METHANOL EXTRACTS AND VOLATILES OF *TELEKIA SPECIOSA* (SCHREB.) BAUMG. FROM BOSNIA AND HERZEGOVINA

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT: The aim of the present study was to analyse the methanol extracts from the aerial and the underground parts of *Telekia speciosa* (Schreb.) Baumg by HPLC and determine their antioxidant capacity. In addition, volatiles from both parts of the plant were analysed. The chlorogenic acid (CGA) and caffeic acid derivatives were present in the extracts. The CGA content was 977.8±85 mg per 100 g extract for underground and 272.0±18 mg per100 g extract for aerial parts of *T. speciosa*. The antioxidant capacity, determined by DPPH and FRAP method, was on average three and a half times higher for the extract of the underground parts in comparison with the extract of the aerial plant parts. The volatile constituents determined by GC-FID/MS were characterized by the presence of a high concentration of oxygenated sesquiterpenes. The obtained results contribute to better knowledge of phytochemical properties of *T. speciosa*, which is traditionally used in bronchial asthma therapy.

KEYWORDS: *Telekia speciosa*, chlorogenic acid (CGA), antioxidant capacity, volatiles

INTRODUCTION

The only representative of the genus *Telekia* in the flora of Bosnia and Herzegovina (BiH) is *Telekia speciosa* (Schreb.) Baumg. It inhabits wet and shady positions in mountain woodlands. It is a perennial herbaceous plant with alternating, wide, whole leaves and large heterogeneous heads which can be individual or in cluster blooms. It is widespread in Eastern and Central Europe and the Balkan Peninsula [1]. The root of *T. speciosa* is traditionally used as a remedy for bronchial asthma in Balkan countries. In BiH, root smoke of this plant is used in inhalations for bronchial asthma [2].

The root of *T. speciosa* contains essential oil, bitter compounds and inulin [3]. Phytochemical investigations have revealed *T. speciosa* as a rich source of sesquiterpene lactones, especially in its underground parts [4]. Isoalantolactone is almost exclusively contained in the essential oil of *T. speciosa* roots. It is a sesquiterpene lactone – an eudesmanolide with an antiproliferative and anti-inflammatory activity [5]. Sesquiterpene lactones have been found to be active bactericidal principles, as well [6].

The aerial part extracts have been found to contain fatty acids, namely palmitic, linoleic, oleic and caproic acids [7]. Reports concerning the sterols of *T. speciosa* extracts can also be found in the literature [8]. Pseudoguaianolide - 2,3-dihydroaromaticin and three thymol derivatives have been isolated as major secondary metabolites from the aerial parts of methanol extract of *T. speciosa*.

Phenol acids derivatives in earlier investigations were isolated from an extract of *T. speciosa* flowers. Those compounds were: one derivative of ferulic acid [(E)-ferulic acid 4-O-β-(6-O-2-hydroxy isovaleryl)-glucopyranoside] and five caffeic acid derivatives [(E)-caffeic acid 4-O-β-(6-O-2-hydroxyisovaleryl)-glucopyranoside, (E)-caffeic acid 4-O-β-(6-O-3-hydroxy-2-methylpropanoyl)-glucopyranoside, 6-O-(E)-caffeoyl-glucopyranose, (E)-caffeic acid 4-O-β-glucopyranoside] and 5-caffeoylquinic acid (chlorogenic acid) [9].

The aim of the present study was to identify and quantify phenol acids in the methanol extracts of *T. speciosa* aerial and underground parts and determine the antioxidant capacity of the extracts. To the best of our knowledge, the chemical composition of the extracts of underground parts as well as the antioxidant capacity of the plant extracts had never been studied before. In addition, we analyzed the volatiles of both, aerial and underground parts.

MATERIAL AND METHODS

PLANT MATERIAL

Aerial and underground parts of *T. speciosa* were collected at their specific location of Zlača, Banovići

municipality (BiH) during the flowering period in August 2015. Geographical coordinates of the location were N44°20'22.3" E18°33'39.6". The plant material was identified according to Flora Croatia by authors [10] and voucher specimen was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, the University of Tuzla. The plant material was cleaned, cut, and dried.

