Antimicrobial Activity of *Cyminum cuminum* Against Biofilm E. coli

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ABSTRACT: Uropathogenic organisms have evolved numerous defense mechanisms against antimicrobial agents, hence resistance to old and newly available drugs are increasing at an unprecedented level. The events of antibiotic resistance have led to screening of several medicinal plants for their potential antimicrobial activity. The aim of this study was to evaluate the antimicrobial efficacy of *Cyminum cuminum* against biofilm Escherichia coli. Cumin is an annual plant of the Umbelliferae family. Cumin is an important medical herb in Asia and has antioxidant, anticholesterol, and antimicrobial properties. Ethanol extracts of seed of *Cyminum cuminum* were tested for antimicrobial activity in vitro by the microdilution method. Ethanol extract of seed exhibited antimicrobial activity against biofilm Escherichia coli. It can be concluded that *Cyminum cuminum* can be used to discover natural products that may serve as lead for the development of new pharmaceuticals, addressing the major therapeutic needs especially for biofilm E. coli.

Key word: Cumin, *Cyminum Cuminum*, Biofilm, Ecoli, Antibacterial Activity

INTRODUCTION

Uropathogenic Escherichia coli (UPEC), which accounts for 85% of urinary tract infections (UTI), assembles biofilms in diverse environments, including the host. Besides forming biofilms on biotic surfaces and catheters, UPEC has evolved an intracellular pathogenic cascade that culminates in the formation of biofilm-like intracellular bacterial communities (IBCs) within bladder epithelial cells. Rapid bacterial replication during IBC formation augments a build-up in bacterial numbers and persistence within the host (Hadji frangiskou et al., 2012). Cuminum cuminum L. is an annual plant of the family Apiaceae. The medicinal component of the plant is Cumin oil extracted from the ripe fruit. In folk medicine, cumin is used as a carminative for stomach disorders, diarrhea, and colic, as well as particularly inveterinary medicine (Gruenwald et al., 2004). Cumin is widely used in medicine for the treatment of dyspepsia, diarrhea, and jaundice, as it has stomachic, diuretic, carminative, and antispasmodic properties (Dhandapani et al., 2002). The aim of this paper was to substantiate the antimicrobial sensitivity of different extracts of *Cyminum cuminum* against Biofilm E. coli strains.

Isolation of bacteria

40 strains of E. coli were isolated from urine culture of hospitalized patients (Amir Al-Momenin Hospital, Zabol, south-eastern Iran) suffered from urinary tract infections during the years 2011-2012.

PLANT MATERIALS

This seed *Cuminum cyminum* was collection in the region of Iran (Zabol and Kerman, south-eastern, Iran) and plant in Kerman Islamic Azad University herbarium received approval and to dry the plant: room temperature and dark place. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Preparation of extracts

Plants were properly dried and pulverized into a coarse powder as reported by Hanafy and Hatem. Each of 20 g grinded powders was soaked in 60 ml ethanol 95%, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman no. 1 filter paper). Then
the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 4°C in air tight screw-cap tube (Hanafy et al., 1999).

**Minimum Inhibitory Concentration (MIC) of plant extracts**

The broth microdilution method was used to determine MIC according to Yu (Yu et al., 2004). All tests were performed in Mueller Hinton Broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 0.3 mg/ml to 10.00 mg/ml. To each well, 10 μl of indicator solution (prepared by dissolving a 10-mg extract in 2 ml of DMSO) and 10 μl of Mueller Hinton Broth were added. Finally, 10 μl of bacterial suspension (10^6 CFU/ml) was added to each well to achieve a concentration of 10^5 CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. The colour change was then assessed visually. The lowest concentration at which the colour change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity.

**Biofilm Formation Assay in Presence of the biocides**

After performing the procedure described above, the microplate was covered and incubated aerobically for 24 h at suitable temperatures. At first, the OD was measured (600 nm) by using an automated ELISA counter, then, the content of each well of the microplate was aspirated and each well was washed three times with 250 μl of sterile physiological saline. The remaining attached bacteria were fixed with 200 μl of 99% methanol per well and after 15 min all of the wells were emptied and left to dry. Then, each well was stained for 5 min with 0.2 ml of 2% crystal violet. Excess stain was rinsed off by washing the plate slowly with distilled water. After the plate was air dried, the dye bound to the adherent cells was resolubilized with 160 ml of 33% (v/v) glacial acetic acid per well. The OD of each well was measured at 492 nm by using an automated ELISA counter.

