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Anti-Staphylococcal Activity of *Zataria multiflora* Boiss on Urinary Tract *Staphylococcus aureus* Infections: A Comparative Study

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Abstract: Urinary Tract Infections (UTIs) are considered when pathogens gain access from urethra to upper parts of urinary tract and cause infections. Evaluation of the anti-microbial activity of *Zataria multiflora* in comparison with antibiotic disks against urine isolates of *Staphylococcus aureus* is the aim of this study. Microbe suspension equal to Mc Farland 0/5 was prepared and then bacterial strains were transferred to the surface of Muller-Hinton agar with a swab and the susceptibility of the mentioned bacteria was determined by embedding the disks of antibiotics, *Zataria multiflora* and Betadine (10%), fallowing incubation of the media the inhibition zone around disks were measured. According to antibiogram results, the inhibition zone produced by *Zataria multiflora* was more than antibiotics and Betadine and it was sensitive in all experiments even in cases that there were no sensitive antibiotics. This comparison showed potential efficiency of anti-*Staphylococcus aureus* activity for *Zataria multiflora* and a significant difference between its antimicrobial activity and antibiotics disks obtained. According to increase of drug resistance, further investigations are needed to determine that if it can be useful in management of urinary tract *Staphylococcus coagulase* positive infections especially when there is no sensitive antibiotic.

Key words: Urinary Tract Infections (UTIs), *Z. multiflora*, *Staphylococcus aureus*, anti-microbia, antibiotics, inhibition

INTRODUCTION

Finding pathogenic microbes in the urine, urethra, bladder, kidney or prostate indicates Urinary Tract Infection (UTI). The second most common infectious problem in community medical practices is urinary tract infection and many micro-organisms can cause UTIs. Each year about 150 million people (Stamm and Norrby, 2001; Stamm, 2005) are diagnosed with UTI and this costs >6 billion dollars globally (Gonzalez and Schaeffer, 1999). Urinary Tract Infections (UTIs) is considered as a cause of morbidity and mortality from neonate to the geriatric age group in all human populations (Malik and Singh, 2010).

UTIs can be divided into catheter associated infections (nosocomial) and non-catheter associated infections (community-acquired). *S. aureus* is a common pathogen in the community and also in hospitals. *S. aureus* is one of the reasons for bacteremia and it causes significant mortality and morbidity but it is an infrequent cause of urinary tract infection (Chihara *et al*., 2010). In Zaree hospital in Iran, *S. aureus* was the 2nd commonest cause of nosocomial UTI and the highest anti-staphylococcal activity was seen for clindamycin and vancomycin. Bacteriuria with *Staphylococcus aureus* occurs through a limited number of mechanisms, primarily by ascending spread after urethral catheterization and urologic procedures or hematogenous spread of the genitourinary tract. Usually, using of precautions such as a sterile closed collecting system, careful attention to aseptic technique during insertion and attention to lessen cross-infection in patients catheterized for <2 weeks prevent catheter associated UTIs.

Additionally, most of the patients catheterized for >2 weeks eventually develop bacteriuria. In Iran, usually Betadine is used as an aseptic agent before catheter insertion. In addition before an empirical treatment begun a quantitative urine culture or a comparable alternative diagnostic test should be preformed to find out infection (except in acute uncomplicated cystitis in women). After susceptibility testing prepared and culture results become available a more sensitive antibiotic can be selected. Despite the presence of varity in antibiotics, UTIs have remained a significant problem in medicine. World wide,

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the indiscriminate consumption of antibiotics has led to the multi-drug resistance in uropathogens and this complicates the therapeutic management of UTIs. Hence, the biological and antimicrobial properties of essential oils have been proved by various researchers. However, reports for their activity against uropathogens are rare. Essential oils are natural products and they also have aesthetic properties, so they may be a suitable alternative treatment of UTIs (Malik and Singh, 2010). There are a lot of herbal formulas in Asia and Middle East regions that are used traditionally. *Zataria multiflora* (from Lamiaceae family) which grows wild in Iran, Afghanistan and Pakistan is one of them which have a global history. It is known for its antiseptic, anesthetic and antispasmodic benefits in Iran. Some medical properties such as stimulant, carminative, diaphoretic and diuretic have been attributed to this plant (Gupta and Gupta, 1972; Malik *et al*., 1987; Aynehchi, 1991).

