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PHYTOCHEMICALS AND ANTIOXIDANT STUDY OF *TEUCRIUM CHAMAEDRYS* (L.) PLANT

SUMMARY

Phytochemicals and antioxidant activity were analysed to the extracts of aerial part of *Teucrium chamaedrys* (L.) plant growing wild in Peja (Western part of Kosovo) in two different altitude levels (790 m and 520 m). In extracts were analysed the total phenols and flavonoids by spectrophotometric methods. Total phenols were determined by Folin-Ciocalteu reagent and were in the range of 21.45 mg/g (in altitude of 790 m) to 237.51 mg/g in altitude of 520 m (expressed as gallic acid equivalent, mg GAE/g). The amounts of flavonoids in the extracts of *Teucrium chamaedrys* (L.) were in the range of 11.85 mg/g (in altitude of 520 m) to 84.50 mg/g in altitude of 790 m (expressed as rutin equivalent, mg RU/g). Antioxidative activity was determined in vitro by using the DPPH test. Experimental values are expressed as IC₅₀ values (µg/mL), and they shows that the tested extracts have high antioxidant activities, which range in scope from 18.40 µg/mL (in altitude 790 m) to 264.22 µg/mL (in altitude 520 m).

Keywords: *Teucrium chamaedrys* (L.), phenols, flavonoids, antioxidant activity.

INTRODUCTION

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 et al., 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 et al., 2007).

Antimicrobial and antioxidant activity, total phenolic and flavonoid concentrations of *Teucrium* species have been studied by a number of researchers in the different parts of the world (Kucuk et al., 2006; Sarker et al., 2007; Gursoy

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et al., 2009; Nevcihan et al., 2009; Stanković et al., 2010; Kundaković et al., 2011; Stanković et al., 2012; Vlase et al., 2014).

Our research group was interested to analyse the chemical profile of different medicinal plants, which are growing wild in the region of Kosovo and Albania (Faiku et al., 2012, 2013, 2015a,b, 2016, 2017a,b, 2018; Haziri et al., 2009, 2010, 2013, 2017a,b).

The aim of this research was to determine the quantity of phenols, flavonoids and antioxidant activity of the different extracts from *Teucrium chamaedrys* (L.) growing wild in Peja (Western part of Kosovo).

MATERIAL AND METHODS

Plant material

The aerial part of *Teucrium chamaedrys* (L.), growing wild in east part of Kosovo, was collected in May 2017. The plants were dried at room temperature.

Preparation of plant organic extracts

A portion of the finely powdered material (200 g) was extracted three times with 70% methanol (methanol, 4 L) during a 24-h period. After removal of methanol under reduced pressure, the aqueous phase was successively extracted with three solvents of increasing polarity, namely ethanol, ethyl acetate and acetone. The extraction was carried out until a colorless extract was obtained. The residue was the aqueous extract. All five extracts (ethyl acetate, methanol, water, ethanol and acetone) were evaporated to dryness and then dissolved in 50% ethanol to make 10% (w/v) solutions. These solutions, either as such or in diluted state, were used in subsequent experiments.

Determination of total phenolic and flavonoid content

The amount of total phenolic contents in the extracts was determined spectrophotometrically with the Folin Ciocalteu (FC) reagent using the method of Fukumoto and Mazza (Fukumoto et al., 2000), with small modifications (Bozin et al., 2008). The reaction mixture contained 1.0% dilution of examined extracts (100 μ L), freshly prepared 0.2 mol/L FC reagent (2.5 mL) and 10% sodium carbonate solution (2 mL). The mixture was incubated in the dark at room temperature for 1 hour to complete the reaction. The absorbance of the resulting solution was measured at 760 nm on a UV/VIS spectrophotometer using distilled water as the blank. The concentration of total phenolic contents was expressed in mg gallic acid equivalents (GAE) per g dried extract (d.e.), using a standard curve of gallic acid (0.1-2.0 μ g/mL). All measurements were replicated five times.

