

## Antibacterial Activity of *Ziziphora Tenuior* Lam. Extract and Essential Oil against Bacteria Isolated from Urogenital Tract Infections

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### ABSTRACT

**Background and Objectives:** Many species of *Ziziphora* have been used in traditional medicine in the Azarbaijan region, northwest of Iran. This study aimed to determine antibacterial activity of essential oil and methanol extract of *Ziziphora tenuior* on some pathogenic bacteria isolated from urogenital tract infections.

**Methods:** The essential oil and methanol extract of *Z. tenuior* were obtained by Clevenger and maceration methods. Under sterile conditions, the required amount of urine was taken from patients referred to a number of clinics in Tabriz during 2014. After identification of the isolates using standard microbiological methods, antimicrobial effects of the essential oil and methanol extract of the plant on the isolates were evaluated by determining minimum inhibitory concentration (MIC), minimum bactericidal concentration, and antibiogram test.

**Results:** The MIC for essential oil of *Z. tenuior* was 250µg/mL for most Gram-negative bacteria except *Pseudomonas aeruginosa*. Moreover, the MIC for *Staphylococcus aureus* was 250µg/mL and 500µg/mL for other strains of *Staphylococcus*.

**Conclusion:** Comparison of the inhibitory and bactericidal effects of the essential oil and methanol extract of *Z. tenuior* showed that the essential oil is able to inhibit growth of the bacteria tested even in low concentrations. Further studies are required in this regard using animal models.

**Keywords:** Antibacterial Agents, *Ziziphora tenuior*, Plant Extracts, Urinary Tract Infection.

## INTRODUCTION

Plant extracts and their constituents have some known antibacterial effects (1). *Ziziphora tenuior* from the *Ziziphora* genus (Lamiaceae family) and breed of mint is a 5-15 cm tall herbaceous plant with a one-year lifetime, short stems and thin sharp leaves. This plant grows wild in several areas of Iran including the mountainous regions of Azerbaijan provinces, especially in mountains of Tabriz. Four species of this plant including *Ziziphora clinopodioides*, *Ziziphora capitata*, *Ziziphora persica* and *Z. tenuior* have been identified in Iran. Mucolytic, carminative and stomach reinforcement activities are among the healing effects of this plant. In some areas, the mixture of its powdered leaves with honey is used to treat dysentery. It is also used as an antiseptic cold remedy, and for treatment of stomach disorders (2, 3). In different regions, the plant's powder is used as garnish on yogurt and dairy products (4). Despite the wide range of traditional applications of this plant for treatment of many infectious diseases, a systematic study has not been yet performed on the antibacterial effects of *Z. tenuior* on pathogenic bacteria. Therefore, this in vitro study aimed to determine the antibacterial activity of essential oil and methanol extract of *Z. tenuior* collected from the hills around the city of Tabriz in East Azerbaijan Province against some pathogenic bacteria isolated from urinary tract infections.

## MATERIAL AND METHODS

Aerial parts of *Z. tenuior* in blooming status were collected from hills located 15-20 km far from Tabriz (1700-1800m) in the East Azerbaijan Province. A voucher specimen of the plant was identified and preserved at the Herbarium of Department of Food Hygiene at Islamic Azad University of Tabriz, Iran. The samples were shade-dried, powdered and stored at 4 °C until in vitro testing (5). For Preparation of plant extraction, in a specialized laboratory at the Department of Pathobiology, one gram of the collected parts mixed with 100ml of 80% methanol (Merck-Germany) was extracted by maceration. The Extracts were filtered by Whatman No.1 filter paper (chm F2042-Spain). The filtrates obtained were dried in a rotary evaporator (Stuart RE300-England) at 40 °C. The extract was

stored at 4 °C. For production of essential oil, Clevenger apparatus (Schott DURAN-Germany) and water distillation were used (6-8). Bacterial strains were isolated from patients with urinary tract infections referred to a number of clinics in Tabriz in 2014. The isolates included nine species. The essential oil and methanol extract of the plant were individually tested against three Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*) and six Gram-negative (*Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Citrobacter freundii* and *Pseudomonas aeruginosa*) bacteria. A 24-hour culture of each bacterium was used for Preparation of bacterial suspension. First, 24 hours before testing, the stored cultures were inoculated into Brain Heart Infusion agar (Merck-Germany) and incubated at 37 °C for 24h. Colonies were washed with normal saline solution and bacterial suspensions were diluted with normal saline to reach turbidity equal to the 0.5 McFarland standards (9, 10). Disk diffusion method was used to evaluate the antibacterial effects of the essential oil and methanol extract of *Z. tenuior*. Disks containing the extract were prepared from sterile blank disks manufactured by Padtan Teb Co. (Iran). The blank disks were placed in tubes containing essential oil and methanol extract of *Z. tenuior* for 30-50 minutes. Following complete absorption, the disks were kept at 44-45 °C until completely dried (11). Then, 100 µl of prepared suspension from each isolated bacterium was cultured separately on the surface of Muller Hinton agar (Merck-Germany). The disks impregnated with the essential oil and methanol extracts of *Z. tenuior* were placed with certain distance from each other and from the edge of the plate on the surface of the medium using sterile forceps. The disks were fixed with little pressure and the plates were incubated for 24h at 37 °C. Antibacterial activity was assessed by measuring the diameter of inhibition zone around the disks. The experiment was repeated three times for each bacterium. The mean inhibition zone diameter obtained from the triplicates was used as the final diameter (12, 13). Moreover, standard antibiotic ampicillin disk (10µg) was used as