REAGENTS AND CHEMICALS

All the reagents used were of analytical grade. Folin-Ciocalteu´s phenol reagent, sodium carbonate, sodium acetate anhydrous, and ferric (III) chloride were obtained from Merck (Germany). HPLC-grade acetonitrile and formic acid were purchased from Merck. Water for HPLC was prepared by Milli-Q Water Purification System. Methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), ferrous (II) sulfate heptahydrate, hydrochloric acid, glacial acetic acid, HPLC-grade chlorogenic acid, caffeic acid, ellagic acid, gallic acid, and *o*-coumaric acid were purchased from Sigma-Aldrich (USA). Rutin and ferulic acid were obtained from Carl Roth (Germany).

PLANT EXTRACTS

The dried plant material was crushed in a grinder until powder formation. The samples were extracted with 98% methanol on a magnetic stirrer under reflux at 50 °C for 1 hour. The mixtures were filtered through a filter paper (Whatman No. 1). The methanol was removed by evaporation. The dried extracts were stored in the fridge at 4 °C, in glass bottles for further investigations.

HPLC ANALYSES

HPLC analyses of extracts (3 mg/mL in methanol) were carried out using an Agilent 1260 Infinity (Agilent Technologies, USA) system equipped with a Agilent 1260 Infinity Quaternary Pump, Agilent 1260 Infinity Standard Autosampler, Agilent 1260 Infinity Diode Array Detector, and Agilent 1260 Infinity Thermostatted Column Compartment. The separations were performed on a Merck LiChroCART$_R$250-4 C18 RP analytical column (250x4.6mm i.d., 5µm). The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). The following gradient was applied: 0-15 min, linear gradient from 10% to 20% B; 15-30 min, linear gradient from 20% to 30% B; 30-35 min, linear gradient from 30% to 40% B; 35-40 min, linear gradient from 40% to 90% B; 40-45 min, then returned to the initial conditions. The injection volume was 10 µL; the flow rate was 0.8 mL/min. The detection wavelength was 325 nm, and the column thermostat was set at 30 °C [11]. Component identification was performed comparing their retention times and UV spectra with those obtained from standards. The calibration curve for chlorogenic acid was obtained by the external standard method in the concentration range of 15.6-500 µg/mL (y=28.93x-220.2, R^2=0.9996).

TOTAL PHENOL CONTENT AND ANTIOXIDANT CAPACITY

The total phenol content was determined by the Folin Ciocalteu spectrophotometric method [12]. The *in vitro* antioxidant capacity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assays [13] and the ferric reducing antioxidant potential (FRAP) [14].

ISOLATION AND GC-FID/MS ANALYSES OF ESSENTIAL OILS

The dried aerial and underground parts of *T. speciosa* were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus, according to the European Pharmacopoeia. The obtained oils were separated, dried over anhydrous sodium sulfate, and stored at -20º C until the analysis.

The volatile constituents were determined by the GC-FID/MS analyses using an Agilent 6890N GC system coupled with an Agilent 5975 MSD, FID, and equipped with a HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm). The oven temperature was programmed linearly rising from 60 to 280 ºC at 3 ° min^{-1} and then isothermal at 280 ºC for 5 min; injector 200 ºC; FID 300 ºC; transfer-line 250 ºC; carrier gas He (1.0 mL min^{-1}, constant flow mode); injection volume 1 µL of essential oil dissolved in ethanol; split ratio 10:1. EI Mass spectra (70 eV) were acquired over the m/z range of 35-550.

The identification of the individual compounds was based on the comparison of their retention times (t_R), retention indices (RIs), and mass spectra with those obtained from authentic samples and/or listed in the NIST, Wiley mass spectral libraries, and the literature [15]. For the quantification, the relative area percentages obtained by FID were used.

RESULTS AND DISCUSSION

The yields of the methanol extracts of *T. speciosa* were 4.31% and 11.52% for the aerial and underground parts, respectively.

