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Antimicrobial activity of *Foeniculum vulgare* Mill essential oil against antibiotic resistance human pathogen

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Abstract

The aim study antimicrobial activity of *Foeniculum vulgare* Mill essential oil against antibiotic resistance human pathogen. All 36 strains of (12 *Klebsiella pneumoniae*, 12 *E. coli* and 12 *Staphylococcus aureus*) isolated from urine culture of hospitalized patients during the years 2011- 2012. In this study, the essential oil of *Foeniculum vulgare* Mill obtained by hydrodistillation and the minimum inhibitory concentrations were investigated to characterize the antimicrobial activities of this essential oil. However, overall, *E. coli* were resistance to 8 of the agent including ceftazidime (50%), tetracyclin (75%), erythromycin (58.3%), cefixime (41.6%) , penicillin (83.3%), ampicillin (58.3%), nalidixic acid (58.3%). Antibiotic susceptibility of *K. pneumoniae* isolates was evaluated for 4 antimicrobial. However *k. pneumoniae* were resistance to 4 of the agent including ceftazidime (33.3%) ,cefixime (58.3%), erythromycin (75%), tetracyclin (50%) and *S. aureus* were resistance to 6 antimicrobial , cefixime (33.3%), trimethoprim-sulfamethoxazol (41.66%), penicillin (50%), oxacillin (83.3%), ceftazidime (66.6%) and vancomycin (8.3%).The highest MIC values of essential oil were found to be 250ppm against four *E. coli* and three *K. pneumoniae* and two of MIC value for *S.aureus* was 10ppm.

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Introduction

The increasing resistance of many clinical strains of bacteria to commonly used antibiotics and chemotherapeutics in pharmacological practice are looking for safe and effective factors that could be used to treat persistent bacterial infection. Medicinal plants are important sources of potentially useful new compounds for the development of chemotherapeutic agents. Plants contain numerous of compounds with antibacterial activity (Branther, 1994; Iwv *et al*., 1993). Certain plant essential oils and or their constituents have a broad spectrum of activity against pre and post harvesting pathogens but they are safe for users and environment. *Foeniculum vulgare* Mill is a biennial medicinal and aromatic plant belonging to the family Apiaceae (Umbelliferaceae). It is a hardy, perennial–umbelliferous herb with yellow flowers and feathery leaves. Essential oil of fennel is used as flavoring agents in food products such as beverages, bread, pickles, pastries, and cheese. It is also used as a constituent of cosmetic and pharmaceutical products (Piccaglia and Marotti, 2003). Herbal drugs and essential oils of fennel have hepatoprotective effects (Ozbek *et al*., 2003). Traditionally in Europe and Mediterranean areas fennel is used as antispasmodic, diuretic, anti-inflammatory, analgesic, msecretomotor, secretolytic, eye lotion, and antioxidant remedy and integrator (Gori *et al*., 2012). Chauche reported the phytochemical test on the stem, roots, and seeds of the plant fennel. The presence of flavonoide, tannins, coumarines saponins, sterols, essential oil and absence of anthocyanes and alkaloids was reported (Chaouche, 2011).

Recently, multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs that are commonly used in the treatment of infectious diseases, making it a global growing problem. In recent years, essential oils of plants and their other products from secondary metabolism have been in high demand by the manufacturers of foods flavoring, fragrance, cosmetics, and pharmaceutical industries due to the growing interest of consumers in ingredients from natural sources.

The aim study antimicrobial activity of *Foeniculum vulgare* Mill essential oil against antibiotic resistance human pathogen.

Material and method

Isolation of bacteria: All 36 strains (12 *K. pneumoniae* and 12 *E. coli* and 12 *S. aureus*) isolated from urine culture of hospitalized patients Hospital (Zabol, south-eastern Iran) suffered from urinary tract infections during the years 2011- 2012 were evaluated. Isolated bacteria were identified by Gram's stain and standard biochemical tests (Forbes *et al*., 2007). Antibiotic susceptibility testing was performed by the Kirby Bauer method on Mueller-Hinton agar according to CLSI protocols (8). The tested drugs (in µg) and their potencies as follow ceftazidim (30 µg), tetracyclin (30 µg), erythromycin (15 µg), ceftazidime (30 µg), trimethoprim-sulfamethoxazol (1.25+23.15 µg), penicillin (10µg),oxacillin (30µg) ,vancomycin (10 µg), nalidixic acid (10 µg) and ampicillin (30 µg).

