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Bioactivity of herbal tea of Hungarian thyme based on the composition of volatiles and polyphenolics

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ABSTRACT

Hungarian thyme (*Thymus pannonicus* All., Lamiaceae) is an aromatic herb used as traditional remedy, a refreshing beverage and a food aromatizer. Herbal teas, *i.e.*, infusions, of Hungarian thyme from eight localities in Serbia were analyzed regarding their polyphenolic and volatile composition, and tested for their antioxidant and antimicrobial activity. The total polyphenolics content, determined by the Folin-Ciocalteu method, ranged from 1122.25 to 1979.93 mg gallic acid/L. HPLC analysis revealed rosmarinic acid (367.42–1199.47 mg/L) and luteolin glucuronides as the main polyphenolics. The volatile fractions of the infusions, analyzed by static headspace extraction coupled with GC and GC–MS analyses, contained citral, 3-octanone, 1-octen-3-ol, linalool and 1,8-cineole as the dominant constituents. The antioxidant activity of the infusions was examined through the ferric reducing antioxidant power (FRAP) (68.09–124.58 mmol Fe^{2+}/L) and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical tests (SC_{50} 1.32–2.96 μL/mL). The antimicrobial activity was tested by the broth microdilution method against standard strains of Gram(+) *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Enterococcus faecalis* and Gram(−) bacteria *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, and one strain of yeast *Candida albicans*. The infusions inhibited microbial growth in the tested concentration range (31.25–500.00 μL/mL) and the strongest activity was exhibited against the strain of *C. albicans* (MIC 31.25–62.50 μL/mL). The antioxidant and antimicrobial properties of the infusions were, to some extent, in correlation with the composition and content of the polyphenolic compounds, whereas the volatiles noticeably influenced the exhibited antimicrobial activity.

1. Introduction

Herbal teas (herbal infusions, hot aqueous extracts of herbal drugs) are one of the most commonly used beverages, either as refreshment drinks or as traditional remedies for various ailments. Their beneficial effects could be partly attributed to polyphenolic compounds, which are known to possess antioxidant and antimicrobial properties (Miguel, 2010; Lu and Foo, 2001; Cowan, 1999; Cushnie and Lamb, 2011). Additionally, some constituents of essential oils are partially soluble in water and present in infusions of aromatic plants, creating a specific aroma and contributing to the medicinal properties of the preparation (Tschiggerl and Bucar, 2010).

Thyme species (*Thymus* spp., Lamiaceae) are aromatic herbs known from the ancient times. The aerial parts of these plants, either wild or cultivated, are commonly used for their seasoning and food-preservation properties, and as medicinal herbs (Teuscher, 2006; Figueiredo et al., 2008; Rey and Sáez, 2002). *Thymus pannonicus* All. (Hungarian thyme, Eurasian thyme) is found in Central and Eastern Europe growing over open dry meadows, grasslands and rocks (Jalas, 1972). In Serbia, the lemon-scented leaves of the citral chemotype of Hungarian thyme are traditionally used as

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; FC, Folin-Ciocalteu; FRAP, ferric-reducing antioxidant potential; GA, gallic acid; HS, headspace; MIC, minimal inhibitory concentration; RA, rosmarinic acid; TPC, total polyphenolics content.

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an aromatizing agent for homemade confectionary products, jams, candies. The herbal tea from the aerial parts of this plant is used as a refreshment drink or as a medicinal agent for the treatment of mild gastrointestinal and respiratory disorders. The antimicrobial activity of the essential oil of *T. pannonicus* was previously confirmed (Maksimović et al., 2008). However, there is little scientific knowledge about the bioactivity and chemical composition of *T. pannonicus* to support its traditional use.

The focus of attention in this study was on a bioactivity and chemical composition of infusions of Hungarian thyme. Based on *a priori* knowledge, the composition of polyphenolic and volatile compounds were analyzed, bearing in mind the possibility of their influence on the overall effect and properties of the consumed herbal teas.

2. Materials and methods

2.1. Chemicals and reagents

All solvents used were of analytical grade. For the HPLC analysis solvents and phosphoric acid were of HPLC gradient grade. Folin-Ciocalteu (FC) reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), ferrous sulfate, and gallic acid (GA), and apigenin were purchased from Sigma Aldrich, rosmarinic acid (RA) and luteolin from Carl Roth, and standard mixture of *n*-alkanes was obtained from Fluka.