Total flavonoid content in the extracts was determined spectrophotometrically according to Zhishen et al. 1999, using a method based on the formation of a flavonoid-aluminium complex with an absorbance maximum at 430 nm. The examined extracts (1mL) were mixed with 2% $\text{AlCl}_3 \times 6\text{H}_2\text{O}$ (0.5 mL). After incubation at room temperature for 30 min, the absorbance of the reaction mixtures was measured. The blank sample was a 1:1 mixture of the examined extracts and distilled water. Flavonoid content was expressed in μ g

rutin equivalent (RE) per g dried extract by using a standard curve of rutin (concentration range 0.5–6.0 µg/mL). All measurements were replicated five times.

Antioxidant activity- DPPH assay

The DPPH assay was performed as described previously (Blois, 1958; Zhou et al., 2004), following the transformation of the DPPH radical to its reduced, neutral form (DPPH-H). The samples of all extracts of *Teucrium chamaedrys* (L.) (from 2.50 to 50.00 µg/mL) were mixed with 90 µM DPPH• solution (1 mL) and made up with 95% methanol to a final volume of 4 mL. The absorbance of the resulting solutions was recorded spectrophotometrically at 515 nm after 1 h at room temperature, against the blank (with the same chemicals, except for the sample). The same procedure was repeated with rutin and *tert*-butyl-4- hydroxyanisole (BHA) as a positive control. For each sample five replicates were recorded.

RESULTS AND DISCUSSION

The concentration of phenolics in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent. Results of the amount of total phenolic contents in *Teucrium chamaedrys* (L.) extracts are given in Table 1.

Table1. Total phenol contents in the plant extract expressed in terms of gallic acid equivalent (mg GAE)

Altitude (m)	Ethanol	Methanol	Ethyl acetate	Acetone	Water
520	237.51±085 ¹	164.12±0.18	24.85±0.21	138.20±0.15	163.15±0.19
790	233.40±0.95	161.50±0.25	21.45±0.15	125.40±0.20	160.52±0.24

¹Each value in the table was obtained by calculating the average of five analysis ± standard deviation

The amount of total phenolics in *Teucrium chamaedrys* (L.) extracts ranged from 21.45 mg GAE/g (ethyl acetate extract to 790 m altitude) to 237.51 mg GAE/g (ethanol extract to 520 m altitude). The highest concentration of phenol was measured in ethanol, methanol and aqueous extract. Somewhat smaller concentration was found in acetone extract, while ethyl acetate extract had considerably smaller concentration of phenolics.

The content of phenolics in the plant extracts of the species *Teucrium chamaedrys* (L.) depends on the type of extract, i.e. the polarity of solvent used in extraction. The extracts obtained using more polar solvents like ethanol; methanol and water have higher concentration of phenolics (Table 1). High dissolubility of phenolics in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction (Zhou et al., 2004).

The concentration of flavonoids in various plant extracts of the species *Teucrium chamaedrys* (L.) was determined using spectrophotometric method

with aluminum chloride. The content of flavonoids was expressed in terms of rutin equivalent (mg RU/g). The amounts of flavonoids in the tested extracts are shown in Table 2.

Table 2. Flavonoid contents in the plant extract expressed in terms of rutin equivalent (mg RU/g)

Altitude (m)	Ethanol	Methanol	Ethyl acetate	Acetone	Water
520	45.55±0.20 ¹	56.0±0.62	82.27±0.75	69.52±0.55	11.85±0.65
790	41.45±0.30	58.50±0.55	84.50±0.74	72.10±0.62	12.15±0.45

¹Each value in the table was obtained by calculating the average of five analysis ± standard deviation

The concentration of flavonoids in plant extracts from *Teucrium chamaedrys* (L.) ranged from from 11.85 mg RU/g (water extract to 520 m altitude) to 84.50 mg RU/g (ethyl acetate extract to 790 m altitude). The concentration of flavonoids in acetone extract was 72.10 mg RU/g, which was very similar to the value of ethyl acetate extract concentration. The lowest flavonoid concentration was found in ethanol and aqueous extract. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Min *et al.*, 2005). Based on the obtained values for the concentration of flavonoids in the examined extracts of *Teucrium chamaedrys* (L.), it was found that the highest concentration of these compounds was in the extracts obtained using solvents of moderate polarity. The amount of total phenolic and total flavonoids in *Teucrium chamaedrys* (L.) extracts were given in Figure 1.

