

Investigation of phenolic compounds and antioxidant activity of *Teucrium polium* L. decoction and infusion

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Abstract

In present study, we report phenolic compounds and antioxidant activity of decoction and infusion of *T. polium*. The quantitative amounts of the phenolic contents were determined by LC-MS/MS. The main compounds and amounts were determined as follow for decoction; fumaric acid, luteolin-7-O-glucoside, luteolin-5-O-glucoside, and pelargonin (2060.1; 1167.0; 835.2; 829.9 mg/kg dried herba, respectively). For the infusion sample main compounds and amounts were as follow; fumaric acid, luteolin-7-O-glucoside, pelargonin, and luteolin-5-O-glucoside (1456.2; 431.1; 312.5; 278.4 mg/kg dried herba, respectively). The antioxidant activities were determined based on three methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, β -carotene linoleic acid assays and cupric (Cu^{2+}) ion reducing power assay (CUPRAC). For all the activity assays, infusion and decoction samples of the *T. polium* showed good activity.

Keywords: *Teucrium polium*, decoction, infusion, phenolic compound, antioxidant activity.

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Teucrium polium L. demleme ve kaynatma örneklerinin fenolik bileşik ve antioksidan aktivitelerinin araştırılması

Özet

Bu çalışmada, T. polium' un kaynatma ve demleme örneklerinin fenolik bileşikleri ve antioksidan aktivitesi rapor edilmiştir. Fenolik içeriğin kantitatif miktarları LC-MS/MS ile belirlendi. Kaynatmada belirlenen ana bileşikler ve miktarları sırasıyla; fumarik asit, luteolin-7-O-glikozit, luteolin-5-O-glikozit ve pelargonin (sırasıyla 2060.1; 1167.0; 835.2; 829.9 mg / kg kuru herba) olarak bulundu. Demleme örneği için ise ana bileşikler ve miktarları; fumarik asit, luteolin-7-O-glikozit, pelargonin ve luteolin-5-O-glikozit (sırasıyla 1456.2; 431.1; 312.5; 278.4 mg /kg kuru herba) olarak belirlendi. Antioksidan aktiviteler üç yöntem esas alınarak belirlendi, bunlar: 2,2-difenil-1-pikrilhidrazil (DPPH) serbest radikal süpürme kapasitesi, β-karoten linoleik asit ve kuprik (Cu²⁺) iyonu indirgeyici antioksidan kapasite (CUPRAC). Tüm analiz sonuçlarına göre, T. polium' un demleme ve kaynatma örnekleri iyi bir aktivite gösterdi.

Anahtar kelimeler: *Teucrium polium, kaynatma, demleme, fenolik bileşik, antioksidan aktivite.*

1. Introduction

Teucrium L. belongs to the family of Lamiaceae (Labiatae), which has more than 150 species from the most common and different plants in the world [1]. *Teucrium* comprises 30 species and eight sections in Turkey [2]. They are mainly found in the mild parts of Asia, Europe and North Africa [2]. *T. polium* named as ‘acı yavşan’ and widely used as herbal tea in folk medicine. Also decoction and infusion of this species is used as treatment diabetes, kidney, liver diseases, stomach and hemorrhoids. In the literatur, the crude extracts of *T. polium* have been investigated by several researchers and these studies have focused on commonly biological activities of the extracts [3-5]. In the previous studies, anti-inflammatory, antibacterial, antihypertensive [6-8], antioxidant [4], fever-reducing, sudorific, antispasmodic, anodyne [9,10] and antidiabetic effects [11] of *T. polium* were reported. Also, there are some reports on total phenolic content and antioxidant activity of aqueous extracts of *T. polium* species from different region of the world [4,12-14]. The results showed that, since locality, climatic and seasonal conditions are effect the chemical constituents of the plants, biological activity results are differ. Plant phenolics were considered to have antioxidant activities due to their behavior such as reducing agents, hydrogen donor antioxidants and singlet oxygen quenchers [15]. For this reason, it is important to determine the phenolic profile of the plants. In present study, we report the phenolic contents and antioxidant capacity (DPPH free radical scavenging activity, β-carotene linoleic acid assays and cupric (Cu²⁺) ion reducing power assay (CUPRAC) of the decoction and infusion of *T. polium*.

2. Materials and methods

2.1. Plant material

The aerial parts of species were collected from Balıkesir-Edremit, Kazdağları, on the road of Pınarbaşı village, square areas, 39 ° 38'14.65 "K, 26 ° 56'47.62" D, 192 m, 15.6.2014. The species were identified by Dr. Selami Selvi at Balıkesir University. The voucher specimens were deposited at the Herbarium of the Altınoluk Vocational School, Balıkesir University, Balıkesir, Turkey (herbarium number SV 1520).

