

Antibacterial Effect of *Teucrium polium* on the Bacteria Causing Urinary Tract Infections

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Article information	Abstract
<p>Article history: Received: 22 July 2012 Accepted: 27 Sep 2012 Available online: 12 Mar 2013 ZJRMS 2014; 16(3): 44-49</p> <p>Keywords: Antimicrobial Effect Teucrium Polium Extract Urinary Tract Infection (UTI)</p> <p>*Corresponding author at: Department of Microbiology, Islamic Azad University, Tehran Sciences and Research, Faculty of Basic Sciences, Tehran, Iran. E-mail: samira62sh@yahoo.com</p>	<p>Background: The present investigation was conducted to study the antibacterial effect of aqueous, ethanolic and ethyl acetate extracts of <i>Teucrium polium</i> plant on the bacteria isolated from urine samples of those with UTI and to compare it with the effect of commonly used antibiotics in treating UTIs.</p> <p>Materials and Methods: The antibiotic resistance of 147 strains of bacteria causing UTIs to the antibiotics selected through Kirby-Bauer disk diffusion method was determined. In the meantime, the aqueous, ethanolic and ethyl acetate extracts of <i>T. polium</i> plant were prepared. The antibacterial activity of these extracts was examined using Disk Diffusion Method. Finally, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of antibacterial were determined using serial dilution method.</p> <p>Results: <i>T. polium</i> extracts were merely effective in enterococcus and pseudomonas bacteria. In general, the MIC rate of aqueous extract in enterococcus was 1.25-5 mg/ml. The MIC rate of ethanolic extract for enterococcus was calculated as 10 mg/ml. The MIC of aqueous and ethyl acetate extracts for pseudomonas bacteria were achieved as 5 and 20 mg/ml, respectively. The MBC contents of aqueous and ethyl acetate extracts of teucrium for pseudomonas bacteria was 10 mg/ml in aqueous and 20 mg/ml in ethyl acetate extracts. The MBC content of extracts for enterococcus bacteria were 10 mg/ml in aqueous extract and 20 mg/ml in ethanolic extract.</p> <p>Conclusion: <i>T. polium</i> extract can be effective in some bacteria causing urinary tract infection, especially enterococcus.</p> <p>Copyright © 2014 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Urinary tract infections (UTIs) are of common infections, especially in women, the elderly and infants. They are considered as the acute problems of the organizations responsible for public health of different countries. As far as frequency is concerned, their prevalence is ranked at the second level after the respiratory diseases [1]. *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Staphylococcus epidermidis*, *Enterobacter*, *Citrobacter* and *Pseudomonas aeruginosa* are among the bacteria causing such infections [2].

The UTIs are usually treated by consumption of antibiotics; however, number of the reports on resistance of pathogen bacteria to antibiotics is increasing [1]. The studies indicate that the antibiotic resistance pattern of the bacteria causing UTI is changeable and unpredictable. On the one hand, it confirms the necessity to continuously monitor these patterns and on the other hand, it shows the need to study the new antibiotics and/or herbal compounds containing antibacterial substances. Therefore, studies on the identification of new antibacterial compounds of natural-origin are ever

increasing. Organic plants have abundant secondary metabolites and they can be considered as one the most important medicinal sources with new antibacterial and antifungal effects [3].

Moreover, considering high cost of producing antibiotics, a special interest in using medicinal plants has been created. One of the plants, which attracted attention of scientists such as Hippocrates and Galen in traditional medicine, was *Teucrium polium* plant. It is an herbal plant of mint family (Labiatae) anti-diabetic, antispasmodic, analgesic, anti-inflammatory and anti-oxidant features of which have been reported during recent years; however, very limited studies have been carried out on its antimicrobial effect [4].

The studies on antimicrobial effects of the plant essence show that it has considerable inhibitory effects on the gram-positive and gram-negative bacteria. It seems that the most antimicrobial feature owes terpene compounds, especially α -pinene, β -pinene and caryophyllene, which have the maximum percentage of chemical composition of the essence [4]. With respect to above items, the present investigation was carried out to compare the

antibacterial effect of aqueous, ethanolic and ethyl acetate extracts of *T. polium* plant and common antibiotics on the bacteria causing UTIs. This is done through disk diffusion method to determine MIC. The results obtained from our studies can be used to substitute effective, natural origin and less risky drugs for controlling and treating bacterial infections. They may lead to reduce consumption of chemical drugs and incidence of complications thereto.

