Antibacterial activity of hydroalcolic Zataria multiflora Boiss extract against *Klebsiella Pneumonia* Invitro

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**ABSTRACT:** Resistance of human and food spoilage pathogens to antimicrobial agents and the side effects of chemical agents or preservative for human is caused for finding natural new antimicrobial agents, especially among the medicinal plants. The present study was carried out to determine the potential antibacterial agent of hydroalcolic extracts of *Zataria multiflora* Boiss against *Klebsiella Pneumonia* isolation of urinary tract infections. The result show *Klebsiella pneumonia* were resistance to 6 of the agent including nalidix acid(18.5%), ciprofloxacin(18.51%), gentamicin(33.3%), ceftazidim (40.74%), trimethoprim-sulfamethoxazol(44.4%), nitrofurantoin (44.4%) and The plant extracts showed inhibitory activity against *Klebsiella pneumonia* with varying magnitudes and these effects were dose dependent manner. In conclusion, it seems that *Z. multiflora* extracts could inhibit the growth of all of the mentioned bacteria. We noticed that the bactericidal effect of *Z. multiflora* extracts was less than its bacteriostatic effects.

**Keyword:** Klebsiella pneumonia, Zataria multiflora Boiss, Antibacterial activity, Antibiotic resistant

**INTRODUCTION**

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 (Cowan, 1999). The replacement of antibacterial agents with herbal medicines may overcome the above-mentioned resistant bacteria. One of these treatment modalities is *Zataria multiflora*, referred as “Avishen-e- Shirazi” in Persian, which is a very famous Iranian folk medicine. *Zataria multiflora* Boiss. is a member of Laminateae family that geographically grows in Iran, Pakistan, and Afghanistan (Ali et al., 2000; Hosseinzadeh et al.,2000). Thisplant has been used as anesthetic, antiseptic,and antispasmodic (Hosseinzadeh et al., 2000; Jafari et al., 2011). This plant is extensively used as a flavor ingredientin a wide variety of food in Iran. The main constituents of the essential oil of this plant are phenolic compounds such as carvacrol and thymol (Li et al., 2000). *Klebsiella pneumonia* is an apportuistic pathogen that cause a significant proportion of community and hospital acquired infections including urinary tract, pneumonia, septicemia and soft tissue infection (Podschun et al., 1998). The present study was carried out to determine the potential antibacterial agent of hydroalcolic extracts of *Zataria multiflora* Boiss against *Klebsiella Pneumonia* isolation of urinary tract infections.

**MATERIALS AND METHODS**

**PLANT MATERIAL**

The leaf of *Zataria multiflora* Boisswas purchased from Municipal market at Zahedan- Iran during February, 2011 and kept in sterilized screw-cap glass container. Samples were crashed and transferred into glass container and preserved it until extraction procedure in the laboratory.
Preparation of seed extracts

The leaf Zataria multiflora Boiss was properly dried and pulverized into a coarse powder as described by Hanafy (Hanafy et al., 1991). Twenty gram of grinded powders from each plant was soaked in 60 ml ethanol (95 %v/v) + water, separately for one day with occasionally shaking. After one day of dissolving materials were filtered through a Whatman no. 1 filter paper. Then the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts was obtained and then stored at 4°C in air tight screw-cap tube.

Isolation of bacteria

All 22 Klebsiella pneumoniae isolated from urine culture of hospitalized patients (Amir Al-Momenin Hospital, Zabol, south-eastern Iran) suffered from urinary tract infections during the years 2010-2011 were evaluated. Isolated bacteria were identified by Gram's stain and standard biochemical tests (Forbes et al., 2007).

Agar disk diffusion assay

The susceptibility of all antibiotics was carried out using disc diffusion method on Muller-Hinton agar as recommended CLSI (Wcll et al., 1989) the procedure followed is briefly described here. S. aureus isolates were grown overnight on blood agar and colony suspension was prepared using the sterile saline water equivalent to a 0.5 Mc Farland standard. Suspension (100 µl) was spread over the media plate and antibiotic disc was transferred aseptically on the surface of inoculated media plate. Isolates were test with different antibiotics and their concentration shown in parenthesis viz. nalidix acid (30), trimethoprim-sulfamethoxazol (1.23+23.15), gentamicin (10), ceftazidim (30), ciprofloxacin (30), nitrofurantoin (30), ampicillin (10).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The broth micro dilution method was used to determine MIC and MBC in 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% solution of bacterial suspension in 2 ml of DMSO) and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension of 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 plated 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 and MBC 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. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed.

RESULT

Antibiotic susceptibility

Antibiotic susceptibility of Klebsiella pneumonia isolates was evaluated for 7 antimicrobial. However, overall, Klebsiella pneumonia were resistance to 6 of the agent including nalidix acid (18.5%), ciprofloxacin (18.51%), gentamicin (33.3%), ceftazidim (40.74%), trimethoprim-sulfamethoxazol (44.4%), nitrofurantoin (44.4%) (Table1).

Assessment of MIC for plant

The antimicrobial activity of the extract and their potency was quantitatively assessed by the presence or absence of inhibition. The plant extracts showed inhibitory activity against Klebsiella pneumonia with varying magnitudes and these effects were dose dependent manner (Table2). The levels of MIC and MBC were observed ranges from 1.25 to 10 and 2.5 to 20 mg/ml in radius respectively (Table 2).

DISCUSSION

Nowadays, the drug resistant bacteria can create the most important infectious diseases. In this study the effect of alcoholic Z. multiflora extract on Klebsiella pneumonia was studied. Moreover, we noticed that the alcoholic Zataria extracts could inhibit the growth of Klebsiella pneumonia strains. The study of Motevasel, thersults showed it inhibited the growth of S. epidermidis, S. saprophyticus and methicillin sensitive S. aureus (MSSA) by about 8-16 µg/mL (Motevasel et al., 2013). The study of Eftekhar, the result show that Z. multiflora essential oil had a considerable in vitro activity against the standard ATCC cultures (except for P. aeruginosa)
as well as the ESBL producing clinical isolates of K. pneumonia (Eftekhar et al., 2011). The study of Sadeghzadeh, the Z. multiflora oil by concentration of 1%, 2.5% and 5% inhibited the growth of Salmonella paratyphi A at 0, 9.3 and 15.66 mm respectively. The growth of Salmonella paratyphi B was inhibited by the 1%, 2.5% and 5% of the oil, at 8, 8.6 and 21.6 mm respectively. 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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<th>Table 1. The percentage of antimicrobial susceptibility of 22 strains of Klebsiella pneumonia</th>
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GM= gentamicin, CAZ=ceftazidim, SXT=trimethoprim-sulfamethoxazol, CP= ciprofloxacin, NA= nalidix acid, Fm= nitrofurantoin, AM= ampicillin

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<th>Table 2. Minimum inhibitory concentration of hydroalcoholic Zataria multiflora Boiss extract against Klebsiella pneumonia</th>
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<td>Concentration</td>
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<td>20 mg/ml</td>
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<td>2.5 mg/ml</td>
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REFERENCES

Sadeghzadeh L, Selidkon F, Owlia P. Chemical composition and antimicrobial activity of the essential oil of Zataria multiflora Boiss. Pajahesh & Sazandegi 71:52-56