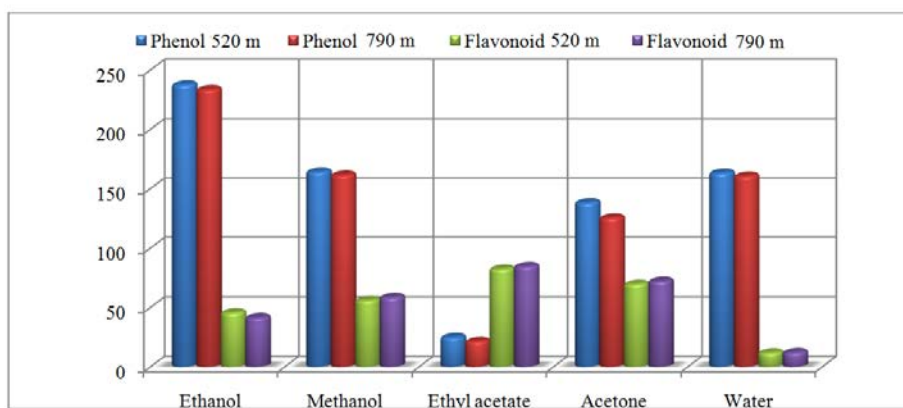


Figure 1. The amount of total phenolic contents and content of total flavonoids in *Teucrium chamaedrys* (L.) extracts

The antioxidant activity of *Teucrium chamaedrys* (L.) extracts has been evaluated in a series of *in vitro* tests. The DPPH radical is one of the most commonly used substrates for fast evaluation of antioxidant activity because of

its stability (in radical form) and the simplicity of the assay. In the DPPH assay, the ability of the investigated extracts to act as donors of hydrogen atoms or electrons in transformation of DPPH into its reduced form DPPH-H was investigated (Table 3). Parallel to examination of the antioxidant activity of plant extracts, the values for three standard compounds were obtained and compared to the values of the antioxidant activity. The standard substances were 3-tert-Butyl-4-hydroxyanisole (BHA) and rutin. Summary display of level for antioxidant activity of tested extracts is shown in Table 3.

Table 3. IC₅₀ values (µg/mL) of the neutralization of DPPH radical with *Teucrium chamaedrys* (L.) extracts

Altitude (m)	Ethanol	Methanol	Ethyl acetate	Acetone	Water	BHA	Rutin
520	21.55±0.84 ¹	24.55±0.85	264.22±0.98	29.95±1.1	26.25±0.65	5.25±0.78	9.27±0.77
790	18.40±0.70	25.60±0.70	259.55±0.80	27.41±0.90	22.41±0.70	5.25±0.78	9.27±0.7

¹Each value in the table was obtained by calculating the average of five analysis ± standard deviation

All of the assessed extracts of *Teucrium chamaedrys* (L.) were able to reduce the stable, purple-colored radical DPPH to the yellow-colored DPPH-H form with IC₅₀ (50% of reduction) values as follows: 21.55 µg/mL in altitude 520 m and 18.40 µg/mL in altitude 790 m for ethanol, 24.55 µg/mL in altitude 520 m and 25.60 µg/mL in altitude 790 m for methanol, 264.22 µg/mL in altitude 520 m and 259.55 µg/mL in altitude 790 m for ethyl acetate, 29.95 µg/mL in altitude 520 m and 27.41 µg/mL in altitude 790 m for acetone, and 26.25 µg/mL in altitude 520 m and 22.41 µg/mL in altitude 790 m for water extract.

