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Antibacterial Effects of *Zataria multiflora* Boiss (Shirazi Avishan Extract) on Urinary Tract *Escherichia coli* Infections

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Abstract: According to increased bacterial resistance to common antibiotics, tendency toward using herbal drugs is increasing. Many researches have been executed about antibacterial and preservative effects of herbal extract like extracts of lamiacceae family (like Shiraz oregano herb). In this study, anti microbial effects of *Zataria multiflora* extract on Urinary Tract *E. coli* Infections in comparison with eleven antibiotic disks in antibiogram pattern is evaluated. After providing microbe suspension and comparing it to Mc Farland 0.5 to obtain 1.5×10^8 bacterium per milliliter the susceptibility of the mentioned bacteria was determined in Muller-Hinton agar by embedding the disks of Betadine, *Zataria multiflora* and antibiotic disks. Fallowing incubation of media for 24 h at 37°C the inhibition zone of Zataria, Betadine and antibiotic disks were measured. Surprisingly, according to antibiogram results, the most sensitivity of *E. coli* was to *Z. multiflora* extract and inhibitory zone produced by eleven antibiotic disks and betadine are significantly less than Zataria induced. According to obtained results and limitations of increasingly usage of chemical antimicrobial substances there is a need to replacement of these substances with natural and herbal extracts.

Key words: *Zataria multiflora*, urinary *Escherichia coli*, antibacterial, herbal medicine, infections

INTRODUCTION

Lamianceae family is one of the biggest herbals' families that have global distribution and has 200 genus and 2000-5000 species of aroma. Shiraz oregano (*Zataria multiflora*) is a member of this family that is basically originated in Iran, Afghanistan and Pakistan with multiple, thin, hard and forked stalks. The extract contains thymol, carvacrol (Mohagheghzadeh *et al*., 2004; Saleem *et al*., 2004), zatrinal, oleanolic acid, betulic acid, rosmarinic acid (Javidnia *et al*., 1999) and monoterpenoids, sesquiter-penoids, p-cymene, y-terpinene (Mohagheghzadeh *et al*., 1999, 2004). Avishan (Persian name of Oregano) is widely used in medicine, alimentary, hygienic and cosmetic industries. Aqueous and alcoholic extracts of *Z. multiflora* have been therapeutically used for relieving nociceptive pain (Hosseinzadeh *et al*., 2000; Ramezani *et al*., 2004), recurrent aphthous stomatitis (Jafari *et al*., 2003) and prevent growth of oral streptococci (Owlia *et al*., 2004), Plasmodiumm falciparum (Ziegler *et al*., 2004) and Trichomonas vaginalis (Abdollahy *et al*., 2004) as well as used as an insect repellent (Saleem *et al*., 2004). Also, Mahmoudabadi *et al*. (2006), showed that methanolic extract of the aerial parts of *Z. multiflora* has anti-Candida effect. According to the recent report (Ettehad and Arab, 2007), Salmonella typhi is another susceptible agent to *Z. multiflora*. Its anti-fungal properties have been also investigated *in vitro* (Fataneh, 1991). The antimicrobial effect of Zataria on gastrointestinal disease is reported such as its beneficial effect on inflammatory bowel disease among mouse (Khalili and Vahidi, 2006; Abdollahy *et al*., 2004).

Clinically Urinary Tract Infection (UTI) is confirmed, while pathogenic microorganisms are Paraclinically detected in the urine, urethra, bladder, kidney, or prostate. In most cases, growth of $>10^5$ organisms per milliliter from a properly collected midstream clean-catch urine sample indicates infection.

In the vast majority of UTIs, bacteria gain access to the bladder via the urethra. Many different microor-ganisms can infect the urinary tract but the most common agent is the gram negative bacilli. *Escherichia coli* cause about 80% of acute infections. Other gram negative rods, especially Proteos and Klebsiella and occasionally Entrobacter, account for a smaller proportion of uncomplicated infections. UTIs are subdivided into catheter associated (or nosocomial) infections and non-catheter-associated (or community-acquired) infections. Catheter associated UTIs can sometimes be prevented in patients catheterized for <2 weeks by use of a sterile closed collecting system, by attention to aseptic technique during insertion and care of the catheter and by

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measures to minimize cross-infection. Despite precautions, the majority of patients catheterized for >2 weeks eventually develop bacteriuria. Colonization of the urine of catheterized or diabetic patients by Candida and other fungal species is common and sometimes progresses to symptomatic invasive infection. Except in acute uncomplicated cystitis in women, a quantitative urine culture or a comparable alternative diagnostic test should be preformed to confirm infection before empirical treatment is begun. When, culture results become available, antimicrobial sensitivity testing should be used to further direct therapy. Above concerns encouraged us to conduct a research study on the effect of zataria on urinary tract *E. coli* infections. Urine *E. coli* positive patients with different cause of UTI selected to test the antibacterial effect of *Z. multiflora* pure extract and and we compared its antimicrobial effects against *E. coli* with betadine and eleven antibiotic disks in Antibiogram pattern.