Materials and Methods

The present study was performed on 147-strain bacteria isolated from urine samples of the patients with UTI who referred to Ali Ebne-Abitaleb and Boo-Ali hospitals of Zahedan during 2009-2010. The samples were prepared for different studies. Table 1 shows the frequency of the bacteria under study. Antibiotic sensitivity test was conducted using the Kirby-Bauer method [5], Mueller Hinton medium and 0.5 McFarland bacterial suspension. Incubating for 16-18 hours, inhibition zone diameter was measured by a ruler, compared with international standards of National Committee on Clinical Laboratory Standards (NCCLS), and reported according to the manufacturer's instruction (MAST) as Resistant (R), Intermediate (I) and Sensitive (S). In this study, the antimicrobial resistance to ciprofloxacin, ceftriaxone, nitrofurantoin, amikacin, ceftazidime, gentamicin, cotrimoxazole and ampicillin antibiotics was measured.

Leaves and flowering shoots of *T. polium* were gathered around Shiraz city in mid-May. After identification in the department of biology of Sistan and Baluchestan University, the aqueous, ethanolic and ethyl acetate extracts were prepared from the plant.

Maceration (soaking) method was used to prepare the extract. For this purpose, 20 g of the plant powder was mixed with 300 ml of distilled water and was poured into a beaker containing magnet. Then, the lid of the beaker was covered by watch glass and it was placed on a stirrer machine to be mixed for 48 hours at the ambient temperature. Then the mixture was passed through Whatman filter paper to separate its waste. Later, it was placed under the hood so that its solvent is baked and a dry substance is obtained. Finally, to provide the concentration of 20000 ppm from the extract, 0.2 g of dry substance was solved in 10 ml of dimethyl sulfoxide (DMSO) solvent. Therefore, the content of *T. polium* extract (20 mg/ml in distilled water) was calculated [6].

The same procedure for preparing the aqueous extract was applied to prepare the ethanolic and ethyl acetate extracts; the only difference was in the use of ethanol 96.9% (made by German company of Merck) and ethyl acetate solvents, respectively.

Disk diffusion method was used to determine sensitivity of the bacteria to the extracts. First, a microbial suspension equal to 0.5 McFarland was prepared from all the bacteria strains. Then spread culture was performed using a sterile cotton swab of the suspensions prepared in Mueller-Hinton agar plate. In the next stage, blank sterile disks (made by Padtan Teb Company) were immersed in the extracts and they disks were placed on an agar surface

with a specific distance between them. With respect to the type of the bacteria, certain antibiotic disks were used as positive control and blank disks were used as the negative control impregnated with distilled water for all the bacteria. The plates were incubated for 24 hours at 37°C. Then growth inhibition zones were evaluated around the extract-containing disks. The diameters of the inhibitory zones were measured in millimeters [7].

Macro-dilution method was used to determine MIC. Concentration of 40,000 ppm was prepared for each extract. For each extract, a 10-piece set of test tubes was used for each microorganism. Eight tubes were considered for different concentrations of each extract, a tube was used as the positive control, and a tube was used as the negative control. One milliliter of the liquid of the Mueller Hinton culture media was poured into all the tubes. Then 1 ml of the extract was added to the first tube and it was mixed well.

One millimeter of the liquid of the first tube was added to the second tube and 1 ml of the liquid of the second tube was added to the third tube. It was continued to the eighth tube and finally 1 ml was discarded from the eighth tube. Then 100 µl of overnight culture suspension of each microorganism, which was diluted for 1/100, was added. The tube with no extract and the tube with no bacteria were considered as the positive control and the negative control, respectively.

The tubes were placed in an incubator at 35°C for 24 hours. They were examined in terms of turbidity caused by growth of microorganisms. Among the tubes through which no bacteria were grown, the tube with the minimum concentration of the plant extract was reported as MIC [8].

In order to determine the MBC, a standard loop of the tubes in which bacteria was not grown (the pipe penultimate MIC tube) was cultured separately on the plates containing Mueller-Hinton agar. After incubation at 37°C for 24 hours, the minimum concentration that prevented formation of colony on agar or in more precise terms, it allowed less than 0.1% of the bacteria to survive, was considered as Minimum Bactericidal Concentration (MBC) [9].

