EVALUATION OF ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *TEUCRIUM CHAMAEDRYS* (L.) GROWING WILD IN KOSOVO

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Abstract. In this study the antibacterial efficiency of different organic extracts from *Teucrium chamaedrys* (L.), growing wild in Kosovo, was examined. Methanol, ethyl acetate, acetone, diethyl ether, water and chloroform extracts were tested against three gram positive bacteria *Staphylococcus aureus* (food isolate), *Staphylococus aureus* (clinical isolate), *Listeria monocytogenes* (clinical isolate) one gram negative bacteria *Escherichia coli* (clinical isolate). The antibacterial activity was determined by using agar disc diffusion method. The inhibition zone of extracts was compared to that of penicillin G as standard. Based on the results, the most intense activity was shown by the plant`s extracts with water and ethyl acetate. The ethyl acetate extract showed activity in all of the concentrations 1, 3 and 5 mg/ml towards *Staphylococcus aureus* (food isolate), *Eshcheria coli* and *Listeria monocytogenes.* Ethyl acetate extract of the plant with concentration 5 mg/ml showed a stronger antibacterial activity towards bacteria *Eshcheria coli* with inhibition zone of 12 mm. Aqueous extract of the plant with concentration 5 mg/ml showed a stronger antibacterial activity against bacteria *Esherichia coli* with inhibition zone of 12 mm. Also aqueous extracts of the *Teucrium chamaedrys* (L.) showed a stronger antibacterial activity as penicillin G against bacteria *Staphylococcus aureus* (clinical isolate). The antibacterial activity of the *Teucrium chamaedrys* (L.) was due to the presence of various secondary metabolites such as phenols and flavonoids. Hence, this plant can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals.

Keywords: Teucrium chamaedrys (L.); antibacterial activity; agar disk diffusion method; organic extracts

Introduction

The propitious geographic position of Kosovo and the diversity of soil types are responsible for the heterogeneousness of the Kosovo flora (Mehmeti et al., 2007).

The products of secondary metabolism are not of essential importance for plants, but are most often the result of adaptation to biotic and abiotic environmental factors. Main secondary metabolites in plants are terpenes, phenolics and nitrogen-containing compounds. Secondary metabolites have important biological and pharmacological activities, such as antibiotic, antioxidative, anti-allergic, hypoglycemic, and anticarcinogenic properties (Stanković et al., 2012; Lysakowska et al., 2011; Anter et al., 2011).

Due to their natural origin, the antibiotics obtained from plants are of greater benefit in comparison to synthetic ones. The use of natural antibiotics from plants does not induce side effects such as abdominal pain, diarrhea, cramping, headache, nausea and vomiting. Plant products may be used as antibiotic alternatives and do not cause resistance in bacteria (Gibbons, 2005).

The genus *Teucrium* L. (Lamiaceae) includes about 200 species and subspecies of herbs and shrubs, often aromatic, with a centre of distribution in the Mediterranean basin (Greuter et al., 1986). In the area of central and west Balkan, nine species of this genus was registered (Tutin & Wood, 1972). The herbs of *Teucrium chamaedrys* (L.) and *Teucrium montanum* (L.) are the most popular traditional remedies in Balkans used as cholagogue, tonic and antianemic, as well for treatment of diarrhea, leucorrhea, wounds and hemorrhoids. The infusion of aerial parts of *Teucrium scordium* was used as bitter aromatic, cholagogue with wound healing and fever reducing properties (Redžić, 2007; Jarić, 2007).

The effect of the plant extracted from microorganisms have been studied by a number of researchers in different parts of the world (Kucuk et al., 2006; Gursoy & Tepe, 2009; Nevcihan & Bektas, 2009; Sarker et al., 2007; Stanković et al., 2012; Vlase et al., 2014; Kundaković et al., 2011).

Our research group was interested to analyze the chemical profile of different medicinal plants, which are growing in the region of Kosovo and Albania (Haziri et al., 2009; 2010; 2013; Faiku & Haziri, 2013; 2015; Faiku et al., 2012; 2015; 2016; 2017a; 2017b).

The aim of this study was to investigate the antibacterial activity of different solvent extracts of *Teucrium chamaedrys* (L.) growing wild in Kosovo.

