Original Article

Evaluation of Antimicrobial Activity of *Cuminum Cyminum* Essential Oil and Extract against Bacterial Strains Isolated from Patients with Symptomatic Urinary Tract Infection

Yasaman Saee[1], Masoud Dadashi[2], Gita Eslami[2*], Hossein Goudarzi[2], Soudabeh Taheri[2], Fatemeh Fallah[2]

[1] Department of Microbiology, Islamic Azad University, Pharmaceutical Sciences Branch, Tehran, Iran
[2] Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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Abstract

Background: Many efforts have been done to find effective agents against resistant pathogens. Cuminum cyminum L. (Cumin) is an aromatic plant within the Apiaceae family. It has a variety of purposes and demonstrates antimicrobial and antioxidant properties. This study evaluated the activity of *C. cyminum* extract and essential oil against bacterial isolates which cause urinary tract infection, including Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus agalactiae, group A streptococci, Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus and Staphylococcus saprophyticus isolated from patients with urinary tract infection.

Materials and Methods: Extract was prepared by maceration and essential oil was prepared by hydrodistillation from *C. cyminum* seeds. The study population was 95 patients with urinary tract infection without malignant diseases, diabetes and immunosupression. After identification of organism, susceptibility testing was carried out by disc diffusion method and MIC values by broth microdilution testing.

Results: *C. cyminum* essential oil can have a better effect on the gram-negative bacteria causing urinary tract infection than gram-positive bacteria. In addition, *C. cyminum* extract have good activity against both gram-positive and gram-negative bacteria. Our findings also showed that essential oil and extract of *C. cyminum* has better antibacterial activity on uropathogen isolates than amoxicillin and the difference was significant (*P value<0.05*) but the activity is not superior to other antibiotics.

Conclusion: These results suggest that the essential oil and extract of *C. cyminum* seeds might be considered as interesting sources of antibacterial components against uropathogenic bacteria.

Keywords: Antimicrobial activity, *Cuminum cyminum L.*, essential oil, extract

***Corresponding Author:** Gita Eslami, Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Science, Tehran, Iran. Email: dreslami@smbu.ac.ir

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Introduction

Urinary tract infection (UTI) is one of the most common bacterial infection in the general population throughout the world[1], affecting 150 million people every year worldwide[2]. Currently, the societal costs of this disease, including health care

prices and time missed from work, are approximately 4 billion dollars per year in the United States alone[3]. In addition, UTIs are a major cause of morbidity among male infants, old men and women of all ages[4]. Serious complications of UTIs include repeated recurrences, pyelonephritis and subsequent sepsis and premature birth can be developed. Important squeal caused by the prolonged use of antibiotics, such as high-level antibiotic resistance and *Clostridium difficile* colitis, are also very probable[5]. In recent years, there has been growing concern about the greater occurrence of antibiotic resistance among bacteria and particularly uropathogens. The uncontrolled overuse of antimicrobial drugs could lead to increased amounts of antibiotic-resistant pathogens, and could eventually compromise treatments of bacterial infections in humans.

Therapeutic herbs and plants have been prescribed for centuries in treatments of various human infections[6]. Recently, the plant-derived extracts and essential oils of herbs are gaining much recognition as a potential source of natural[7] and safer antibacterial, antioxidant, anticarcinogenic, antifungal, analgesic, insecticidal, and anticoccidial agents[8]. *Cuminum cyminum* (commonly known as cumin) is an aromatic plant within the Apiaceae family, which is used in foods, perfume, and medical preparations such as toothpastes, mouthwashes and soaps. It is cultivated in India, China, Saudi Arabia and in the countries bordering the Mediterranean sea[9]. It is also used as carminative, appetite stimulating agents and antispasmodic in medicine. In addition, *Cuminum cyminum* seeds have been demonstrated to possess significant biological properties, such as antibacterial and antifungal activity[10]. The aim of the present study was to evaluate the antibacterial activity of the methanolic extract and essential oil of *Cuminum cyminum* seeds against some uropathogenic bacteria isolated from patients with urinary tract infection.

Methods

Samples: In this study, 95 urine samples were collected from patients with symptomatic urinary tract infection. Primary isolation of uropathogens was performed by a surface streak plate technique on blood agar, MacConkey agar and EMB agar and then incubation of the petri dishes for 24 hours at 37°C. Then suspected colonies that colony counts of them were ≥105 CFU/ml further identified by conventional biochemical methods (oxidase, catalase, coagulase, fermentation of lactose, indole, citrate, production of H2S, lysine decarboxylase, urea hydrolysis, gas production, , mannitol fermentation and susceptibility testing Novobiocin).

Essential oil preparation: The essential oil of the cumin seeds was made by the Clavenger system, using the hydrodistillation technique. The dehydrated powdered seeds of cumin (100g) were located in a distillation apparatus containing one liter of distilled water and hydrodistilled about for 3 hours. Afterward, the oil was gently removed and kept at 4°C in sterile dark glasses until being tested.