MATERIALS AND METHODS

E. coli isolated from the urine of the patients, was cultured in Muller-Hinton agar for 24 h. About 3-4 colonies of species of *E. coli* were suspended in a tube containing 0.5 mL of saline. In order to standardize the number of bacteria, Mc Farland 0.5 standard was prepared by adding 99.5 mL of 1% sulfuric acid to 0.5 mL of 1.175% barium sulfate solution. Then, the suspension of bacterium was compared to Mc Farland 0.5 to obtain 1.5×10^8 bacterium per milliliter ($CFUmL^{-1}$).

To determine the susceptibility of the mentioned bacteria, Muller-Hinton agar (Merk) was prepared and the bacterial strains were transferred on to the surface of the medium using sterile cotton swabs. Blank disks were loaded with one drop (2.3 mg Thymole and 12.7 mg carvacrol manufactured by Barig Essance Co. Iran) and also one drop of Betadine 10% solution (manufactured by Behsadin Co. Iran) were put on the surface of the plates left 1 h at 4°C to allow better diffusion of the extract in to the media prior then simultaneously, these disks and 11 antibiotic disks such as Nitrofurantoin, Ceftriaxone, Ciprofloxacin, Cephalexin, Nalidixic acid, Co-trimoxazole, Cefixime, Cefotaxime, Gentamycin, Cefotizoxime, Amikacin (manufactured by pad tan Co. Iran) were embedded in two separate plates of Muller-Hinton agar. Following incubation of media for 24 h at 37°C, the inhibition zone of Zataria and antibiotic disks and also Betadine were measured and the average of results were recorded. Note that the measurements of the antibiotics were compared to NCCLS Standard Tables and the results were recorded as Sensitive, Intermediate and Resistance for each antibiotic disk.

RESULTS

Surprisingly, according to antibiogram results (Table 1), the most inhibition zone and sensitivity obtained for *E. coli* was produced by *Z. multiflora* extract and the inhibitory zone produced by the most sensitive antibiotics Ciprofloxacin (Cp) and Ceftizoxime (Ct) in antibiogram pattern are significantly less than Zataria induced and by using ANOVA-Tukey test Zataria was more significant when compared to betadine and eleven antibiotics that were used in antibiogram pattern ($p = 0.00$)

As Table 2 shows, the most sensitivity percentage obtained for *E. coli* was to *Z. multiflora* (100%), while Cefotizoxime (Ct) (77.77%), Ciprofloxacin (Cp) (75.92%), Cefotaxime (Ctx) (62.96%) and Ceftriaxone (Cro) (57.4%), ranked next, whereas it was resistance to Cephalexin (Cn) (79.62%) and Co-trimoxazole (Sxt) (70.62%) followed by Nalidixic acid Na (37.03%) Amikacin (An) (61.11%) Gentamycin (Gm) (55.55%) Nitrofurantoin (Fm) (53.7%) Cefixime (Cfm) (46.29%) were the most antibiotics that placed in the intermediate part. The results of this experiment shows that the antimicrobial effects of *Zataria multiflora* pure extract on UTI derived *E. coli* was more than Betadine and antibiotics and zataria was sensitive in all experiments.

Table 1: Average of inhibition zone and comparison the significancy for *E. coli*

Antibiotics	X· (mm)±SE	p-values
Zataria	37.85±0.73	-
Betadine	8.24±0.32	0.00
Nitrofurantoin	13.20±0.90	0.00
Ceftriaxone	17.16±1.07	0.00
Ciprofloxacin	20.66±1.09	0.00
Cephalexin	6.48±0.95	0.00
Nalidixic acid	12.31±1.08	0.00
Co-trimoxazole	8.27±1.02	0.00
Cefixime	13.01±0.89	0.00
Cefotaxime	18.38±1.16	0.00
Gentamycin	11.55±0.73	0.00
Cefotizoxime	20.33±0.91	0.00
Amikacin	14.62±0.91	0.00

The mean difference is significant at the 0.05 level

Table 2: Comparison the sensitivity percentage of *Z. multiflora* with eleven antibiotics against *E. coli*