positive control. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): Broth microdilution method was used for this purpose. In order to determine the MIC for essential oil and methanol extract of *Z. tenuior*, a series of 10 test tubes was used. Eight tubes were used for testing different concentrations of essential oil and methanol extract (according to Tables 2-5) and two tubes were used as controls. All test tubes were incubated at 37 °C for 24 hours. After the incubation period, the turbidity of tubes was studied for bacterial growth. This method was repeated three times for each bacterium. The lowest concentration of extract and essential oil that inhibited bacterial growth was considered as the MIC. A sample from the lowest concentration of the methanol extract and essential oil at which no bacterial growth was observed was cultured by pure plate method to determine the MBC (14, 15).

Tukey's test was used for comparing the samples. ANOVA with equal frequency was used to determine significant differences between the means. Descriptive statistics was used to compare MBC and MIC of plant extract and essential oil.

## RESULTS

At 95% confidence level, there was significant difference between the

antibacterial effects of essential oil and methanol extract of *Z. tenuior* against all bacteria except for *E. coli*, *C. frundii* and *E. aerogenes*. The highest antibacterial effect was observed against *K. pneumoniae*. Compared with the positive control (ampicillin), the essential oil and methanol extract showed higher antibacterial activity in most cases. This effect was more evident in the case of essential oil. The MIC of essential oil for *Z. tenuior* was  $\leq 125$  mcg/ml for most Gram-negative bacteria (Table 2). In addition, the MBC of essential oil for bacterial isolates tested was equal to the MIC value. On the other hand, the MIC for Gram-positive bacteria including *S. aureus* was 250 mcg/ml, and 500 mcg/ml for other Staphylococcus strains (Table 2). The results also showed that the essential oil and methanol extract of *Z. tenuior* have no effect on *P. aeruginosa* at the tested concentrations. Among the Gram-positive bacteria, *S. aureus* was the most sensitive to the concentrations of *Z. tenuior* essential oil tested in our study. Among the six Gram-negative bacteria, *K. pneumoniae* showed more sensitivity to the essential oil compared to the other species. The extract had inhibitory and bactericidal effects at all concentrations tested against all bacterial isolates except *P. aeruginosa* (Table 2). The MBC of extract for most isolates was equal to their MIC values (Table 3).

Table 1- in vitro antibacterial activity of essential oil (10  $\mu$ l/disk) and methanol extract (10  $\mu$ l/disk) of *Z. tenuior* compared to ampicillin (10 $\mu$ g/disk) as the standard antibiotic

	Essential oil of <i>Z. tenuior</i>	Methanol extract of <i>Z. tenuior</i>	Ampicillin
Bacterial isolates	Mean diameter of inhibition zone (mm) $\pm$ SD	Mean diameter of inhibition zone (mm) $\pm$ SD	Mean diameter of inhibition zone (mm) $\pm$ SD
<i>S. aureus</i>	22 $\pm$ 0.1	19 $\pm$ 0.1	13 $\pm$ 0.1
<i>S. epidermidis</i>	20 $\pm$ 0.1	19.5 $\pm$ 0.2	14 $\pm$ 0.1
<i>S. saprophyticus</i>	21 $\pm$ 0.1	19 $\pm$ 0.1	14 $\pm$ 0.1
<i>E. coli</i>	21 $\pm$ 0.1	20 $\pm$ 0.1	13 $\pm$ 0.1
<i>K. pneumonia</i>	29 $\pm$ 0.1	26 $\pm$ 0.1	19 $\pm$ 0.1
<i>P. vulgaris</i>	19 $\pm$ 0.1	17 $\pm$ 0.1	9 $\pm$ 0.1
<i>E. aerogenes</i>	19 $\pm$ 0.1	17.5 $\pm$ 0.2	11 $\pm$ 0.1
<i>C. frundii</i>	20 $\pm$ 0.1	19 $\pm$ 0.1	4 $\pm$ 0.1
<i>P. aeruginosa</i>	0	0	0