Material and methods

Plant material

The aerial part of *Teucrium chamaedrys* (L.), growing wild in east part of Kosovo, was collected in 23.06. 2015 (Fig. 1). Voucher specimens were deposited in the herbarium of the Department of Biology, University of Prishtina. The plants were dried at room temperature.

Fig. 1. *Teucrium chamaedrys* (L.) growing wild in Kosovo (Photo taken from Arben Haziri)

Preparation of plant organic extracts

The aerial part of *Teucrium chamaedrys* (L.) was air-dried and then milled with a mixer. A piece of finely powdered material (200 g) was extracted three times with 70% methanol (CH_3OH, 4 L) during a 24h period. After removing the CH_3OH under reduced pressure, the aqueous phase is extracted with four consecutive increasing polarity solvents, ether ($C_4H_{10}O$), chloroform ($CHCl_3$), etilacetate ($CH_3COOCH_2CH_3$) and acetone (CH_3COCH_3). Extraction is done until colorless extracts are taken. The remaining was water extract. Five extracts ($C_4H_{10}O$, $CHCl_3$, $CH_3COOCH_2CH_3$, CH_3COCH_3 and H_2O) were evaporated by vacuum rotary evaporator (EYELA N-1000, Japan) and then dissolved in DMSO to prepare the 10% solution (w/v). These solutions, either as such or diluted, are used in subsequent experiments for testing for antibacterial and antifungal activity. Solvents (analytical grade) for extraction were obtained from commercial sources (Sigma–Aldrich, Merck).

Antibacterial activity

The antibacterial activity of the extracts methanol, ethyl acetate, acetone, diethyl ether, water and chloroform of *Teucrium chamaedrys* (L.) are determined

applying the Kirby-Bayer (Barry, 1991) method or disk method (d = 5.5 mm, maximum capacity 10 mg). Organic extracts samples were tested *in vitro* against bacterial strains; *Staphylococcus aureus* (food isolate with code 3321), *Staphylococus aureus* (clinical isolate with code 3319), *Listeria monocytogenes* (clinical isolate with code 2653) and *Escherichia coli* (clinical isolate with code 2813). Discs are previously wetted with DMSO solution of the organic extracts with three different concentrations, 1, 3 and 5 mg/ml and then placed in a Petri dish (d=15 cm). The disks were incubated at 37 ^{0}C for 48 h; the control was also maintained with penicillin dissolved in DMSO in a similar manner.

Results and discussion

During the antibacterial activity, we have tested the extracts from the plant *Teucrium chamaedrys* (L.) against four bacteria: *Staphylococus aureus* (positive Gram bacteria), *Listeria monocytogenes* (positive Gram bacteria), *Escherichia coli* (negative Gram bacteria) and *Staphylococcus aureus* (positive Gram bacteria). Antibacterial activity of these extracts were compared with Penicillin G- a substance, which can be found in the market and have been used with concentration 1, 3 and 5 mg/ml as a standard. The comparison is done in accordance to the measurements of the diameter of the inhibition zone that is formed. These results are shown in Table 1.

Extracts of methanol (3 and 5 mg/ml), ethyl acetate (1, 3 and 5 mg/ml), acetone (3 and 5 mg/ml), diethyl ether (3 and 5 mg/ml), chloroform (1, 3 and 5 mg/ml) and water with concentration 1, 3 and 5 mg/ml shows antibacterial activities against *Escherichia coli* (Table 1). The extracts of methanol, acetone and diethyl ether with concentration of 5 mg/ml resulted in a lower activity than the penicillin G of the same concentration. The extracts of acetone and methanol with concentration of 3 mg/ml have the same inhibition zone as the standard (6 mm). The extract of ethyl acetate with concentration of 1 mg/ml resulted in lower activity than the penicillin G with the same concentration. Extracts of ethyl acetate (3 and 5 mg/ml), chloroform (1 and 3 mg/ml) and water (1, 3 and 5 mg/ml) created an inhibition zone higher than the penicillin G with the same concentrations. The other extracts such as methanol, acetone and diethyl ether with concentration 1 mg/ml do not create any inhibition zone, in other words they do not show activity (Table 1, Fig. 2).