Table 1. Frequency of the bacteria under study

Name of Bacteria	Quantity (%)
Enterococcus	15 (10.2)
Escherichia coli	81 (55.1)
Enterobacter	15 (10.2)
Morganella	3 (2)
Staphylococcus	3 (2)
Klebsiella	8 (5.4)
Acinetobacter	6 (4)
Serratia	3 (2)
Proteus	2 (1.3)
Pseudomonas	11 (7.4)
Total	147 (100)

Results

Generally, 31% of the whole samples showed resistance to ciprofloxacin, whereas this percentage for ceftriaxone was 50%. In addition, 28% of the whole samples showed resistance to nitrofurantoin; however, the rate of resistance to amikacin was 7%. On the other hand, 47% of the whole samples revealed resistance to ceftazidime, but the same percentage for gentamicin was 31%. In addition, 75% and 83% of all the samples showed resistance to cotrimoxazole and ampicillin, respectively.

Studying the sensitivity of the isolations under study to the extracts indicated that generally 6 samples as 5% of the whole samples (5 enterococcus and one pseudomonas) were sensitive to the aqueous extract. Twenty percent of the whole samples (2 samples of enterococcus) were sensitive to ethanolic extract and only 1% of the whole samples (1 sample of pseudomonas) were sensitive to the ethyl acetate extract. On the other hand, all the samples of *Escherichia coli*, enterobacter, staphylococcus, proteus, klebsiella, acinetobacter, serratia, and morganelia showed resistance to all the (aqueous, ethanolic and ethyl acetate)

extracts (Table 2). Shows resistance frequency distribution of the isolations under study to the aqueous, ethanolic and ethyl acetate extract of *T. polium*.

In addition, the average diameters of inhibition zone for 5 items of enterococcus and ethanolic extract were calculated as 13.5 mm and 12 mm, respectively. The average diameter of inhibition zone of the aqueous extract in pseudomonas sample and ethyl acetate extract were calculated as 12 mm and 11 mm. Table 3 shows the diameter of inhibition zone of the bacteria sensitive to aqueous, ethanolic extract and ethyl acetate extract of *T. polium*. Generally, the MIC of the aqueous extract in enterococci was calculated as 1.25-5 mg/ml and MIC amount for the ethanolic extract for enterococcus was equal to 10 mg/ml. In addition, MIC contents for the aqueous and ethyl acetate extract for pseudomonas bacteria were 5 and 20 mg/ml, respectively. Table 4 shows the MIC of the aqueous, ethanolic, and ethyl acetate extracts on the bacteria under study. Generally, the MBC results of the aqueous, ethanolic and ethyl acetate extracts for the sensitive bacteria were calculated as 10, 20, and 20 mg/ml.

Table 2. Resistance frequency distribution of the isolations under study in aqueous, ethanolic extract and ethyl acetate extract of *Teucrium polium*

Name of Bacteria	N	Aqueous Extract		Ethanolic Extract		Ethyl Acetate Extract	
		Resistant N (%)	Sensitive N (%)	Resistant N (%)	Sensitive N (%)	Resistant N (%)	Sensitive N (%)
Enterococcus	15	10 (67)	5 (33)	13 (87)	2 (13)	15 (100)	0
Escherichia coli	81	81 (100)	0	81 (100)	0	81 (100)	0
Enterobacter	15	15 (100)	0	15 (100)	0	15 (100)	0
Enterobacter	3	3 (100)	0	3 (100)	0	3 (100)	0
Staphylococcus	3	3 (100)	0	3 (100)	0	3 (100)	0
Klebsiella	8	8 (100)	0	8 (100)	0	8 (100)	0
Acinetobacter	6	6 (100)	0	6 (100)	0	6 (100)	0
Serratia	3	3 (100)	0	3 (100)	0	3 (100)	0
Proteus	2	2 (100)	0	2 (100)	0	2 (100)	0
Pseudomonas	11	10 (90)	1 (10)	11 (100)	0	10 (90)	1 (10)
Total	147	141 (95)	6 (5)	145 (100)	2 (2)	146 (99)	1 (1)

Table 3. Diameter of inhibition zone for aqueous, ethanolic and ethyl acetate extracts of sensitive bacteria

Bacteria under Study	Diameter of inhibition zone for aqueous extract	Diameter of inhibition zone for ethanolic extract	Diameter of inhibition zone for ethyl acetate extract
Pseudomonas	12 mm	-	11 mm
Enterococcus faecalis	9 mm	12 mm	-
Enterococcus faecalis	17 mm	-	-
Enterococcus faecalis	10 mm	-	-
Enterococcus faecalis	16 mm	-	-
Enterococcus faecalis	15 mm	12 mm	-

Table 4. Determination of Minimum Inhibitory Concentration of aqueous, ethanolic and ethyl acetate extracts for the bacteria under study