Plant materials

The seed *Foeniculum vulgare* Mill.**,** was collection in the region of Iran (Sistan , south-eastern, Iran) and plant in Zabol university herbarium received approval and dried at room temperature .Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Distillation of essential oil

Plant materials were subjected to steam distillation for 3 h using a Clevenger-type apparatus. Essential oils were collected after decantation. The distilled essential oil was dried over anhydrous sodium sulfate, filtered and stored at 4°C.

Minimum Inhibitory Concentration (MIC) of essential oil

The broth microdilution method was used to determine MIC. 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 250ppm, 100ppm, 50ppm and 10ppm. To each well, 10 µl of indicator solution 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 10^4 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 color change was then assessed visually. The lowest concentration at which the color 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.

Results

Antibiotic susceptibility of *E. coli* isolates was evaluated for 8 antimicrobial. however, overall, *E. coli* were resistance to 8 of the agent including ceftazidime (50%), tetracyclin (75%), erythromycin (58.3%), cefixime (41.6%), penicillin (83.3%), ampicillin (58.3%), nalidixic acid (58.3%)(Table 1), Antibiotic susceptibility of *K. pneumoniae* isolates was evaluated for 4 antimicrobial. However *k. pneumoniae* were resistance to 4 of the agent including ceftazidime (33.3%), cefixime (58.3%), erythromycin (75%), tetracyclin (50%) (Table 2) and *S.aureus* were resistance to 6 antimicrobial, cefixime (33.3%), trimethoprim-sulfamethoxazol (41.66%), penicillin (50%), oxacillin (83.3%), ceftazidime (66.6%) and vancomycin (8.3%) (Table 3). The highest MIC values of essential oil were found to be 250ppm against four *E.coli* and three *K. pneumoniae* and *two* of MIC value for *S.aureus* was 10ppm (Table 4, 5).

Table 1. Antimicrobial susceptibility of 12 strains of *E. coli* (%).

	E	CN	CAZ	TE	P	AM	NA
S	16.6	50	50	25	16.6	8.3	41.6
I	25	8.3	0	0	0	33.3	0
R	58.3	41.6	50	75	83.3	58.3	58.3

S= Sensitive, I= Intermediate, R= Resistant
CAZ= Ceftazidime, TE=Tetracyclin,E= Erythromycin, CN= cefixime, P=penicillin, AM=ampicillin, NA=nalidixic acid

Table 2. Antimicrobial susceptibility of 12 strains of *k.* pneumoniae (%).

	CAZ	E	CN	TE
S	50	8.3	33.3	33.3
I	16.6	16.6	8.3	16.6
R	33.3	75	58.3	50

CAZ= Ceftazidime, TE= Tetracyclin, E= Erythromycin, CN= cefixime .

Table 3. Antimicrobial susceptibility of 12 strains of *S.aureus* (%).

	CN	SXT	V	CAZ	P	OX
S	58.3	50	50	25	25	0
I	8.3	8.3	41.6	8.3	25	16.6
R	33.3	41.66	8.3	66.6	50	83.3

CAZ= Ceftazidime, CN= cefixime, SXT= trimethoprim-sulfamethoxazol, P= penicillin, OX= Oxacillin,V= Vancomycin.

Table 4. Antimicrobial susceptibility, MIC against *E. coli*.

Bacterial	MIC(ppm)
1	NO
2	NO
3	NO
4	NO
5	NO
6	250
7	100
8	50
9	250
10	250
11	100
12	50

Table 5. Antimicrobial susceptibility, MIC against *k.* pneumoniae.