The qualitative and quantitative analysis of the extracts was performed by the RP-HPLC method. The chlorogenic acid (CGA) and caffeic acid deriva-

tives (CA derivatives) were present in the extracts. In the aerial parts of *T. speciosa* except CGA, two CA derivatives (Rt - 22.464 min and Rt - 35.631min) were detected. In the underground parts, except CGA, four CA derivatives (Rt - 22.464 min, Rt - 25.433 min, Rt - 35.627 min and Rt - 37.694 min) were also present. Caffeic acid and other reference substances used in the analyses (gallic acid, o-coumaric acid, ferulic acid, ellagic acid and rutin) were not detected.

The content of chlorogenic acid was determined in the extracts (Table 1). In the extracts from the underground parts of *T. speciosa* the CGA content was on average three and a half times higher than the CGA content in the extracts from the aerial parts of *T. speciosa*.

Table 1. Chlorogenic acid content in the methanol extracts of *T. speciosa* aerial and underground parts

Extract	Chlorogenic acid (mg per 100 g extract)[a]
T. speciosa aerial parts summer Zlača	272.0±18
T. speciosa underground parts summer Zlača	977.8±85

[a] mean ± SD (n=3)

There were no available literature data about phenol acids in *T. speciosa* underground and aerial parts except some derivatives of phenol acids from a fraction of extract from *T. speciosa* flowers. Research studies have shown that chlorogenic acid demonstrates a wide range of pharmacological activities including antioxidant, antiobesity, anti-edematogenic and antinociceptive activities [16], [17].

Phenol compounds contribute to the overall antioxidant capacity of plants, which is why the extracts of *T. speciosa* were analyzed for their total phenol content by the Folin Ciocalteu method. DPPH and FRAP assays were used to evaluate the antioxidant capacity of those extracts (Table 2).

Table 2. Total phenol content, IC_{50} and FRAP values of *T. speciosa* methanol extracts

Sample	Total phenol content (mg GAE g^{-1} extract)[a]	IC_{50} (µg mL^{-1})[a]	FRAP µmol Fe^{2+} g^{-1} extract[a]
TSH	63.02±1.17	497.79±31.86	384.32±9.54
TSR	162.84±7.48	129.57±3.93	1337.48±48.03

[a] mean ± SD (n=3)
TSH = *T. speciosa* extract of the aerial parts
TSR = *T. speciosa* extract of the underground parts

To the best of our knowledge, the data about the antioxidant capacity of this plant have not been found by now. The extract of the underground parts of *T. speciosa* had on average three and a half times higher antioxidant capacity in comparison with the extract of the plant aerial parts. IC_{50} for rutin used as standard was 12.42 µg mL^{-1}. The content of chlorogenic acid indicates positive correlation existence between its content and antioxidant capacity in analysed extracts. The obtained results also confirm the well-known positive correlation between the total phenol content and antioxidant capacity.

The aerial parts of *T. speciosa* contained 0.04% (v/w) of yellow, liquid fragrant essential oil. The identified 69 constituents from the aerial parts of *T. speciosa* accounting for 87.9% of the oil are presented in Table 3. The oil of *T. speciosa* was characterized by the presence of a high concentration of oxygenated sesquiterpenes (51.8%). The major components were (*E*)-nerolidol (10.3%), caryophyllene oxide (8.2%), (*Z,E*)-farnesol (7.7%) and prenopsan-8-ol (4.9%). Sesquiterpene hydrocarbons constituted (5.8%) of the oil. Thymol derivative 10-isobutyryloxy-8,9-epoxythymyl isobutyrate (3.4%) was the major representative of oxygenated monoterpenes (12.0%). Non-terpene compounds presented an appreciable amount of essential oil (18.3%) with dominant (*E*)-phytol (4.9%) and hexadecanoic acid (3.3%).