The compostion of *Zataria multiflora* essential oil contains rosmarinic acid, oleanolic acid, betulic acid (Javidnia *et al*., 1999) monoterpenoids, sesquiterpenoids, p-cymene, y-terpinene (Mohagheghzadeh *et al*., 1999; Javidnia *et al*., 1999). Its most effective anti-bacterial components are thymol and carvacrol (Abdollahy *et al*., 2004; Saleem *et al* ., 2004). The RA another component of *Z. multiflora* essential oil is also known to have anti-bacterial, anti-viral, antioxidant and anti-inflammatory activities (Mohagheghzadeh *et al*., 2004; Parnham and Kesselring, 1985; Osakabe *et al*., 2004). Anti-nocicptive effects of *Z. multiflora* fractions in mice were reported (Ramezani *et al*., 2004). Anti-Candida effect of methanolic extract of the aerial parts of *Z. multiflora* has been shown (Mahmoudabadi *et al*., 2006). Moreover, *Zataria multiflora* prevents growth of oral streptococci (Owlia *et al*., 2004). Anti-microbial effect of Zataria on *E. coli*, *S. enteritis* and *S. dysantreria* in gastrointestinal disease was shown by Khalili and Vahidi (2006). *Salmonella typhi* is also susceptible to *Z. multiflora* (Ettehad and Arab, 2007). In the recent study, researchers suggested *Zataria multiflora* as a potential bactericide in urinary tract *E. coli* infections especially in the cases that there was no significant antibiotic against *E. coli* (Alizadeh *et al*., 2009).

Also, *Zataria multiflora* boiss essential oil showed synergism with vancomycin and displayed the ability to enhance the activity of vancomycin and it was suggested that it might be useful in controlling MRSA infections. According to the high antimicrobial resistance, alternative nonantibiotic agents are required (Malik and Singh, 2010). Thus, the purpose of this study was to investigate the anti-urinary tract *Staphylococcus aureus* infection activity of *Zataria multiflora* and we compared its anti-microbial effects with antibiotics and Betadine.

MATERIALS AND METHODS

Antibiotic disks were purchased from pad tan Co. Iran *Z. multiflora* extract containing 2.3 mg thymole and 12.7 mg carvacrol was prepared from Barig Essence Co. Iran (The essential oil was obtained by hydrodistillation from aerial parts of the plant). Betadine 10% solution was from Behsadin Co. Iran and Muller-Hinton agars were purchased from Merk Co., Germany.

Bacterial strains and culture condition. Microbes (*Staphylococcus aureus*) were isolated from the urine of patients and they were cultured in Muller-Hinton agar for 24 h. Then Mc Farland 0.5 was prepared by adding 99.5 mL of 1% sulfuric acid to 0.5 mL of 1.175% barium sulfate solution. Colonies (3-4) of *Staphylococcus aureus* were suspended in a tube containing 0.5 mL of saline.

Then in order to standardize the number of bacteria to obtain $1.5.10^8$ bacterium mL^{-1} (CFU mL^{-1}), the suspension was compared to Mc Farland 0.5. Sterile cotton swabs were inoculated by the suspension and bacterial strains were transferred on to the surface of the Muller-Hinton agar.

Blank disks which were loaded with one drop of *Z. multiflora* (containing 2.3 mg thymole and 12.7 mg carvacrol) and also one drop of Betadine 10% solution were embedded on the surface of the Muller-Hinton agar and left 1 h at 4°C to allow better diffusion of the extract into the medium, antibiotic disks such as Ciprofloxacin (Cp), Cefixime (Cfm), Penicillin-G (P), Erythromycin (E), Amoxycillin (Amx) Amoxycillin/Clavulanic acid (Amc), Ampicillin (Am), Gentamycin (Gm), Cephalexin (Cn), Cloxacillin (Cx), Cefotaxime (Ctx) and Cefotizoxime (Ct) were embedded in two separate plates of Muller-Hinton agar.

Bacterial growth determinatio: Following incubation of the media for 24 h at 37°C the inhibition zone around disks of antibiotics, Zataria and also Betadine were measured and the susceptibility of the *Staphylococcus aureus* was determined, the same procedure was repeated 17 times. These measurements around antibiotic disks were compared to NCCLS standard tables and the results for each antibiotic disk recorded as sensitive, intermediate and resistance. Then to determine the susceptibility of *Staphylococcus aureus*, the sensitivity percentage was measured for each antibiotic.

