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Invitro evaluation of some Iranian plants against antibiotic resistance *Klebsiella pneumonia*

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ABSTRACT

This study confirms the invitro evaluation of some Iranian plants against antibiotic resistance k. pneumonia. All 12 Klebsiella pneumonia were isolated from urine culture of hospitalized patients during the years 2012- 2013. In this study, the essential oil of Cuminum cyminum, Zataria multiflora Bioss., Coriandrum sativum and Anethum graveolens dhi obtained by hydrodistillation and the minimum inhibitory concentrations were investigated to characterize the antimicrobial activities of this essential oil. The results show that the highest MIC values of essential oil from Z. multiflora were found to be 50ppm against K. pneumoniae and the least MIC values of essential oil from Z. multiflora were10ppm. The highest MIC values of essential oil from A.graveolens, A.graveolens and C.cyminum were found to be 250ppm against K.pneumoniae.

Keyword: *Klebsiella pneumonia***,** Iranian Plants, Antibiotic resistance, Urinary tract infection.

INTRODUCTION

The medicinal plants being used for treatment of infections is an age-old practice especially in developing countries. Plants generally act to stimulate and supplement the healing forces and are the natural food for human beings [1]. The medicines of plant origin are used for a variety of diseases [2, 3]. *Zataria multiflora* is a plant that belongs to the Laminaceae family and grows only in Iran, Pakistan and Afghanistan. This plant, known as Avishan-e-shirazi (in Iran), is used as a flavor agent in a variety of Iranian foods [4, 5]. It is used as a traditional cure for its antiseptic, analgesic and carminative specifications. Cumin (*Cuminum cyminum* L.) is aromatic plants included in the *Apiaceae* family and is used to flavor foods, added to fragrances, and for medical preparations. In folk medicine, cumin is used as a carminative for stomach disorders, diarrhea, and colic, as well as particularly in veterinary medicine [6]. Moreover, cumin oil shows a high antifungal activity against various pathogenic fungi, and effective high antibacterial activity. Dill (*Anethum graveolens*), also known as Shapt or dill-weed, belongs to family *Umbelliferae*, and is an annual herb growing to a height of 1.5 m. Dill originates from Mediterranean and West Asia. Its medicinal uses are as an antispasmodic, carminative, diuretic, stimulant and stomachic [7]. Coriander (*Coriandrum sativum*), first is called "coriandrum". Its origin countries are the shores of the Mediterranean and Central Asia, and it is cultivated all over the world. Coriander can be used as condiment, at the same time it has preservative function. It has a good prospect of application as a natural food preservative. This study also confirms the invitro evaluation of some Iranian plants against antibiotic resistance *k. pneumonia*.

MATERIALS AND METHODS

Isolation of bacteria

All 12 *Klebsiella pneumonia* 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.

Plant materials

The seeds of *Cuminum cyminum* and leaves of *Anethum graveolens* , *Coriandrum sativum* and *Zataria multiflora* were collected in the different regions of Iran (Zabol, Sherazi and Kerman, south-eastern, Iran) and the specimens were deposited in Zabol University Herbarium and dried at room temperature . The Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Distillation of essential oil

The seeds of *Cuminum cyminum* and leaves of *Zataria multiflora* , *Coriandrum sativum* and *Anethum graveolens* were ground before operation and then 300 g of ground rosemary was submitted to water distillation for 4 h using a Clevenger apparatus. 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 (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 10^4 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 was 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.

Statistical Analysis

All experiments and measurement were repeated at least three times. Statistical analyses were performed using SPSS and Excel 2010 software. All experimental results were analyzed using mean descriptive statistics and the correlation-coefficient. A value of $P<0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Overall, *k. pneumoniae* was resistance to 4 of the agent including ceftazidime (33.3%), cefixime (58.3%), erythromycin (75%) and tetracycline (50%) (Table1).

Inhibitory effects of essential oil of *Cuminum cyminum*, *Zataria multiflora* , *Coriandrum sativum* and *Anethum graveolensi* against *K. pneumoniae* demonstrate in table 2. The results in table 2 show that essential oil of *Cuminum cyminum*, *Zataria multiflora* , *Coriandrum sativum* and *Anethum graveolens* had inhibitory effect against *K. pneumoniae*. The highest MIC values of essential oil of *Zataria multiflora* were found to be 50ppm against *K. pneumoniae* and the leas MIC values of essential oil from *Z. multiflora* were10ppm. The highest MIC values of essential oil from *A.graveolens*, *A.graveolens and C.cyminum* were found to be 250ppm against *K.pneumoniae*. In the study the highest MIC values of essential oil of *Z. multiflora* were found to be 50ppm against *K. pneumoniae* and the leas MIC values of essential oil from *Z. multiflora Bioss* were10ppm. The highest MIC values of essential oil of *A.graveolens*, *A.graveolens* and *C.cyminum* were found to be 250ppm against *K.pneumoniae.* According *to* the study of Rahman, the MIC and MBC values for alcoholic extracts of *Z. multiflora* were in the range of 1.718-6.25 and 2.832-6.25 mg/ml, respectively. Significant (p=0.05) synergistic effect of combination of EtOH and MeOH extracts was recorded against *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 25923 while additive effect against rest of the bacterial strains [8]. The study of Ghasemi showed that the minimum inhibitory concentration of *Z.multiflora* for *S. enteritidis* and *E. coli* were enumerated at 6250 ppm. The minimum inhibitory concentration of antimicrobial agent for *L. monocytogenes* and *St. aureus* were found at 3125 ppm [9]. It was important to observe that *E. coli*, which is the main causative agent for gastroenteritis in Iran, showed the highest sensitivity following application of Zataria flower extracts [10, 11]. The oil showed inhibitory effect against *B. subtilis*, *P. vulgaris*, *A. flavus* and *A. niger*. The fungi were resistant to the oil than that of *C. albicans* [12]. The study of Motevasel showed that it inhibited the growth of *Z.multiflora* extract against *S. epidermidis*, *S.*

saprophyticus and methicillin sensitive *S. aureus* (MSSA) by about 8-16 µg/mL [13]. According to the study of Dahiya, seed oil of Dill (*Anethum graveolens*) showed broad antibacterial activity against both Gram-positive bacteria such as *Staphylococcus aureus*, *S. aureus* MRSA, *Enterococcus* sp. and Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*. The highest *in vitro* inhibitory activity was observed for MDR *Enterococcus* sp. with wide inhibition zone diameters (15±0.11 mm) followed by standard *S. aureus* ATCC 25923 (14±0.12 mm) [14].

Table 1: 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.
S= sensitive I= intermediate R= resistance

Table2: Antimicrobial susceptibility, MIC extracts plant for *k.* pneumonia (mg/ml).

Bacterial cods	MIC *Z.multiflora* (ppm)	MIC *A.graveolens* (ppm)	MIC *C.sativum* (ppm)	MIC *C.cyminum* (ppm)
1	50	250	NO	100
2	50	250	250	100
3	50	250	250	NO
4	50	100	NO	NO
5	50	NO	NO	NO
6	50	NO	NO	NO
7	50	NO	NO	NO
8	50	NO	NO	NO
9	50	NO	NO	100
10	50	NO	250	NO
11	50	250	250	250
12	10	250	100	10

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