Luteolin 7-*O*-diglucuronide, apigenin 7-*O*-glucuronide, salvianolic acid H (3′-*O*-(8′′-*Z*-caffeoyl)-rosmarinic acid) and methyl derivative of salvianolic acid H were isolated from the aerial parts of *T. pannonicus* by fractionation of water and methanol extracts by means of column chromatography, solid phase extraction, preparative thin layer chromatography (TLC) and semipreparative HPLC. Luteolin 7-*O*-diglucuronide and apigenin 7-*O*-glucuronide were identified by their TLC properties (Markham, 1982), UV spectra recorded in methanol and in the presence of shift reagents (Mabry et al., 1970), as well as by comparison of ESI–MS data with those found in the literature (Marczak et al., 2010; Kozics et al., 2013; Pereira et al., 2013). UV, ^{1}H NMR and ESI–MS data of salvianolic acid H were in agreement with those published by Dapkevicius et al. (2002) and Pereira et al. (2013). The structure elucidation of methyl salvianolic acid H was performed by the analysis of UV, ^{1}H NMR and ESI–MS spectra, but the precise position of the methyl group could not be determined by used techniques (Appendix A in the Supplementary material).

2.2. Plant material and preparation of the infusions

The aerial parts of *T. pannonicus* (samples **1**–**8**) were collected during the flowering period in May 2011 from eight localities in Serbia (Table 1). The plant material was identified according to Flora Europaea (Jalas, 1972) and voucher specimens were deposited at the Herbarium of the Department of Botany, University of Belgrade—Faculty of Agriculture. The samples were air dried and comminuted immediately before use.

For the preparation of the infusions (**I1**–**I8**), 25.00 mL of boiling water was poured over 1.25 g of plant material, left for 20 min, then quickly filtered into 25.00 mL volumetric flasks and filled with water to the volume. All further experiments were performed during the same day. The infusions were analyzed by high performance liquid chromatography (HPLC) and headspace-gas chromatography (HS-GC), as well as for the content of total polyphenolics. The antioxidant and antimicrobial activities of the infusions were tested *in vitro*.

Table 1
Collection sites of *Thymus pannonicus*.

Sample	Locality	Coordinates
	Southern Banat	
1	Mt. Vršačke planine—Djakov vrh	N 45° 07′ 18.5” E 21° 21′ 10.0”
2	Mt. Vršačke planine—village Sočica	N 45° 05′ 31.3” E 21° 27′ 23.5”
3	Mt. Vršačke planine—tower	N 45° 07′ 12.0” E 21° 19′ 12.0”
4	Devojački bunar	N 45° 00′ 10.8” E 20° 57′ 03.6”
5	Grebenac	N 44° 54′ 10.8” E 21° 13′ 48.0”
	Mountains in E and S-E Serbia	
6	Mt. Stol	N 44° 10′ 15.8” E 21° 07′ 25.2”
7	Mt. Ozren	N 43° 35′ 28.8” E 21° 53′ 15.8”
8	Mt. Rtanj	N 43° 43′ 34.4” E 21° 50′ 45.6”

2.3. HPLC analysis

HPLC analysis of the infusions was performed on an Agilent 1100 Liquid Chromatograph under the following conditions: Zorbax Eclipse XDB-C18 analytical column (250 × 4.6 mm; particle size 5 μm), flow rate 0.8 mL/min, temperature 25 °C and injection volume 20 μL. The binary mobile phase consisted of solvent A, 0.03% phosphoric acid, and solvent B, 10% of A and 90% acetonitrile. Elution was commenced with 10% of B raised to 25% over 5 min, then kept constant until 15 min, 15–20 min raised to 30%, 20–25 min increased to 50%, 25–30 min increased to 70% of B, returned to the initial conditions until 35 min, and kept constant for 3 min. The diode–array detector was operating at 210, 250, 320, 350 and 370 nm. Identification of compounds was performed by comparing their UV spectra and retention times with those obtained for available standards and isolated compounds (Appendix A in Supplementary material). The UV spectra were also compared with the literature (Mabry et al., 1970).

The composition of the infusions, presented through the relative amounts of the identified constituents, was ascertained by calculating the relative percentage area of a peak in relation to the sum of all the peak areas in the chromatogram recorded at 350 nm.

The content of RA was determined by external calibration at 320 nm in the concentration range 0.10–1.00 mg/mL ($y = 32{,}001x - 27.56$; $r^2 = 0.9993$). The content of luteolin heterosides was calculated from the peak areas obtained at 350 nm using luteolin as the reference compound (concentration range 0.005–0.020 mg/mL; $y = 59{,}410.82x - 51.34$; $r^2 = 0.9999$). The quantification of apigenin heterosides was performed in similar way and the results are expressed as apigenin equivalents (concentration range 0.005–0.020 mg/mL; $y = 61{,}304.21x - 68.58$; $r^2 = 0.9999$).

2.4. Determination of total polyphenolics content

The total polyphenolics content (TPC) was determined by the Folin-Ciocalteu (FC) spectrophotometric method (Velioglu et al., 1998). Briefly, 100 μL of the suitably diluted infusion was mixed with 750 μL of the FC reagent (10-fold diluted) and 750 μL of sodium carbonate solution (60 g/L). After 90 min, the absorbance was measured at 725 nm. TPC was calculated from the calibration curve obtained in the same way with GA as the standard ($y = 110.05x + 0.008$; $r^2 = 0.9999$), and expressed as GA equivalents. Following the same procedure equivalents of GA were determined for the RA as the reference compound.