Table 2- MIC of different concentrations of essential oil and methanol extract of *Z. tenuior* against bacterial isolates

Bacterial isolates	Essential oils concentration (mcg/ml)								Methanol extract concentration (mcg/ml)							
	62.5	12	25	50	1000	2000	4000	8000	62.5	125	250	500	1000	2000	4000	8000
<i>S. aureus</i>	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-
<i>S. epidermidis</i>	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-
<i>S. saprophyticus</i>	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
<i>E. coli</i>	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-
<i>K. pneumonia</i>	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
<i>P. vulgaris</i>	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-
<i>E. aerogenes</i>	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-
<i>C. frundii</i>	+	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-
<i>P. aeruginosa</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ indicates the growth of bacteria and - indicates the absence of bacterial growth.

Table 3- MBC of different concentrations of essential oil and methanol extract of *Z. tenuior* against bacterial isolates

Bacterial isolates	Essential oils concentration (mcg/ml)								Methanol extract concentration (mcg/ml)							
	62.5	12	25	50	1000	2000	4000	8000	62.5	125	250	500	1000	2000	4000	8000
<i>S. aureus</i>	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-
<i>S. epidermidis</i>	+	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-
<i>S. saprophyticus</i>	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-
<i>E. coli</i>	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-
<i>K. pneumonia</i>	+	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-
<i>P. vulgaris</i>	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-
<i>E. aerogenes</i>	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-
<i>C. frundii</i>	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-
<i>P. aeruginosa</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ indicates the growth of bacteria and - indicates the absence of bacterial growth.

## DISCUSSION

The inhibitory effect of herbal extracts and essential oils against bacteria have been known for thousands of years. In recent years, the effect of herbal compounds on pathogenic bacteria have attracted a lot of attention in order to replace chemical antibiotics with natural antibacterial agents derived from plants (16,17). The effect of these substances on important food-borne pathogens such as *E. coli*, (18), *Salmonella enteritidis* (19-21), *Bacillus cereus* (22, 23), *S. aureus* (20, 21) and *Listeria monocytogenes* have been demonstrated (24). Analysis of essential oils from different plants shows the presence of different compounds. Thymol and carvacrol are the main components of essential oils of plants from the mint family. The strong antimicrobial activity of carvacrol has been shown in previous studies (25, 26). Review of

Ozturk and Ercisli in 2006 showed that the extract of *Z. tenuior* is consisted of 31.86% poligon, 12.21% senion, 10.48% limonen, 9.13% menthol, 6.88% beta-pinene, 6.73% menton, 3.5% peperitnon and 4.18% peperitnon. Poligon is the main component of the essential oil of some plants from the mint family such as *Z. tenuior*. Poligon has antibacterial and antifungal properties and is particularly effective against different Salmonella strains (27). According to the present study, essential oil of *Z. tenuior* has more antibacterial effects than the methanol extract. This could be because of the higher polygon content in the essential oil since the antibacterial activity is more associated with poligon. Study of Salehi et al. assessed the antimicrobial effect of *Z. tenuior* extract and showed that the extract can inhibit the growth of Gram-negative bacteria including

*K. pneumoniae* and *E. coli*. Similar to the result of this study, the *Z. tenuior* methanol extract had no antibacterial activity against *P. aeruginosa* in the mentioned studies (8, 28). Results of Salehi et al. also suggest that the extract can inhibit the growth of *S. epidermidis* and *B. subtilis*. Studies of Ercili and Ozturk in 2006 and 2007 showed that mountains' Kakoty and *Z. persica* extracts are able to inhibit growth of a wide range of Gram-positive and Gram-negative pathogenic bacteria. In the present study, the essential oil of *Z. tenuior* had inhibitory and bactericidal effects on most Gram-negative bacteria except *P. aeruginosa*. These results are in agreement with the results of Baser et al. study in Turkey (2). Their results showed that the essential oil of Kakoty can prevent the growth of *E. coli* and *E. aerogenes* but not *P. aeruginosa*. The above findings are similar to the results of Salehi et al. (29, 30). The present study showed that the essential oil of *Z. tenuior* has favorable anti-bacterial effect on the Gram-negative bacteria tested. According to the study of Baser et al. in 1991, the extract of Kakoty

native to Turkey have antibacterial effects on Gram-positive bacteria including *S. aureus* and *B. subtilis* (2). Study of Salehi et al. also showed that the extract of mountains' Kakoty could prevent the growth of *B. subtilis* and *S. aureus* (8).

## CONCLUSION

This study showed that the essential oil and methanol extract of *Z. tenuior* (Kakoty) have antibacterial effects against all the bacterial isolates tested except *P. aeruginosa*. Therefore, they can be used as antibacterial agents against a broad spectrum of bacteria causing urogenital tract infections. It is suggested to conduct further studies in this regard using animal models.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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