Antibiotics	S	I	R
Zataria	100.00	0.00	0.00
Fm	18.51	53.70	27.77
Cro	57.40	18.51	24.07
Cp	75.92	3.70	20.37
Cn	11.11	9.25	79.62
Na	29.62	33.33	37.03
Sxt	27.07	5.55	70.37
Cfm	12.96	46.29	40.74
Ctx	62.96	12.96	24.07
Gm	12.96	55.55	31.48
Ct	77.77	9.25	12.96
An	14.81	61.11	24.07

S: Sensitive; I: Intermediate; R: Resistance; Fm: Nitrofurantoin; Cro: Ceftriaxone; Cp: Ciprofloxacin; Cn: Cephalexin; Na: Nalidixic acid; Sxt: Co-trimoxazole; Cfm: Cefixime; Ctx: Cefotaxime; Gm: Gentamycin; Ct: Cefotizoxime; An: Amikacin

DISCUSSION

Comparison between results reported about antibacterial effects of different essences is very difficult. Differences in various methods for evaluating of antibacterial effect of different plant essences, resources of essences and different genus of tested bacteria are from its reasons (Ettehad and Arab, 2007). Iranian scientists, Avicenna and Razi have published several books on herbal medicine a few centuries ago that are still in use in many libraries in Europe.

In Iranian traditional herbal medicine *Z. multiflora* is used for antiseptic, analgesic and carminative properties (Mohagheghzadeh *et al*., 2004; Hosseinzadeh *et al*., 2000; Zargari, 1990). It is also, used for treatment of 'Women disease' (Candidiasis vagina) in Iranian folklore (Rojhan, 2000). Also, the leaf powder of *Z. multiflora* is used as nutritional flavoring. Various studies has been performed about antibacterial effects of essence of herbs belong to Lamiaceae family and some important compounds in essence of this family like Carvacrol and Thymol. Karaman *et al*. (2001) showed powerful bacteriostatic effect of Thymus Revolatus essence on gram positive bacteria among *Staphylococcus aerous*. They illustrated high level of Carvacrol in essence as possible reason of this effect. Similar study by Rasooli and Mirmostafa (2004) about bactericidal effects of Thymus pubescus essence (with high amount of Carvacrol) on geram positive and geram negative and *E. coli* was executed and like Karaman *et al*. (2001), high amount of Carvacrol in essence was mentioned as reason of powerful antibacterial effect of *Z. multiflora*. The anti-Candida activity of methanolic extract of *Z. multiflora* indicated against isolates of Candida recently (Mahmoudabadi *et al*., 2006). They also mentioned that this anti-candida activity is due to both rosmarinic acid and thymol that extracted only into methanol (Mahmoudabadi *et al*., 2006; Mohagheghzadeh *et al*., 2004). Fataneh (1991) have shown that *Z. multiflora* has anti-fungal activity. They tested several isolates of dermatophytes and saprophytic fungi against *Z. multiflora* extract. Amanlou *et al*. (2006) have shown that *Z. multiflora* has antierythma in denture stomatitis compared to miconazol gel. Ramezani *et al*. (2004) reported six fractions of the extracts of aerial parts of *Z. multiflora* that have anti-nociceptive activity. Abdollahy *et al*. (2004) showed anti oxidative stress potential of *Z. multiflora* in rats, Present study shows that the antimicrobial effects of *Zataria multiflora* pure extract on UTI derived *E. coli* was more than Nitrofurantoin, Ceftriaxone, Ciprofloxacin, Cephalexin, Nalidixic acid, Co-trimoxazole, Cefixime, Cefotaxime, Gentamycin, Cefotizoxime and Amikacin that were used in antibiogram pattern. Surprisingly, according to antibiogram results, the most sensitivity of *E. coli* was to *Z. multiflora* extract (100%) and the inhibitory zone produced by Betadine that is normally used as an antiseptic solution before catheterization is significantly less than Zataria induced.

Zataria was sensitive in all experiments and it played a major roll in an experiment that the most sensitive antibiotics were Amikacin (16 mm) and Co-trimoxazole (13 mm) and according to NCCLS tables, they placed in intermediate part and the inhibitory zone produced by *Z. multiflora* for *E. coli* was (42 mm). In addition, high potency of *E. coli* in mutation against drugs may be another reason, which supports the study. According to obtained results and limitations of increasingly usage of chemical antimicrobial substances there is a need to replacement of these substances with natural and herbal extracts.

CONCLUSION

According to obtained results from this research, previous publications and increasing limitations of chemical and synthetic antibacterial agents usage due to drug resistance and some side effects, we recommend *Zataria multiflora* as a potent bactericide and it can have a potent anti urinary tract *E. coli* infections activity, especially in the cases that there is no significant antibiotic against *E. coli*.

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