2.2. Preparation of decoction and infusion samples

Infusion; 2 g of the plant, dried in the shade and chopped into small pieces, were added to 98 mL of distilled boiling water and allowed to stay for 15 minute. The tea was filtered with an ashless filter paper. The filtrate (25 mL) was diluted with 25 mL of distilled water.

Decoction; 2 g of the plant, dried in the shade and chopped into small pieces, were added to 98 mL of distilled water and heated together in a steel kettle and allowed to stay for 15 minute after it boiled. It was filtered with an ashless filter paper. The filtrate (25 mL) was diluted with 25 mL of distilled water.

The determinations were performed using LC-MS/MS.

2.3. Chemicals

Standard compounds used for LC-MS/MS analysis were as follow: fumaric acid (99%, Sigma-Aldrich), pyrogallol (98%, Sigma-Aldrich), rutin (94%, Sigma-Aldrich), chlorogenic acid (95%, Sigma-Aldrich), gallic acid (99 %, Merck), syringic acid (95%, Sigma-Aldrich), t-ferulic acid (99%, Sigma-Aldrich), caffeic acid (98%, Sigma-Aldrich), pelargonin chloride (98%, Sigma-Aldrich), quercitrin (97%, Sigma-Aldrich), salicylic acid (99 %, Sigma-Aldrich), *p*-coumaric acid (98 %, Sigma-Aldrich), luteolin-7-*O*-glu (99%, AppliChem), rosmarinic acid (96%, Sigma-Aldrich), pyrogallol (98 %, Sigma-Aldrich), apigenin (95 %, Sigma-Aldrich), kaempferol (96 %, Sigma-Aldrich) and isorhamnetin (98 %, ExtraSynthese, Genay-France). Stock solutions were prepared as 10 mg/L in methanol. HPLC grade methanol was purchased from Merck (Darmstadt, Germany). Calibration solutions were prepared in methanol in a linear range. Dilutions were performed using automatic pipettes and glass volumetric flasks (A class). 100 mg/L curcumin solution was freshly prepared, from which 50 µL was used as an Internal Standard (IS) in all experiments.

2.4. Liquid chromatography-mass spectrometry

LC-MS/MS experiments were performed by a Zivak® HPLC and Zivak® Tandem Gold Triple quadrupole (Istanbul, Turkey) mass spectrometry equipped with a Synergy Max C18 column (250 x 2 mm i.d., 5mm particle size). The mobile phase was composed of water (A, 0.1 % formic acid) in methanol (B, 0.1 % formic acid), the gradient programme of which was 0-1.00 minute 55 % A and 45 % B, 1.01-20.00 minutes 100 % B and finally 20.01-23.00 55 % A and 45 % B. The flow rate of the mobile phase was 0.25 mL/min, and the column temperature was set to 30 °C. The injection volume was 10 mL. The detailed information on preparation of test solution and evaluation of uncertainty has been reported in the literature [16,17].

2.5. Antioxidant Activity

The antioxidant activities were measured based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, β -carotene linoleic acid assays and cupric (Cu^{2+}) ion reducing power assay (CUPRAC) [16-24].

3. Results and discussion

3.1. Phenolic contents

Phenolic components and quantity analyses of *T. polium* decoction and infusion were analyzed by LC-MS/MS. Phenolic compounds were analyzed in three groups: flavonoids and derivatives; coumaric acid and derivatives; and simple phenolics and others. Total 20 compounds, composed of 9 phenolic acids, 9 flavonoids and 2 flavonoid glycoside were determined in the *T. polium* decoction and infusion samples. The results were given in Table 1. While the decoction was found to be rich in flavonoids, the infusion was rich in phenolic acids. Luteolin-7-*O*-glucoside was mostly founded flavonoid in *T. polium* teas. In addition this, fumaric acid was determined in a high ratio in decoction and infusion samples (2060.1; 1456.2 mg/kg dried herb, respectively). The main compounds and amounts were determined as follow for decoction; fumaric acid, luteolin-7-*O*-glucoside, luteolin-5-*O*-glucoside and pelargonin (2060.1; 1167.0; 835.2; 829.9 mg/kg dried herba, respectively). For the infusion samples main compounds and amounts were as follow; fumaric acid, luteolin-7-*O*-glucoside, pelargonin and luteolin-5-*O*-glucoside (1456.2; 431.1; 312.5; 278.4mg/kg dried herba, respectively). Both of them (decoction and infusion) consisted of luteolin-7-*O*-glucoside, luteolin-5-*O*-glucoside, pelargonin and fumaric acid as dominant compounds in our study.