Statistics: One way ANOVA followed by Tukey post-test was used to determine significant differences between groups using SPSS software. Means of results were

recorded and $p<0.05$ was considered significant. Sensitivity percentages of each antibiotic for Staphylococcus aureus were measured.

RESULTS

According to Table 1, the most inhibition zone and sensitivity obtained for *Staphylococcus aureus* was produced by *Z. multiflora* extract. Ciprofloxacin and Cefotaxime produced the most inhibition zone when compared to other antibiotics and the inhibition zone for Cefotizoxime, Cephalexin and Erythromycin were placed in the next ranks. Cloxacillin had the least inhibition zone followed by Penicillin-G and Cefixime. The experiment shows a potential anti-*Staphylococcus aureus* activity for *Zataria multiflora* and t here was a significant difference between *anti-Staphylococcus aureus* activities of *Zataria multiflora* in comparison with Betadine and 12 antibiotics that were used in antibiogram pattern ($p = 0.00$). Table 2 shows that *Z. multiflora* has the most sensitivity

Table 1: Mean of inhibition zone and comparison the significancy for *Staphylococcus coagulase* positive

Antibiotics	p-values	X (mm)±SE
Zataria	0.00	33.35±1.2600
Betadine	0.00	8.64±0.4600
Ciprofloxacin	0.00	18.70±1.9100
Penicillin-G	0.00	2.35±1.0800
Erythromycin	0.00	11.23±2.0000
Amoxycillin	0.00	7.58±2.0200
Amoxycillin/Clavulanic acid	0.00	6.58±2.1000
Cefixime	0.00	4.88±1.5900
Cefotaxime	0.00	18.47±2.0900
Cefotizoxime	0.00	16.17±2.4200
Ampicillin	0.00	6.17±1.9500
Gentamycin	0.00	11.64±1.8100
Cephalexin	0.00	12.11±1.9000
Cloxacillin	0.00	1.76±0.9500

The mean difference is significant at the 0.05 level

Table 2: The sensitivity percentage for *Z. multiflora* and antibiotics for *Staphylococcus coagulase* positive

	Sensitivity percentage for *Staphylococcus coagulase* positive		
Antibiotics	S	I	R
Zataria	100.00	0.00	0.00
Cp	64.70	23.52	11.76
P	0.00	0.00	100.00
E	11.76	41.17	47.05
Amx	11.76	0.00	88.23
Amc	11.76	0.00	88.23
Cfm	5.88	0.00	94.11
Ctx	52.94	29.41	17.64
Ct	47.05	17.64	35.29
Am	5.88	0.00	94.11
Gm	29.41	29.41	41.17
Cn	23.52	35.29	41.17
Cx	0.00	0.00	100.00

S: Sensitive; I: Intermediate; R: Resistance; P: Penicillin-G; E: Erythromycin; Cp: Ciprofloxacin; Amx: Amoxycillin; Amc: Amoxycillin/Clavulanic acid; Cfm: Cefixime; Ctx: Cefotaxime; Gm: Gentamycin; Ct: Cefotizoxime; Am: Ampicillin; Cn:Cephalexin; Cx: Cloxacillin

prcentage for *Staphylococcus aureus* and it was sensitive in all experiments (100%) whereas, the sensitivity percentage for Ciprofloxacin was 64.70% followed by Cefotaxime 52.94%, Cefotizoxime 47.05%, Gentamycin 29.41%, Cephalexin 23.52% and the sensitivity percentage for Erythromycin, Amoxycillin and Amoxycillin/Clavulanic acid was 11.76% and it was 5.88% for Ampicillin and Cefixime more over, Penicillin-G and Cloxacillin were resistant in all experiments.

DISCUSSION

Plants products have been used for relieving pain in human's diseases. Several books on herbal medicine have been published by Iranian scientists like Avicenna and Razi a few centuries ago. The inhibitory activity of essential oils and their components have been reported against bacteria, fungi, viruses and cancer by many researchers. Natural products such as berry juices, fermented milk products containing probiotic bacteria and some herbal formulations are favoured for treatment of UTIs (Malik and Singh, 2010).