Table 3. Chemical composition of essential oils from *T. speciosa* aerial and underground parts

No.	Compound	RIE[a]	Aerial parts (%)	Undergr. parts (%)
1	linalool	1101.4	**1.4**	0.2
2	nerol oxide	1155.3	0.4	-
3	*α*-terpineol	1192.5	0.3	-
4	*n*-decanal	1206.2	0.6	-
5	nerol	1229.5	0.7	-
6	geraniol	1255.1	0.3	-
7	dihydroedulan I	1294.1	**1.3**	-
8	silphiperfol-5-ene	1326.2	-	0.3
9	7-*epi*-silphiperfol-5-ene	1345.0	-	0.4
10	decanoic acid	1371.9	0.2	-
11	silphiperfol-6-ene	1377.3	0.3	0.2
12	modheph-2-ene	1381.0	-	0.2
13	*α*-isocomene	1387.8	-	0.2
14	*β*-elemene	1394.0	0.3	0.2
15	*β*-ionol	1406.2	0.7	-
16	*β*-isocomene	1407.2	-	0.2
17	(*E*)-*β*-caryophyllene	1421.4	**1.3**	0.5
18	*epi*-*β*-santalene	1448.2	-	0.2
19	geranyl acetone	1453.5	0.6	-
20	*α*-humulene	1455.7	0.2	-
21	4,5-di-*epi*-aristolochene	1472.9	0.2	-
22	*β*-chamigrene	1477.6	0.3	0.1

No.	Compound	RIE[a]	Aerial parts (%)	Undergr. parts (%)
23	thymol isobutyrate	1485.6	0.3	0.3
24	(*E*)-*β*-ionone	1487.8	0.7	-
25	neryl isobutanoate	1491.4	**1.7**	**1.3**
26	*α*-selinene	1498.3	0.7	0.2
27	*β*-bisabolene	1509.3	-	0.2
28	cameroonan-7*α*-ol	1512.1	0.7	-
29	modhephen-8*β*-ol	1515.5	0.3	0.4
30	(*E*)-dihydro-apofarnesal	1521.0	0.3	-
31	*δ*-cadinene	1525.8	**1.1**	-
32	*cis*-calamenene	1536.7	0.1	-
33	*α*-calacorene	1545.6	0.6	-
34	isocaryophyllene oxide	1556.5	**1.5**	-
35	*epi*-longipinanol	1562.1	0.2	-
36	(*E*)-nerolidol	1567.8	**10.3**	-
37	neryl (*S*)-2-methylbutyrate	1576.3	-	0.5
38	prenopsan-8-ol	1577.7	**4.9**	-
39	caryophyllene oxide	1587.6	**8.2**	**1.3**
40	humulene epoxide II	1612.4	0.9	0.2
41	*cis*-isolongifolanone	1614.3	0.5	-
42	muurola-4,10(14)-dien-1*β*-ol	1631.6	0.4	-
43	*α*-acorenol	1634.3	-	0.1
44	caryophylla-4(12),8(13)-dien-5- *α*-ol	1636.6	**1.1**	0.1
45	caryophylla-4(12),8(13)-dien-5- *β*-ol	1640.6	**2.6**	-
46	*β*-eudesmol	1645.8	**2.8**	**1.6**
47	*α*-eudesmol	1653.1	0.3	-
48	atractylone	1656.3	-	0.4
49	selin-11-en-4α-ol	1657.7	0.8	0.4
50	14-hydroxy-(*Z*)-caryophyllene	1667.9	0.7	0.2
51	*trans*-calamenen-10-ol	1670.2	0.3	-
52	14-hydroxy-9-*epi*-(*E*)-caryophyllene	1674.5	**2.0**	0.7
53	cadalene	1677.7	0.6	-
54	*epi*-*α*-bisabolol	1685.2	0.5	-
55	6-methoxythymyl isobutirate	1687.5	0.6	0.4
56	*n*-heptadecane	1698.4	0.7	0.2
57	*δ*-dodecalactone	1707.8	0.5	0.7
58	(*E,Z*)-farnesal	1716.3	0.7	-
59	3-methoxy-cuminyl isobutyrate	1719.7	-	0.3
60	6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphtalen-2-ol	1723.0	-	0.3
61	(*Z,E*)-farnesol	1725.0	**7.7**	-
62	(*E,E*)-farnesal	1743.8	**1.1**	-
63	fukinone	1749.1	0.4	-
64	cyclocolorenone	1753.8	0.4	-
65	tetradecanoic acid	1765.0	**1.1**	-
66	*α*-costol	1775.4	0.6	-
67	*n*-octadecane	1798.4	0.3	-
68	hexahydrofarnesyl acetone	1845.3	**1.9**	-
69	benzyl salicylate	1870.1	0.4	-
70	alantolactone	1902.1	-	**2.4**
71	10-isobutyryloxythymyl isobutyrate	1917.2	1.5	0.5
72	10-isobutyryloxy-8,9-dehydrothymyl isobutyrate	1921.5	0.7	0.4
73	isoalantolacton	1945.9	**1.9**	**77.2**
74	hexadecanoic acid	1969.5	**3.3**	-
75	10-isobutyryloxy-8,9-epoxythymyl isobutyrate	2034.6	**3.4**	**3.7**
76	(*E*)-phytol	2114.8	**4.9**	-
77	10-isovaleryloxy-8,9-epoxythymyl isobutyrate	2119.1	0.6	0.2
78	pentacosane	2498.1	0.5	-
79	heptacosane	2698.0	0.3	-
80	nonacosane	2899.9	0.2	-
Total			**87.9**	**96.9**
Oxygenated monoterpenes			12.0	8.1
Sesquiterpene hydrocarbons			5.8	3.2
Oxygenated sesquiterpenes			51.8	85.6
Others			18.3	0.0