Table 1. Antibacterial activities of *Teucrium chamaedrys* (L.) organic extracts

Extract	Concentration (mg/ml)	Inhibition zones diameters (mm)			
		E. coli (c. i.)	*L. monocytogenes* (c. i.)	*S. aureus* (f. i.)	*S. aureus* (c. i.)
Methanol	1	-	-	5	-
	3	6	-	6	-
	5	6	-	8	-
Ethyl acetate	1	3	5	6	-
	3	8	6	7	4
	5	12	10	9	10
Acetone	1	-	-	-	-
	3	6	-	6	-
	5	7	5	8	5
Diethyl ether	1	-	-	-	-
	3	5	-	-	4
	5	7	4	6	8
Water	1	7	-	-	6
	3	8	-	6	9
	5	12	6	7	10
Chloroform	1	5	-	4	-
	3	7	-	6	-
	5	8	3	7	5
Penicillin	1	4	2	4	2
	3	6	6	8	6
	5	8	8	10	10

(-) no inhibition zone

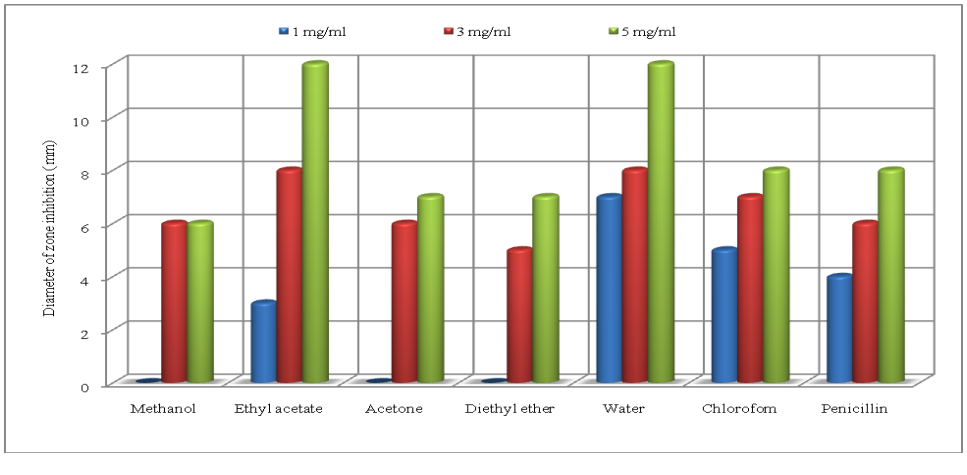

Fig. 2. Antibacterial activity of different extract of *Teucrium chamaedrys* (L.) against bacteria *Escherichia coli* (clinical isolate)

The extract of ethyl acetate with concentration of 1 and 5 mg/ml (inhibition zone 5 mm respectively 10 mm) resulted in a higher activity against *Listeria monocytogenes* (isolated by clinical way – positive Gram bacteria) than the penicillin G (inhibition zone 2 mm respectively 8 mm). The extract of ethyl acetate with concentration of 3 mg/ml have the same inhibition zone as the standard (inhibition zone 6 mm). The extracts of acetone, diethyl ether, chloroform and water with concentration of 5 mg/ml resulted in a lower activity than the penicillin G of the same concentration with inhibition zone of 8 mm. The other extracts such as methanol (1, 3 and 5 mg/ml), acetone (1 and 3 mg/ml), diethyl ether (1 and 3mg/ml) water (1 and 3 mg/ml) and chloroform with concentration 1mg/ml and 3 mg/ml did not show any inhibation activity on bacteria *Listeria monocytogenes* isolated by clinical way as was shown in Table 1 and Fig. 3.