Plant extraction: Seeds of the cumin were pulverized to powder using an electric blender. For extraction, we used Soxhlet extraction method. In this way, 200g of the powder was percolated in 1600ml of absolute methanol. At the end, samples were placed at 50°C in oven to remove the solvent. After controlling for pH, the extractions were stored in sterile dark vials at 4°C until used.

Disc diffusion method: This method was used for the screening of antibacterial effect of the methanolic extract on the bacteria and comparing the result with some antibiotics. Bacterial suspension (equivalent to a 0.5 McFarland standard) was spread over the plates containing Mueller- Hinton agar using a sterile cotton swab in three directions in order to get a uniform microbial growth. In aseptic conditions, blank sterile discs (9mm in diameter) were impregnated with 15µl of the methanolic extract. Discs were left for 5 min at room temperature for better oil absorption and were then placed on the inoculated agar surface. In addition, standard antibiotic discs of ciprofloxacin, amoxicillin, clotrimazole, and gentamicin (MAST Co., UK) placed on the agar surface. The Petri dishes were then placed in an incubator at 37°C for 24 hrs. After an incubation period, diameters of inhibition zones around the discs were measured.

Broth microdilution testing: To determine of minimum inhibitory concentration (MIC), broth microdilution testing was carried out based on document M07-A9 for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus*

agalactiae, group A streptococci, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Staphylococcus saprophyticus* (CLSI, 2012). Briefly, a series of twofold dilutions of extract and essential oil of cumin ranging from 10mg/mL to 0.075mg/ml was prepared in a 96-microtiter plate, with a final concentration of 0.001% (v/v) Tween 80 to enhance essential oil solubility. After inoculation of isolating suspensions (equivalent to a 0.5 McFarland standard), microtiter plates were incubated at 37°C for 24 hours. MICs were determined visually with the assistance of a reading mirror, according to CLSI guidelines. Minimal bactericidal concentration (MBC) was determined by using 0.01ml from each well of the microtiter plates used in the MIC assay without observable growth onto blood agar plates. MBCs were defined as the lowest concentration resulting in no growth on subcultures.

Statistical analysis: Variance (ANOVA) of each isolated bacterial species (agar disc-diffusion assay) was analyzed ($P<0.05$) and differences between means were determined by Tukey's HSD test ($P<0.05$).

Results

Bacterial isolation: Out of 95 samples, 60 bacterial strains were isolated from patients with symptomatic urinary tract infection. Of 60 that were studied, *E. coli* had the highest incidence among bacteria and after that, *Klebsiella pneumoniae* had the second place. Other detected bacteria were *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae* and *Staphylococcus saprophyticus*, group A streptococci.

Results of disc diffusion susceptibility testing: The antibacterial effect of *C. cyminum* extract and essential oil against some important uropathogens studied in the present study and their potencies were qualitatively assessed by the presence or the absence of inhibition zone diameter. The results indicated that the essential oil of *C. cyminum* had substantial antibacterial activity against *E. coli*, *K. pneumoniae*, *E. faecalis*, *P. aeruginosa*, *S. agalactiae*, *S. saprophyticus* and group A streptococci (≥20 mm inhibition zone diameter). *S. aurous* and *S. epidermidis* were resistant to the essential oil with a diameter of inhibition less than 10mm (Table 1). Ninety percent of isolates were susceptible to ciprofloxacin followed by gentamicin (74.9%). In addition, *C. cyminum* extract showed suitable antimicrobial activity against isolates except *S. agalactiae* isolates.

Results of broth microdilution testing: The MIC values of *C. cyminum* essential oil against uropathogenic isolates varied from 0.015 to 0.25 mg/ml. The data obtained, through the determination of MIC are revealed in Table 2. MIC and MBC values demonstrated that the cumin essential oil was effectual against tested uropathogenic bacteria with MIC about 0.25 mg/ml for Gram-negative bacteria, from 0.015 to 0.25 mg/ml for Gram-positive bacteria. The MIC and MBC values obtained with cumin oil for *P. aeruginosa*, *S. epidermidis*, *S. saprophyticus* and *E. faecalis* strains were similar to those obtained with cumin extract.

Table 1: Results of disc diffusion susceptibility testing for isolates.

Bacterial strain	The mean of inhibition zone diameter (mm)					
	CIP	SXT	GM	AMX	C. cyminum extract	*C. cyminum* essential oil
P. aeruginosa	28	8	14	7	20	20
E. coli	28	22	22	17	22	23
K. pneumoniae	14	14	16	7	22	22
S. agalactiae	14	7	9	23	7	21
E. faecalis	38	30	20	7	23	20
S. aurous	20	22	20	7	20	7
S. epidermidis	19	20	23	7	25	10
S. saprophyticus	26	26	25	7	23	20
group A streptococci	19	16	15	7	23	20

Table 2: Antibacterial activity of *C. cyminum* essential oil and extract against clinical isolates.