**Statistical analyses**

The growth was compared at each experiment using analysis of variance (ANOVA) repeated measures (SPSS 16.0 for Windows). The level of statistical significance was set at P< 0.01.

**RESULT**

The highest MIC of alcoholic extract of CCL were 5 mg/ml respectively. In vitro antibacterial activities of leaves of CCL have been investigated against biofilm and ESBL producing - E. coli and results are shown in table 1,2

**DISCUSSION**

Cuminumcuminum Linn. (Cumin) is an annual plant of the Umbelliferae family. Cumin is an important medical herb in Asia and has antioxidant, anticholesterol and antimicrobial properties. inhibitory effect of cumin extract on E. coli 0:157 demonstrated in vitro (Sagdic et al., 2002). According to Shetty et al. (Shetty et al., 1994), fungi and yeast were more sensitive to cumin essential oil as compared to bacteria. Cumin increased activity and excretion content of bile acids and also increased pancreas and small intestine digestive enzymes such as amylase, tripsine, chymotripsine therapy of some diseases for a long time. Many Plantsandlipase in rats (Muthamma et al., 2008). In the present study the MIC of Cyminum cuminum ranged from 1.25 to 10 mg/ml against the strains of E. coli, as 15 strains of E. coli showed MIC of 5 mg/ml (Table 1). The another study, essential oil of Cyminum cuminum was biofilm formation preventive properties, is found against streptococcus mutans and streptococcus pyogenes(Shayegh et al., 2008). Cumin seed oil and alcoholic extract inhibited the growth of Klebsiella pneumonia and its clinical isolates and caused improvement in cell morphology, capsule expression and decreased urease activity, this property is attributed to cuminldehyde (Derakhshan et al., 2010). The study of Derakshan, the essential oil of Cyminum cuminum decreased biofilm formation (Derakhshan et al., 2010). C. cuminum oil exhibited stronger antimicrobial activity against E. coli, S. aureus and L. monocytogenes. The study of HosseiniJazani, Cumin essential oils possessed antibacterial effect against all isolates of Pseudomonas aeruginosa, with MIC and MBC values in the range of 0.015 to 0.25 ml/ml (Hosseini Jazani et al., 2008). In conclusion results of this study showed that the Cyminum cuminum seed have exhibited varied antimicrobial activities against the biofilm E. coli.
Table 1. Minimum inhibitory concentration (MIC) for plant extract used against isolates of Ecoli. (%)

<table>
<thead>
<tr>
<th>Extract plant concentration</th>
<th>CCL</th>
</tr>
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<tbody>
<tr>
<td>0.3</td>
<td>0</td>
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<tr>
<td>0.62</td>
<td>0(3.7)</td>
</tr>
<tr>
<td>1.25</td>
<td>4(14.81)</td>
</tr>
<tr>
<td>2.5</td>
<td>15(55.5)</td>
</tr>
<tr>
<td>5</td>
<td>7(25.92)</td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Antimicrobial activity extract of Cyminumcuminumseed against Biofilm E. coli

<table>
<thead>
<tr>
<th>Time(h)</th>
<th>Concentration(mg/ml)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>2h</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4h</td>
<td>0.005</td>
<td>0.009</td>
</tr>
<tr>
<td>6h</td>
<td>0.052</td>
<td>0.088</td>
</tr>
<tr>
<td>24h</td>
<td>0.010</td>
<td>0.023</td>
</tr>
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REFERENCES


Gachkar L, Yadegari D, Rezaei MB, Taghizadeh M, Astaneeh SA, Rasooli I. 2006. Chemical and biological characteristics of Cuminumcyminumand Rosmarinusofficinalis essential oils. Food Chemistry DOI:10.1016/j.foodchem. 06.035