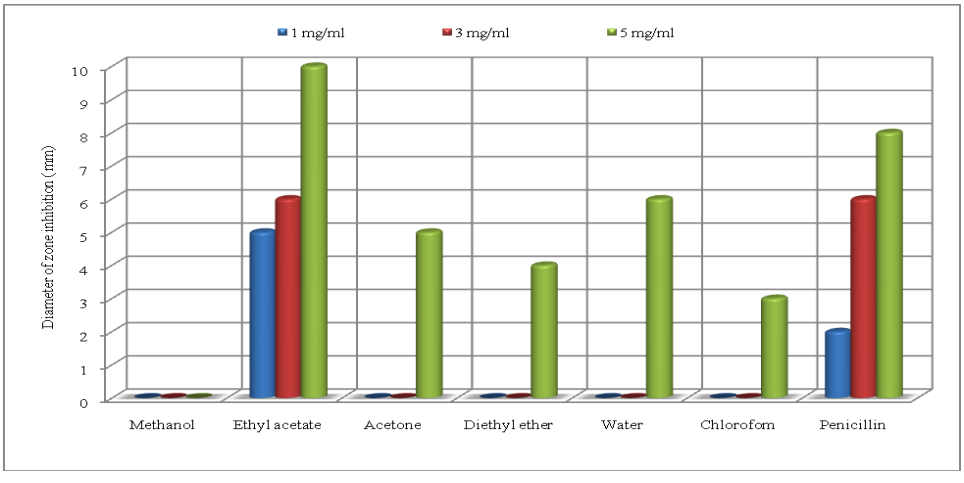

Fig. 3. Antibacterial activity of different extract of *Teucrium chamaedrys* (L.) against bacteria *Listeria monocytogenes* (clinical isolate)

The extracts of methanol, ethyl acetate and chloroform showed antibacterial activity against *Staphylococus aureus* (isolated in food) in all of the concentrations 1, 3 and 5 mg/ml. The extract of chloroform with concentration of 1 mg/ml has the same inhibition zone as the standard 4 mm. The extracts of methanol and ethyl acetate with concentration of 1 mg/ml (inhibition zone 5 mm respectively 6 mm) resulted in a higher activity against *Staphylococus aureus* (positive Gram bacteria) than the penicillin G (inhibition zone 4 mm). The extracts of methanol, acetone, water and chloroform with concentration of 3 mg/ml (inhibition zone of 6 mm) resulted in a lower activity than the standard of the same concentration with inhibition zone of 8 mm. The extracts of methanol (8 mm), ethyl acetate (9

mm), acetone (8 mm), diethyl ether (6 mm), water (7 mm) and chloroform (7 mm) with concentration of 5 mg/ml resulted in a lower activity than the standard of the same concentration with inhibition zone of 10 mm. The extracts of diethyl ether (1 and 3 mg/ml), acetone (1 mg/l) and water with concentration 1 mg/ml did not show activity on bacteria *Staphylococcus aureus* isolated in food (Table 1 and Fig. 4).

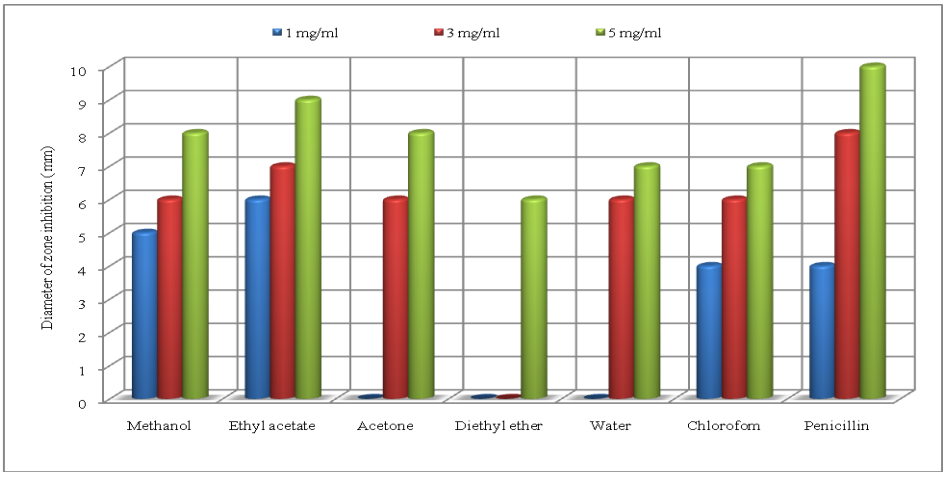

Fig. 4. Antibacterial activity of different extract of *Teucrium chamaedrys* (L.) against bacteria *Staphylococus aureus* (food isolate)

