>E. coli</i>	14	14	15	10
<i>P. vulgaris</i>	10	10	15	12
<i>K. pneumoniae</i>	16	17	17	13
<i>S. aureus</i>	9	11	11	8

Table 4: Zones of inhibition (ZOI) for cumin seed extracts across methanol and durations against selected UTI pathogens.

Microorganism	Zone of Inhibition (mm)			
	24 hours	48 hours	72 hours	+ve control
<i>E. coli</i>	13	13	14	10
<i>P. vulgaris</i>	11	11	13	10
<i>K. pneumoniae</i>	14	14	16	13
<i>S. aureus</i>	11	11	13	8

Time-Dependent and Solvent-Dependent Activity

Antimicrobial activity increased with increased extraction durations. Out of all four solvents, methanol consistently yielded the largest ZOIs against *E. coli* and *P. vulgaris* (19 mm), indicating higher phytochemical extraction. Ethanol and acetone extract also showed significant antimicrobial activity but lower than the methanol extracts. Distilled water showed less antimicrobial activity and its results were consistent across all pathogens.

Notable Antimicrobial Trends

- Methanol extract at 72 hours showed the highest antimicrobial activity due to better extraction of phytochemicals from the cumin seed.
- Extract prepared in distilled water was least effective but showed significant activity at 72 hours extraction period.
- The antimicrobial effect of cumin extract was proved to be time-sensitive and solvent-dependent.

The findings from this study are consistent with previous researches indicating that methanol is often effective in extraction of phenolic compounds, flavonoids, and other secondary metabolites from plants, which are directly responsible for antimicrobial activity (Nadeem and Riaz 2012). In a study, 31 bioactive compounds were identified from methanolic extracts including Adamantane methanol, Octanal dimethyl acetal, and S, S-dimethyl 1,2-hydrazine, which have the potential to contribute to the observed antimicrobial effects (Sharifi et al., 2021). Ethanol extracts also showed significant activity which indicates that semi-polar solvents effectively draw out compounds with antimicrobial potential.

The essential oil of *C. cyminum* contains cumin aldehyde, β -pinene, γ -terpinene, and p-cymene which are known to exhibit antibacterial activity. The effectiveness of ethanol could be linked to its ability to extract these essential oil components along with other bioactive molecules. Previously, *Cuminum cyminum*

essential oil (CcEO) has shown antibacterial activity against multidrug-resistant *Staphylococcus aureus* by inhibiting biofilm formation. (Al Dosary 2023; Saeed et al., 2016). Acetone and distilled water extracts displayed moderate to lower activity. Acetone extract showing a 17 mm zone against *K. pneumoniae* at 72 hours and 11mm zone of inhibition against *S. aureus* at 72 hours indicates that antimicrobial effects depend on different extraction capabilities of the extracts and the selected organism as well. Distilled water extracts had the lowest inhibition zones but still exhibited some activity, suggests that some water-soluble compounds with antimicrobial properties are present in *C. cyminum*. The variations in antimicrobial activity across different extracts based on their polarity, resulted in different phytochemical extraction and varying biological activities.

These alcoholic extracts disrupt bacterial cell membranes which increase the permeability of the membranes resulting in leakage of intracellular compounds, inhibiting bacterial enzymes, interfere with metabolic processes and eventually cell death (Sharifi et al., 2021; Abbaszadegan et al., 2015).

Acetone extracts contain high levels of phenolics and tannins that shows higher antioxidant effects, oxidative stress and antimicrobial properties (Petretto et al., 2018). Aqueous extracts contain polar phenolics like rutin and ellagic acid, which exhibit mild antibacterial effects by enzyme inhibition and free radical scavenging but their efficiency highly depends on extraction conditions. These bioactive compounds proves that cumin seed extracts have broad-spectrum antimicrobial activity against uropathogens and could be used in the treatment for urinary tract infections (Mohammed et al., 2024; Saeed et al., 2016; Al-Shawi et al., 2017).

Conclusion

This study showed the potential of *Cuminum cyminum* seed extract as promising candidates for treating infections with increased antimicrobial resistance. Further studies will be required to explore the reaction pathways, side effects and potential doses so these compounds could be practically used as therapeutic agents and help in combating antimicrobial resistance.

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