Microorganisms	*C. cyminum* essential oil		C. cyminum extract	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
P. aeruginosa	0.25	0.5	0.25	0.5
E. coli	0.25	0.5	0.125	0.25
K. pneumoniae	0.25	0.5	0.125	0.25
S. agalactiae	0.25	0.5	-	-
E. faecalis	0.125	0.25	0.125	0.25
S. aurous	-	-	0.125	0.25
S. epidermidis	0.25	0.5	0.125	0.25
S. saprophyticus	0.25	0.5	0.25	0.5
group A streptococci	0.015	0.03	0.125	0.25

Discussion

UTI is one of the most common bacterial infections, resulting in billions of dollars in health care expenditure yearly. The spread of antibiotic resistance threaten the effective treatment option[11]. In addition, high rates of recurrent UTIs suggest that antibiotic therapy is not an effective treatment for all UTIs[12]. The spread of antibiotic resistance among uropathogenic bacteria remains a significant medical problem, which stimulates researches about new methods for fighting with drug resistance or renews interest in traditionally used and forgotten ways, such as treatment with antimicrobial essential oils and plant extracts[13]. In the present study, we evaluated antibacterial activity of *Cuminum cyminum* extract and essential oil against some important uropathogenic bacteria isolated from urine of patients with symptomatic urinary tract infection.

Some studies about herbal medicine have been reported an enhanced antimicrobial activity of essential oil in comparison with hydro-extract, methanolic extract and powder of plants. This antimicrobial activity of essential oils is associated with major components of them[14-18]. These ingredients, with a specific chemical structure including aromatic rings, are capable to decompose the outer membrane of gram-negative bacteria and increase the permeability of the inner cytoplasmic membrane to various compounds[15]. In our study, cumin essential oil had significant antibacterial effect on gram-negative bacteria and cumin extract had excellent activity against gram-positive and gram-negative bacteria.

These results of the cumin essential oil was in agreement with values reported from other studies[16]. In numerous investigations, the antibacterial effect of *C. cyminum* on various bacteria such as, Vibrio spp., *P. aeroginosa*, *K. pnomoniae*, *E. coli*, *Y. enterocolitica*, *S. aureus* and *E. feacalis*[17-20] and others have surveyed and concluded that cumin essential oil and extract alone or combined with the coadministration of broad-spectrum antibiotics inhibit the growth of pathogenic bacteria[18]. Jazani et al. surveyed the effect of cumin essential oil on burn isolates of *P. aeroginosa*. They reported that essential oil of cumin possessed antibacterial activity against *P. aeroginosa* isolates with MIC values in the range of 0.015 to 0.25 mg/ml. In our study, MIC values of cumin essential oil were in the range of 0.25 to 0.5 mg/ml. Özcan and Erkmen conducted a study to determine antimicrobial effect of nine Turkish herb species including *C. cuminum*[21-23].

The results obtained indicated that the cumin essential oil tested varied in its antibacterial effect. The essential oil at 1% had potent bactericidal activity against *E. coli*, while at 10% inhibited *Y. enterocolitica*, *S. aureus* and *E. feacalis*. Mekawey and colleagues have tested the in vitro antibacterial activity of 1-(2-Ethyl, 6-Heptyl) Phenol (EHP), a biologically active compound, which extracted by benzene from cumin[19]. Higher activity of the component was detected against Gram-positive bacteria than Gram-negative ones where *S. aureus*, *S. pneumoniae*, *B. subtilis* and *B. thuringiensis* were more sensitive than *S. typhi*, *E. coli*, *S. marcescens* and *P. aeruginosa*. Some researches examining the effect of whole essential oil of herbs against food decay microorganisms[20] and food-borne pathogens agree that, commonly, essential oils are slightly more effective against Gram positive than Gram-negative

bacteria[21]. Derakhshan et al. also reported that growth of *K. pneumonia* strains exposed to subminimum inhibitory concentrations of cumin seed essential oil and alcoholic extract resulted in cell elongation and repression of capsule expression[18].

Gachkar and collogues have indicated that the essential oil extracted by hydrodistillation from Iranian *C. cyminum* displayed good to moderate antimicrobial activities against tested *E. coli*, *S. aureus* and *L. monocytogenes*[22]. In fact, *E. coli* was the most susceptible bacterium with the lowest MBC value (1µl/ml) and *L. monocytogenes* required higher essential oil concentration (2µl/ml) for complete elimination.

Our results revealed variability in the inhibitory concentrations of the essential oil and extract for staphylococci. Cumin extract was more effective than essential oil against examined Staphylococcus spp. Our findings also showed that essential oil of *C. cyminum* has better antibacterial activity on uropathogen isolates than amoxicillin and the difference was significant ($P<0.05$) but the activity is not superior to other antibiotics. In this context, *C. cyminum* essential oils and extract, gave interesting results, being one of the promising performing essential oil and extract of herbs in terms of antibacterial activity against common uropathogenic bacteria. These results suggest that the essential oil and extract of *C. cyminum* seeds might be considered as an interesting source of antibacterial components against uropathogenic bacteria. The findings indicated in the study may also contribute to knowledge of the antimicrobial potentials of these species reported elsewhere.

Conclusion

These results suggest that the essential oil and extract of *C. cyminum* seeds might be considered as interesting sources of antibacterial components against uropathogenic bacteria.

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Conflict of interest

The authors declare no conflicts of interest.

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