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# Antibacterial activities of *Cuminum cyminum* Linn Essential Oil Against Multi-Drug resistant *Escherichia coli*

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**Background:** Development of antibiotic resistance among pathogenic bacteria motivates the researchers to search for newer antimicrobial agents.
**Objectives:** In the present study, antibacterial effects of *Cuminum cyminum* Linn. essential oil against multidrug resistant (MDR) *Escherichia coli strains* isolated from urinary tract infections were studied, using microdilution method.
**Materials and Methods::** A total of 12 *E. coli strains* were isolated from urine cultures of hospitalized patients (Zabol, southeastern Iran) suffering from urinary tract infection during 2011-2012. After bacteriological confirmatory tests, minimum inhibitory concentrations (MICs) of the essential oil of *C. cyminum* Linn. were determined using microdilution method. Essential oil of *C. cyminum* Linn. was obtained by hydro-distillation and the MICs were investigated to characterize the oil antimicrobial activities.
**Results:** All of *E. coli* isolates were resistant to four of the antibiotics including ceftazidime (50%), cefixime (41.6%), tetracycline (75%) and erythromycin (58.3%). The highest MIC value (250 ppm) was observed against two antibiotics and the lowest (10 ppm) against one antibiotic.
**Conclusions:** *C. cyminum* Linn essential oil has a potent antimicrobial activity against *E. coli* MDR strains. Our study confirms the use of this essential oil as an antibacterial agent. However, further research will be required before its therapeutic application.

*Keywords:Escherichia coli*; Oils, Volatile; *Cuminum*

## 1. Background

The currently used feed additives such as antibiotics, probiotics and prebiotics in broiler diets to enhance nutrient utilization, play important roles in antibiotic resistance development among pathogens and saprophyte bacteria (1). The search for components with antimicrobial activities has recently gained increasing importance, due to the growing worldwide concern about the alarming increase in the rate of infections by antibiotic-resistant microorganisms. Due to their antibacterial activities against bacterial pathogens, medicinal plants are very important in human health. *Cumin* (*Cuminum cyminum* L.) originates from Egypt and Ethiopia and is much cultivated in Arabia, Malta, Sicily, India and China. *Cumin* seeds are used to flavor foods and liquors and its oil is utilized in perfumes and cosmetics. *C. cyminum* Linn. is an annual plant of the Umbelliferae family with antioxidant, anticholesterol and antimicrobial properties. Urinary tract infections (UTIs) are the second most common type of infections in the body and *E. coli* is the most common bacterial pathogen causing UTI (2). Different antimicrobial agents with high levels of activity against Gram-negative bacilli, including amikacin, ciprofloxacin, fosfomycin, gentamicin and nitrofurantoin, have shown acceptable levels of activities against this bacterium. Unfortunately, rapid appearance and development of drug resistance among these bacteria have caused a lot of difficulties in the modern world.

## 2. Objectives

In the present study, antibacterial activities of *C. cyminum* Linn. essential oil against multidrug resistant (MDR) *E. coli* strains isolated from UTIs were determined, using microdilution method.

**Implication for health policy/practice/research/medical education:**
The present study evaluated the antibacterial effects of *Cuminum cyminum* Linn seed extract and represented a potential template for design of antibacterial agents to decrease bacterial resistance and overcome the drug resistance problem. *C. cyminum* Linn has potent antimicrobial activities against antibiotic-resistant *E. coli* strains. However, further research is required to evaluate the practical values before therapeutic usage.

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## 3. Materials and Methods

### 3.1. Isolation of Bacteria

A total of 12 strains of *E. coli* were isolated from urine cultures of hospitalized patients (Zabol, southeastern Iran) suffering from UTIs, during 2011-2012. The isolated bacteria were identified and evaluated by Gram staining and standard biochemical tests (3).