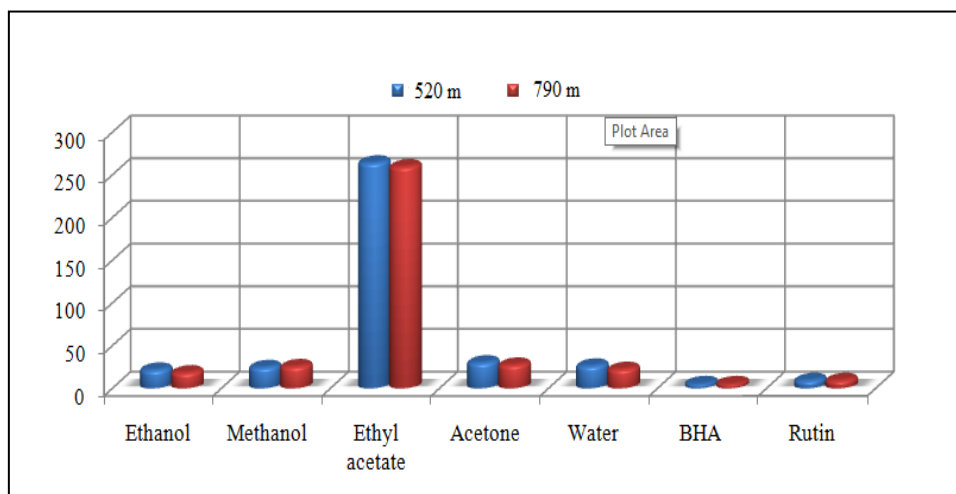


Figure 2. The IC₅₀ values (µg/mL) of the neutralization of DPPH radical with *Teucrium chamaedrys* (L.) extracts

The obtained values for antioxidant activity examined with DPPH radical are in the range of 264.22 to 18.40 $\mu\text{g/ml}$. The largest capacity to neutralize DPPH radicals was found for ethanol extract, which neutralized 50% of free radicals at the concentration of 18.40 $\mu\text{g/ml}$. A similar activity was found for water, methanol and acetone extracts whose IC_{50} values were 22.41, 24.55 and 27.41 $\mu\text{g/ml}$ respectively. The minutest capacity to inhibit DPPH radicals was determined for ethyl acetate extract. In comparison to IC_{50} values of BHA and rutin, ethanol, methanol, water and acetone extracts from *Teucrium chamaedrys* (L.) manifested the strongest capacity for neutralization of DPPH radicals. The IC_{50} values ($\mu\text{g/mL}$) of the neutralization of DPPH radical with *Teucrium chamaedrys* (L.) extracts were given in Figure 2.

The extracts that perform the highest antioxidant activity (Table 3) have the highest concentration of phenolics (Table 1). Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants may contribute directly to their antioxidant action (Tosun *et al.*, 2009).

Flavonoids are class of secondary metabolites with significant antioxidant and chelating properties. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups (Sharififar *et al.*, 2009). Ethyl acetate extract has a very high concentration of flavonoids as well, but it manifests less intense antioxidant activity, while aqueous extract has smaller content but manifests the antioxidant activity similar to methanol and acetone extracts. This difference is a result of different dissolubility of flavonoids in the solvents of different polarities.

CONCLUSIONS

The aim of this research was to determine the concentration of phenols, flavonoids and *in vitro* antioxidant activity of the different extracts from *Teucrium chamaedrys* (L.) growing wild in Peja (Western part of Kosovo). To conduct this type of research, we took two samples of the plant *Teucrium chamaedrys* (L.) at different altitudes 520 m and 790 m. The amount of total phenols in organic extracts of *Teucrium chamaedrys* (L.) was in the region of 21.45 mg GAE/g (ethyl acetate extract to 790m altitude) to 237.51 mg GAE/g (ethanol extract to 520 m altitude). Also, great amount of these compounds was found in the water extract (160.15 mg GAE/ g 790 m to 163.52 mg GAE/ g de 520 m). The amount of total flavonoids in organic extracts of *Teucrium chamaedrys* (L.) was in the region of 41.45 mg RU/g (ethanol extract to 790 m altitude) to 84.50 mg RU/g (ethyl acetate extract to 790 m altitude). Also, the amount of these compounds was found in the water extract (11.85 mg RU/ g 590 m to 12.15 mg RU/ g de 790 m).

Investigation of five different plant extracts from *Teucrium chamaedrys* (L.) showed high concentration of total phenols, especially flavonoids. Investigation of antioxidant activity of extracts and comparison with the activity of reference substances revealed intense antioxidant activity of plant extracts,

which was related to high concentration of phenolics in the examined extracts. In addition to its intensive use in the treatment of digestion disorders, gout and various infectious diseases, wall germander (*Teucrium chamaedrys* (L.)) is a potential source of natural antioxidant substances of high value.

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