Bacteria (Sample Code)	Extract Dilution	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	+ Control	- Control
Pseudomonas (135)	Aqueous	-	-	-	+	+	+	+	+	+	-
Pseudomonas (135)	Ethyl Acetate	-	+	+	+	+	+	+	+	+	-
Enterococcus (173)	Aqueous	-	-	-	-	+	+	+	+	+	-
Enterococcus (173)	Ethanolic	-	-	+	+	+	+	+	+	-	-
Enterococcus (178)	Aqueous	-	-	-	+	+	+	+	+	+	-
Enterococcus (187)	Aqueous	-	-	-	-	-	+	+	+	+	-
Enterococcus (130)	Aqueous	-	-	-	+	+	+	+	+	+	-
Enterococcus (150)	Aqueous	-	-	-	-	+	+	+	+	+	-
Enterococcus (150)	Ethanolic	-	-	+	+	+	+	+	+	+	-

Discussion

In the present study, the resistances of the bacteria under study were calculated for ciprofloxacin as 31%, ceftriaxone 50%, nitrofurantoin 28%, amikacin 7%, ceftazidime 47%, gentamicin 31%, cotrimoxazole 75% and ampicillin 83%.

Of course, this is a situation where there were a few samples for some strains such as *Serratia*, *Proteus*, *Staphylococcus*, *Morganella* and *Acinetobacter*. As far as statistics is concerned, it might be incorrect to make a judgment about them.

Antibiotic resistance pattern of *E. coli* which constituted the majority of samples in our study can be compared with the results of other studies. In our studies, *Escherichia coli* had the most sensitivity to amikacin and nitrofurantoin; however, they showed the maximum resistance to ampicillin (84%) and cotrimoxazole (81%).

Alaei and Salehzadeh studied the results of antibiogram in 510 children with UTI. The results obtained in their studies are consistent with those of our study. Ampicillin, with 78.9% was at the top level, whereas cotrimoxazole with 66% and cephalexin with 62.8% were standing at the next levels. Resistance of *Escherichia coli* to gentamicin was 15.8% as higher than the rest of bacteria. Its resistance to ceftriaxone was 8% and lower than that of the rest of bacteria. According to the results, the pharmaceutical resistance of common antibiotics used for treating UTIs including ampicillin, cotrimoxazole (similar to the results of our study) was high [10].

In the study conducted by Madani et al., the most cases of resistance to *Escherichia coli* was respectively related to ampicillin (91.4%), cotrimoxazole (61.1%), cefixime (46.8%), gentamicin (43.3%), nalidixic acid (38.5%) antibiotics. The most sensitivity was related to ciprofloxacin (66.7%), cefotaxime and ceftriaxone (both are 62.2%) and nitrofurantoin (48.8%) antibiotics, which were consistent with the results of our study [11].

Nateghian et al. studied prevalence of acute UTI with *Escherichia coli* resistant to gentamicin and ceftriaxone and its risky factors among the children admitted to Ali Asghar (AS) hospital. In this study, resistant of ceftriaxone, gentamicin, both antibiotics (ceftriaxone, gentamicin) were 38.4%, 24% and 20%, respectively which was almost similar to the results of our study [12].

According to the studies conducted by Savadkouhi et al., *Escherichia coli* was the most common factor which causes UTI; strains showed the most resistance to ampicillin and the least resistance to amikacin. The results obtained from their study were similar to our study in which the resistance of the bacteria under study to amikacin and ampicillin were 7% and 83%, respectively [13].

The study of Imam Ghoreishi and Kohan Teb showed that gentamicin had the minimum effect (72.1%) on *Escherichia coli*. Among oral medications, cotrimoxazole had the most resistance (71.4%) and among injection drugs, gentamicin had the most resistance. Similar result was achieved in our study for cotrimoxazole (81%); however, for the injection drugs, maximum resistance was

related to ampicillin (84%) [14]. With respect to an increase in the resistance to the antibiotics, the effects of many plants on the prevalent bacteria on UTIs have been studied; however, a few studies are available on the antibacterial effect of *T. polium*.

In our investigation, we studied the effects of the aqueous, ethanolic and ethyl acetate extracts of *Teucrium polium*.