### 3.2. Antibiotic Susceptibility Test

Susceptibility to all antibiotics was tested using standard disc diffusion method, as recommended by Clinical and Laboratory Standards Institute (CLSI) (4). Briefly, a colony suspension was prepared using sterile normal saline, equivalent to the 0.5 McFarland standard, and spread over the Mueller Hinton agar plate. Afterwards, the antibiotic discs were transferred aseptically to the surfaces of the inoculated media plates. Antibiotics and their concentrations were as follows: ceftazidim: 30 μg, tetracycline: 30 μg, erythromycin: 15 μg and ceftazidime: 30 μg. *E. coli* ATTCC 25922 was used as the control strain.

### 3.3. Plant Materials

Seeds of *C. cyminum* Linn. were collected from suburban areas of Kerman (southeastern Iran) and dried at room temperature in Kerman Azad University herbarium. Afterwards, the samples were crashed, transferred into a glass container, and preserved until the extraction process was performed in the laboratory.

### 3.4. Distillation of Essential Oil

The seeds were ground prior to the operation, and then, 300 g of the ground powder was submitted to water distillation for four hours, using a Clevenger apparatus. The distilled essential oil was dried over anhydrous sodium sulfate, filtered, and stored at 4°C.

### 3.4. Minimum Inhibitory Concentration of Essential Oil

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC). Briefly, serial double dilutions of the extract in Mueller Hinton broth, containing 0.5% (V/V) tween 80 over the ranges of 250, 100, 50 and 10 ppm, were prepared and added to a 96-well microtiter plate. To each well, 10 μL of indicator solution and 10 μL of Mueller Hinton broth were added. Finally, 10 μL of bacterial suspension (106 CFU/mL) was added to each well to achieve a concentration of 104 CFU/mL of the bacteria. The plates were wrapped loosely with cling film to prevent the bacteria dehydration. The plates were prepared in triplicates, and placed in an incubator at 37°C for 18–24 hours. MIC was defined as the lowest concentration of the essential oil, at which the microorganism did not demonstrate any visible growth. Average of the three values was calculated to provide the MIC values for the tested extract.

## 4. Results

### 4.1. Antibiotic Susceptibility

Antibiotic full resistance profile of the *E. coli* isolates was as follows: tetracycline (75%), erythromycin (58.4%), ceftazidime (50%) and cefixime (41.7%). Moderate resistance was observed in only 25% and 8.3% of the isolates against erythromycin and cefixime, respectively (Table 1). About 25% of the *E. coli* isolates showed resistance to all the antibiotics, whereas 8.3% and 25% showed resistance to three and two antibiotics, respectively (Table 2).

### 4.2. Minimum Inhibitory Concentration Assessment for Essential Oil

Different inhibitory effects of essential oil against most *E. coli* isolates were demonstrated in Table 2. The essential oil had inhibitory effects against most of the isolates. About 8.3% and 16.6% of the *E. coli* isolates showed the lowest MICs (10 and 50 ppm, respectively), while moderate (100 ppm) and highest (250 ppm) MIC values were seen in 41.6% and 16.6% of the isolates, respectively.

**Table 1.** Antimicrobial Susceptibility of *Escherichiacoli* isolates [a]

| | Ceftazidime | Erythromycin | Cefixime | Tetracycline |
|---|---|---|---|---|
| **Sensitive** | 6 (50) | 2 (16.6) | 6 (50) | 3 (25) |
| **Intermediate** | 0 | 3 (25) | 1 (8.3) | 0 |
| **Resistant** | 6 (50) | 7 (58.4) | 5 (41.7) | 9 (75) |

[a] Data are presented in No. (%).