The extracts of ethyl acetate and diethyl ether with concentration of 3 mg/ml (4 mm) resulted in a lower activity against *Staphylococus aureus* (positive Gram bacteria) isolated in the clinical way, than the standard with the same concentration (inhibition zone of 6 mm). The extract of water with concentration of 1 and 3 mg/ml (inhibition zone 6 mm respectively 9 mm) resulted in a higher activity than the penicillin G (inhibition zone 2 mm respectively 6 mm). The extracts of water and ethyl acetate with concentration of 5 mg/ml have the same inhibition zone as the standard (10 mm). The extracts of acetone, diethyl ether and chloroform with concentration of 5 mg/ml resulted in a lower activity than the penicillin G of the same concentration (inhibition zone10 mm). Aqueous extract of the *Teucrium chamaedrys* (L.) showed a stronger antibacterial activity to bacteria *Staphylococus aureus* isolated in the clinical way. The other extracts such as methanol (1, 3 and 5 mg/ml), ethyl acetate (1 mg/ml), acetone (1 and 3 mg/ml), diethyl ether (1 mg/ml) and chloroform with concentration 3 and 5 mg/ml do not create any inhibition zone, in other words they do not show activity (Table 1 and Fig. 5).

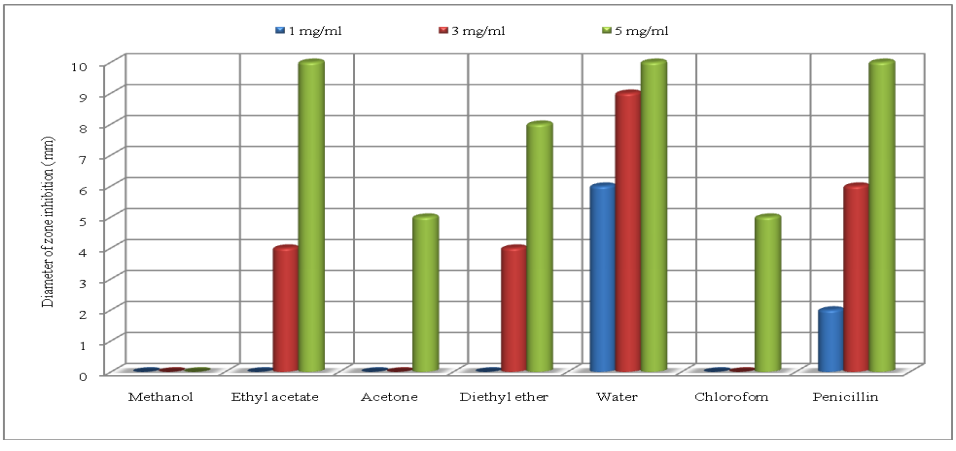

Fig. 5. Antibacterial activity of different extract of *Teucrium chamaedrys* (L.) against bacteria *Staphylococus aureus* (clinical isolate)

Conclusion

Teucrium chamaedrys (L.) is a plant that is used in traditional medicine for various purposes. The aim of this study was to evaluate the antibacterial activities of different extracts from *Teucrium chamaedrys* (L.) plant growing wild in Kosovo. Based on the results, the most intense activity was shown by the plant`s extracts with water and ethyl acetate. The three concentrations of the extract with ethyl acetate showed a very good activity by inhibiting the growth of all the tested bacteria. Ethyl acetate extract of the plant with concentration 1, 3 and 5 mg/ml showed a stronger antibacterial activity towards bacteria *Staphylococcus aureus* (food isolate), *Escherichia coli* and *Listeria monocytogenes*. Water and ethyl acetater extracts of the plant with concentration 5 mg/ml showed a stronger antibacterial activity (inhibition zone 12 mg) against bacteria *Eshcheria coli*. Thus, these extracts showed higher activity than all other extracts. Results obtained from water and ethyl acetate extracts for antibacterial activity are logical, based on numerous of studies where these extracts were analyzed in the content of flavonoids, phenols, terpenes, alkaloids, etc., and these components are responsible for the biological activity.

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