RIE[a] – experimental retention indices
n/a – not available
„-„ - not detected

In the previous investigations, aerial parts collected during the flowering time of *T. speciosa* from Serbia contained 0.06% (v/w) of essential oil. Dominant compounds were sesquiterpenes, non-terpene components and oxigenated monoterpenes [4]. Furthermore, leaf, stem, flower and root essential oils were also studied. The qualitative composition of the essential oils from the examined plant material was similar, whereas the quantities of individual components of the oils varied widely depending on the kind of the plant material [18]. The results of essential oils components obtained from the literature were similar to our results.

The amount of the essential oil found in the underground parts of *T. speciosa* (0.29% (v/w)) was higher than in the aboveground parts. The oil was yellowish with needle crystals, and of aromatic odor. The identified 35 constituents from the underground parts of *T. speciosa* accounting for 96.9% of the oil are presented in Table 3. The oil was characterized by the presence of high concentration of oxygenated sesquiterpenes (85.6%) with isoalantolactone being the major component (77.2%). *β*-Eudesmol (1.6%) and caryophyllene oxide (1.3%) were also the representatives of oxygenated sesquiterpenes in the oil of the underground parts. Sesquiterpene hydrocarbons constituted only (3.2%) of the oil. Thymol derivatives [10-isobutyryloxy-8,9-epoxythymyl isobutyrate (3.7%), 9-isobutyryloxythymol isobutyrate (2.4%)] and neryl isobutanoate (1.3%) were the oxygenated

monoterpenes found in appreciable amounts. They constituted (8.1%) of the oil.

In the previous investigations, the underground parts collected during the flowering time of *T. speciosa* from Poland and Montenegro contained 0.4% - 1.7% of essential oils. The dominant compound of those oils was also isoalantolactone (62.3% - 95%) [1], [18].

CONCLUSION

To the best of our knowledge, the extracts from the aerial and underground parts of *Telekia speciosa* (Schreb.) Baumg were analysed on the presence of phenol acids by HPLC for the first time. The chlorogenic acid and caffeic acid derivatives were detected in the extract from the aerial and underground parts, with a higher amount of caffeic acid derivatives and three and a half times higher amount of the chlorogenic acid in the underground parts.

The presence of phenol compounds contributes to the antioxidant capacity, which was also evaluated in the extracts for the first time, as we know. On average the three and half times higher antioxidant capacity was determined for the underground parts than for the aerial parts of *T. speciosa* extracts. The results for the chemical composition of the essential oils of *T. speciosa* were similar to the previously published data.

The obtained results contribute to better knowledge of phytochemical properties of *T. speciosa*, which is traditionally used in bronchial asthma therapy. The recommendation for further investigations refers to the isolation of caffeic acid derivatives using the column chromatography and the determination of their structures as known or new compounds by comparison of their spectral data (1H NMR, UV) with those found in the literature.

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