Bacterial	MIC(ppm)
1	50
2	100
3	NO
4	250
5	50
6	100
7	250
8	50
9	100
10	250
11	250
12	100

Table 6. Antimicrobial susceptibility, MIC against *S. aureus*.

Bacterial	MIC(ppm)
1	50
2	NO
3	NO
4	50
5	NO
6	50
7	100
8	10
9	10
10	NO
11	NO
12	NO

Discussion

In recent years, the appearance of antibiotic resistant bacteria and fungi to antimicrobial agents has been an important issue for researchers. This resistance to antibiotics increases the morbidity rate in communities**.**

In the study the highest MIC values of essential oil were found to be 250ppm against four *E. coli* and three *K. pneumoniae* and two of MIC value for *S.aureus* was 10ppm. The essential oil extracted from the fruits of *F. vulgare* exhibited antibacterial effect against foodborne pathogens such as *Escherichia coli*, *Bacillus megaterium* and *Staphylococcus aureus* (Mohsenzadeh, 2007), *E. coli* 0157:H7, *Listeria monocytogenes* and *S. aureus* (Cantore, 2004; Dadalioglu and Evrendilek, 2004). Aqueous and organic extracts of *F. vulgare* have been reported to show antibacterial activity against some bacterial strains (Kaur and Arora, 2008). The study of Manonmani, the seed extracts of *Foeniculum vulgare* Mill exhibited activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Enterobacter aerogeus*, *Salmonella typhi*, *Bacillus cereus* and *Staphylococcus aureus*. In addition, the effectiveness of plant was not due to one main active constituent, Earlier reports also support the view that antibacterial activity was due to different chemical constituents including flavanoids, alkaloids, terpenoids and other compounds which are classified as active antimicrobial compounds (Rojas *et al.*, 1992). The antimicrobial activity of *Foeniculum vulgare* against *E. coli*, *S. aureus*, *Enterococcus sp* and *K.pneumoniae* has also been reported by Saviue (Saviu *et al.*, 2012). In the study *E. coli* resistance were to 8 of the agent including ceftazidime (50%), tetracyclin (75%), erythromycin (58.3%), cefixime (41.6%), penicillin (83.3%), ampicillin (58.3%), nalidixic acid (58.3%). The study of Akond, 88%, 82%, 80%, 76%, 70%, 68%, 64%, 58%, 52%, and 20% of the tested Escherichia coli strains from poultry sources were found resistant respectively to penicillin, ciprofloxacin, riphampicin, kanamycin, streptomycin, cefixine, erythromycin, ampicillin, tetracyclin and chloramphenicol and neomycin (Akond *et al.*, 2009). Tricia reported 43% isolates of *E. coli* were resistant to ampicillin but no isolate was found resistant to gentamicin (Tricia *et al.*, 2006). In the study *k. pneumoniae* were resistance to 4 of the agent including ceftazidime (33.3%), cefixime (58.3%), erythromycin (75%), tetracyclin (50%). The study of Zamani, the most effective antibiotics against the isolates were tobramycin (79.05%), ceftazidime (79.05%), ceftizoxime (78.09%), ciprofloxacin (76.19%), ceftriaxone (76.24%) and amikacin (74.29%) (Zamani *et al.*, 2013). The study of Sikarwar 60 % strains was resistant to chloramphenicol and tetracycline. We also found that 28 to 76 % of them were resistant to cephalosporins (ceftizoxime and cefotaxime) (Sikarwar and Batra, 2011). In our study S.aureus were resistance to 6 antimicrobial, cefixime (33.3%), trimethoprim-sulfamethoxazol (41.66%), penicillin (50%), oxacillin (83.3%), ceftazidime (66.6%) and vancomycin (8.3%). The study in Mashhad, *Staphylococci* isolates were highly resistant

against ceftazidime (94%), followed by penicillin (91%), ampicillin (82%), cefotaxime (65%), erythromycin (60%), and oxacillin (43%) (Zarifian *et al.*, 2012). However, further studies about the isolation of active compounds and the absence of toxicity of plant extracts are necessary to propose these plants as alternative approaches to resistance management.

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