3.2. Antioxidant activity

The antioxidant activities were determined applying DPPH free radical scavenging activity, β -carotene linoleic acid assays and CUPRAC assays. Inhibition of lipid peroxidation and DPPH free radical scavenging effect were determined at 2, 5, 10, and 20 μL . The results were given in Table 2, Table 3. Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) were used as standard compounds in DPPH and β -carotene linoleic acid assays. In DPPH free radical scavenging activity assay, except infusion samples at volume of 2 μL , decoction and infusion samples at other concentrations showed good activity. In β -carotene linoleic acid assays, decoction and infusion samples at 20 μL concentrations had good activity results. Especially, decoction at 20 μL , have more effective than BHT. For the CUPRAC method, decoction sample has better activity than curcumin, which is used as standard. We observed that the infusion sample has moderate activity. In the literature, *T. polium* has been evaluated in terms of antioxidant potential several times. When the results, obtained from antioxidant activity assay, are compared with those of found in the other studies in the literature, phenolic compounds seem to have important role in antioxidant properties. Our results are compatible with other studies in the literature [12-14].

Table 1. Phenolic contents of *T. polium* decoction and infusion.

	Parent ion	Daughter ion	Collision energy (V)	Decoction	Infusion
Flavonoids and derivatives					
pelargonin	271.2	121	34	829.9±84.4	312.5±15.9
penduletin	345.2	311	25	241.4±2.5	65.3±6.6
quercitrin	471.9	309.9	16	-	36.2±2.3
luteolin	285	132	30	356.4±91.5	112.6±14.5
apigenin	269	151	22	614.0±49.5	160.4±12.9
isorhamnetin	315	300	15	97.5±8.6	5.4±0.5
quercetagenin-3,6-dimethylether	345.1	329.5	16	29.9±5.6	-
luteolin-7-O-glucoside	447	284.5	14	1167.0±118.7	431.1±21.9
luteolin-5-O-glucoside	447	289.5	20	835.2±53.7	278.4±17.9
kaempferol	287	152.3	30	682.9±48.2	178.3±12.6
rutin	609	301	16	41.4±2.7	71.2±4.7
Total (mg/kg dried herb)				4895.6	1651.4
Coumaric acids and derivatives					
caffeic acid	179	135	10	45.5±9.0	18.5±3.7
<i>t</i> -ferulic acid	193	133	15	18.7±1.3	10.2±0.7
chlorogenic acid	353	191	14	23.2±3.2	23.5±3.3
rosmarinic acid	359.2	160.5	15	194.5±14.9	17.3±1.3
<i>p</i> -coumaric acid	163.2	118.7	14	13.9±2.1	1.2±0.9
Total (mg/kg dried herb)				101.3	70.7
Simple phenolics and others					
gallic acid	168.6	124	13	-	4.4±0.3
syringic acid	196.7	181.4	12	388.3±26.1	232.3±15.6
fumaric acid	115	71	8	2060.1±142.9	1456.2±101
pyrogallol	125	80	16	34.2±2.3	36.3±2.4
Total (mg/kg dried herb)				2482.6	1729.2
Curcumin*	369.3	176.9	20		
				7478.9	3451.1

* Used as internal standard

Table 2. Inhibition (%) of DPPH and lipid peroxidation of the extracts, BHA and BHT.

		2 µl	5 µl	10 µl	20 µl
DPPH	infusion	43.5±18.2	63.9±0.8	61.8±3.0	62.6±0.8
	decoction	64.0±0.6	65.3±0.7	63.9±0.5	61.9±0.8
	BHA	22.7±2.1	30.9±4.1	48.2±3.9	62.4±2.9
	BHT	73.1±2.6	77.7±0.7	78.8±0.8	80.8±1.6
β-carotene linoleic acid	infusion	38.2±4.8	43.9±0.5	53.4±0.7	71.5±0.1
	decoction	46.0±6.3	47.8±0.2	63.2±5.1	71.9±2.6
	BHA	81.9±1.9	85.5±1.7	85.9±2.4	79.5±4.1
	BHT	82.6±5.0	72.4±11.8	77.1±2.9	71.0±1.0

Table 3. Antioxidant activity of extracts (CUPRAC).

	CUPRAC (mmol TR g-1)
infusion	0.8±0.1
decoction	2.2±0.4
curcumin	0.9

4. Conclusion

In conclusion, we examined and reported the main phenolic components and antioxidant activity of decoction and infusion of *T. polium*. We observed that fumaric acid is the main compound of *T. polium* decoction and infusion samples. LC-MS/MS results indicated that the highest proportion of phenolic content in decoction samples (7478.9 mg/kg), is an important factor for the antioxidant capacities of *T. polium*. Decoction and infusion samples are consisted of fumaric acid, luteolin-7-*O*-glucoside, pelargonin and luteolin-5-*O*-glucoside as major components in our study. This study supported that, the plants which are rich in phenolic compounds, can be a good source of antioxidants.

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