**Table 2.** Antimicrobial Susceptibility and Minimum Inhibitory Concentration of the Essential Oil for *Escherichiacoli*[a, b]

| Bacterial Code | Antibiotic Susceptibility | | | | MIC for Essential Oil, ppm | Resistance Pattern |
|---|---|---|---|---|---|---|
| | $A_1$ | A2 | $A_3$ | $A_4$ | | |
| **1** | R | R | R | R | 100 | $A_1, A_2, A_3, A_4$ |
| **2** | R | I | R | S | 250 | $A_1, A_3$ |
| **3** | S | R | R | R | NO | $A_2, A_3, A_4$ |
| **4** | R | R | R | R | 100 | $A_1, A_2, A_3, A_4$ |
| **5** | R | R | R | R | 50 | $A_1, A_2, A_3, A_4$ |
| **6** | S | S | I | S | 50 | - |
| **7** | S | S | S | S | 10 | - |
| **8** | I | S | S | R | 100 | $A_4$ |
| **9** | R | S | S | R | 250 | $A_1, A_4$ |
| **10** | R | S | I | R | 100 | $A_1, A_4$ |
| **11** | S | S | S | R | NO | $A_4$ |
| **12** | R | R | R | R | 100 | $A_1, A_2, A_3, A_4$ |

[a] Abbreviations: I, intermediate; MIC, minimum inhibitory concentration; NO, Any inhibition; R, resistant; S, sensitive.
[b] $A_1$, erythromycin; $A_2$, cefixime; $A_3$, ceftazidime; $A_4$, tetracycline.

## 5. Discussion

In the present study, *E. coli* strains were resistant to four of the agents, including tetracycline (75%), erythromycin (58.3%), ceftazidime (50%), cefixime (41.6%). Different results were reported by other investigators in different geographical areas. For example, Shayan et al. reported antibiotic susceptibility of the AmpC-producing *E. coli* isolates as follows: erythromycin (92.3%), tetracycline (92.2%) nalidixic acid (84.6%), cefixime (84.6%), difloxacin (84.6%) azithromycin (76.9%), amoxicillin (76.9%), trimethoprim-sulfamethoxazole (76.9%) and gentamicin (76.9%) (5). Madani et al. reported antimicrobial resistance to ampicillin (91.4%), cotrimoxazole (61.1%), cefixime (46.8%), gentamicin (43.3%), ceftazidime (38.8%) and nalidixic acid (38.5%) (6). In the study of Heidari-Soureshjani et al. the highest resistance was reported to ampicillin (85.71%), nalidixic acid (78.78%), and ciprofloxacin (46.51%) (7). In the recent years, essential oils of plants have been in high demand from the manufacturers of foods flavoring, fragrance, cosmetics, and pharmaceutical industries, due to the growing interest of consumers to ingredients from natural sources. In our study, 8.3% and 16.6% of the *E. coli* isolates showed the lowest MICs (10 and 50 ppm respectively), while moderate (100 ppm) and highest (250 ppm) MIC values were seen in 41.6% and 16.6% of the isolates, respectively. Inhibitory effects of *Cumin* extract on *E. coli* 0:157 has been demonstrated previously (8). Other authors have also shown the antimicrobial activities of hexane extract and volatile components (9), water extracts or juices (10), and methanolic extracts of *C. cyminum* against different bacterial strains. For example, in the study of Vaishnavi et al. *Cumin* seeds were effective at lower concentration against *Salmonella typhi* and *E. coli* O:157 isolates (11). Soniya et al. reported the largest diameter of inhibition zone related to methanol extracts of *C. cyminum* against Bacillus *subtilis*, *E. coli* and *Proteus* sp. (12). As the study of Steffanini et al. reported, essential oil of *C. cyminum* was active against different Gram-negative bacteria, including *E. coli*, *Pseudomonas. aeruginosaand Salmonella* sp. with inhibitory zones of 18 mm, 10 mm and 23 mm, respectively (13). Con et al. reported that *Cumin* had inhibitory effect against *Staphylococcus. aureus* and *Micrococcus luteus* (14). Akgul and Kivanc reported that *Cumin* exhibited an inhibitory effect against *S. aureus*, *Klebsiella pneumonia* and *P. aeruginosa* (15). essential oil of *C. cyminum* can be used for protection against some bacteria.

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## Authors' Contributions

All authors had equal roles in design, work, statistical analysis and manuscript writing.

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There was no conflict of interest.

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