Plants of Labiatae family have various effects such as analgesic and anti-inflammatory, antioxidant, hepatoprotective and hypoglycemic action (Hernandez-Perez *et al*., 1995; Cuppett and Hall, 1998; Wasser *et al*., 1998; Hosseinzadeh *et al*., 1998). *Zataria multiflora* is a plant from Labiatae family. In Iranian traditional herbal medicine, *Zataria multiflora* has been used for antiseptic, analgesic and carminative properties (Mohagheghzadeh *et al*., 2004; Hosseinzadeh *et al*., 2000). The composition of *Z. multiflora* and its anti-bacterial activities has been reported (Jafari *et al*., 2003). Important compounds in *Z. multiflora* which have anti-bacterial activity are Thymol and carvacrol (Saleem *et al*., 2004; Mohagheghzadeh *et al*., 2004). Methanolic extract of *Z. multiflora* has anti-Candida activity and this is due to both rosmarinic acid and thymol (Mohagheghzadeh *et al*., 2004; Mahmoudabadi *et al*., 2006). *Zataria multiflora's* activity against *Trichomonas vaginalis* has been shown (Abdollahy *et al*., 2004). Additionally, it has been reported that 6 fractions of the extracts of aerial parts of *Z. multiflora* have anti-nociceptive activity (Ramezani *et al*., 2004).

Benefits of *Z. multiflora* on inflammatory bowel disease in mouse and its anti-oxidative stress activity in rats has been reported (Nakhai *et al*., 2007; Babaie *et al*., 2007). *Z. multiflora* has anti-microbial effects on *E. coli*, *S. enteritis* and *S. dysanteria* in Gastrointestinal disease (Khalili and Vahidi, 2006). Other studies showed that *Z. multiflora* oil has strong antimicrobial activity against *E. coli*, *Staphylococcus aureus and Salmonella*

typhimurim (Rasooli and Rezaei, 2002; Basti *et al*., 2004a, b). World wide, Urinary Tract Infection (UTI) remains a therapeutic problem not only as a catheter associated infection but also as a community acquired infection (Stamm and Norrby, 2001; Gonzalez and Schaeffer, 1999; Stamm, 2005; Gupta and Gupta, 1972; Malik *et al*., 1987).

One of the major causes of infections in communities and hospitals is *Staphylococcus aureus*. In addition, antibiotics are also associated with adverse effects on host which include depletion of beneficial gut flora and mucosal micro-organisms, immunosuppression, hypersensitivity and allergic reactions showed powerful bacteriostatic effect of Thymus Revolatus essence on *Staphylococcus aureus*. They illustrated high level of Carvacrol in essence as possible reason of this effect. Recently, the essential oil of *Zataria multiflora* Boiss synergy with vancomycin displayed its ability to induce the activity of vancomycin.

In addition by considering the importance of increasing drug resistance against *Staphylococcus aureus* and in order to the latest results that showed high potential bactericide activity of *Z. multiflora* on urinary tract *E. coli* infections we were encouraged to examine the same procedure for *Staphylococcus aureus* urinary tract infection. This study reveals anti-microbial effect of *Zataria multiflora* pure extract against *Staphylococcus aureus*. The finding confirms that *Z. multiflora* is sensitive for urinary tract *Staphylococcus aureus* infections and it has more anti-*Staphylococcus aureus* activity (100% experiments) than antibiotic disks such as Ciprofloxacin, Cefixime, Erythromycin, Amoxycillin, Amoxycillin/Clavulanic acid, Gentamycin, Ampicillin, Cephalexin, Penicillin-G, Cloxacillin, Cefotaxime and Cefotizoxime in anti-biogram patterns.

In addition, the inhibitory zone produced by Betadine is significantly less than Zataria induced (Malik and Singh, 2010). In Iran, Betadine is an antiseptic solution that is routinely used before catheterization. It should be noticed that in one experiment there was not any sensitive antibiotic for urinary tract *Staphylococcus aureus* infection and the most inhibition zone was produced by Erythromycin that was 14 mm and it was placed in intermediate part according to NCCLS tables whereas the inhibition zone produced by *Z. multiflora* was 32 mm.

CONCLUSION

According to the limitations for the usage of chemical and synthetic anti-bacterial agents and due to the increasing of drug resistance and some side effects; researchers recommend *Zataria multiflora* as a potent anti-microbial agent against *Staphylococcus aureus*. *Z. multiflora* is valuable plant that may be useful for urinary tract infections caused by *Staphylococcus aureus*. Further, *in vivo* studies are recommended especially in cases that there is a drug resistance and there is no sensitive antibiotic for this microbe.

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