In the study conducted, 67%, 87% and 100% of the enterococci were resistant to the aqueous extract, the ethanolic extract and ethyl acetate extract, respectively. Here, resistance means the complete growth around the disks containing *T. polium* extract. In other words, 5 items of the 15 enterococci samples, which are equal to (33%) of the whole enterococci, showed resistance to the aqueous extract and 2 items of the whole enterococci samples, which are equal to 13%, showed resistance to the ethanolic extract. However, all 15 items showed resistance to the ethyl acetate extract. On the other hand, all *E. coli*, *Enterobacter*, *Staphylococcus*, *Proteus*, *Klebsiella*, *Acinetobacter*, *Serratia*, and *Morganella* samples were resistant to aqueous, ethanolic and ethyl acetate extracts.

Pseudomonas was different; as one out of 11 samples (equal to 10%) was sensitive to the aqueous and ethyl acetate extract. However, none of them was sensitive to the ethanolic extract.

In an investigation carried out by Esmaeili et al., the results of the antimicrobial effects of *T. polium* showed considerable inhibitory effects of this plant on most gram-positive and gram-negative bacteria and it was more effective than antibiotic, as the mean of inhibition zone diameter of *Staphylococcus epidermidis*, *E. coli*, and *Salmonella* were 32, 30, 35 mm, respectively. However, in our study, *E. coli* samples were resistant to all the three (aqueous, ethanolic, ethyl acetate) extracts [15].

In another research, Darabpour et al. studied the antimicrobial effect of ethanolic and methanolic extracts of leaves of the plant on pathogenic bacteria (*Bacillus anthracis*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Yersinia enterocolitica*, *Escherichia coli*, *Salmonella typhimurium*, *Bordetella bronchiseptica*, *Proteus mirabilis*, and *Antinomyces pyogenes*). The results obtained from the ethanolic extract indicated that *S. epidermidis* is the most sensitive bacteria and *Salmonella typhimurium*, *S. aureus* and *E. coli* bacteria are the most resistant species to this extract. In our study, *Staphylococcus* and *Escherichia* also showed resistance to all the three types of (aqueous, ethanolic and ethyl acetate) extracts [16]. Shakibaei et al. studied antimicrobial (anti-plasmid) effects of 5 different pharmaceutical plants (myrtle, savory, *Teucrium*, licorice, eucalyptus) on the strains containing plasmid of *Klebsiella pneumoniae* bacteria, which are resistant to the different antibiotics. These plants used for treating infections in Iranian traditional medicine.

The study of minimal inhibitory concentration of the taken extracts compared to the strains resistant to *Klebsiella pneumoniae* showed that all the above extracts have antimicrobial properties and they were able to

prevent the growth of strains resistant to *K. pneumonia*, whereas all 8-strain klebsiella in the study were resistant to the three (aqueous, ethanolic, ethyl acetate) extracts [17].

Our study examined the effects of *T. polium* extracts on the bacteria causing UTI. Another study showed that in case these extracts are mixed with some antibiotics, it might reduce resistance of bacteria to antibiotics [18].

Sarac and Ugur extracted ethanolic extract of *T. polium* with other species of the Laminase family. The standard strain of *E. coli* and other bacteria were used in this study indicating that the plants of this family had no effects on the gram-negative bacteria (*E. coli*) and *Candida albicans*; however, they prevent the growth of some gram-positive bacteria. These results are somehow consistent with those of our research. For instance, *T. polium* extract affected Enterococcus (gram-positive bacteria), but *E. coli* showed 100% resistance.

In the same study, species and sub-species of teucrium had certain anti-staphylococcus effect, most of which was on the *S. epidermidis*. This bacteria was not studied in our study, but all 3 *Staphylococcus aureus* samples were resistant to *T. polium* extracts [19].

For *E. coli* and generally all the bacteria, poor response of *T. polium* extracts might be due to the type of extraction and the concentration used in different ethanolic and ethyl acetate varieties, which should be taken into consideration. In addition, it should be noted that several factors are effective in the antimicrobial effects of a plant. Factors such as plant essence, extraction method and type of the solvent, type of medium and its ingredients, and extract concentration can affect the antimicrobial effects of the plant. Such important factors should be considered in the following studies [20].

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Therefore, it can be concluded that *T. polium* extract is effective in some bacteria causing UTI, especially enterococcus. Clearly, enterococcus is of those bacteria whose antibiotic resistance is considered as a major problem. In our investigation, the effect of *T. polium* extract, especially its aqueous extracts, was as much as that of ciprofloxacin, gentamicin and amikacin antibiotics and exceeded cotrimoxazole, ampicillin and nitrofurantoin. Yet, this finding requires further studies and with more samples. It is suggested for the next studies especially on this plant to be carried out on the bacteria which provided relative responses in this study.

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Authors' Contributions

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Conflict of Interest

The authors declare no conflict of interest.

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