Karadeniz Fen Bilimleri Dergisi, 9(2), 289-296, 2019. DOI: 10.31466/kfbd.597153

KFBD

Karadeniz Fen Bilimleri Dergisi
The Black Sea Journal of Sciences
ISSN (Online): 2564-7377

Araştırma Makalesi / Research Article

Antioxidant Activity of Decoction and Infusion of *Teucrium chamaedrys* subsp. *chamaedrys* Grown in Turkey

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Received: 26.07.2019
Accepted: 04.11.2019

Abstract

In this study, antioxidant activity of aerial parts of *Teucrium chamaedrys* subsp. *chamaedrys* decoction and infusion were studied. The antioxidant activities were analyzed based 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, *β*-carotene linoleic acid assays, and cupric (Cu^{2+}) ion reducing power assay (CUPRAC). Infusion and decoction extracts at the concentration of 100 µg/mL, demonstrated higher antioxidant activity (86.5% and 84.1%, recpectively) than synthetic antioxidants (81.1%, BHA and 78.8%, BHT) in DPPH scavenging assay. Our results indicate that *T. chamaedrys* subsp. *chamaedrys* tea can be a promising source of natural antioxidant agent.

Keywords: Lamiaceae, *Teucrium chamaedrys* subsp. *chamaedrys*, Antioxidant activity, Decoction, Infusion.

Türkiye' de Yetişen *Teucrium chamaedrys* subsp. *chamaedrys* Türünün Demleme ve Kaynatma Örneklerinin Antioksidan Aktivitesi

Öz

Bu çalışmada, *Teucrium chamaedrys* subsp. *chamaedrys* türünün toprak üstü kısımlarının demleme ve kaynatma örneklerinin antioksidan aktivitesi çalışılmıştır. Antioksidan aktivite analizinde, 2,2-difenil-1-pikrilhidrazil (DPPH) radikal giderme aktivitesi, *β*-karoten linoleik asit ve kuprik (Cu^{+2}) iyonu indirgeme antioksidan kapasite (CUPRAC) yöntemleri kullanılmıştır. 100 µg/mL konsatrasyonda, DPPH radikal süpürücü kapasite yöntemi sonuçlarına göre, sentetik antioksidanlara (%81.1, BHA ve %78.8, BHT) kıyasla, demleme ve kaynatma örneklerinin (%86.5 ve %84.1, sırasıyla), daha yüksek antioksidan aktivite gösterdiği belirlenmiştir. Elde edilen bu sonuçlar, *T. chamaedrys* subsp. *chamaedrys* türünün çay örneklerinin doğal antioksidan kaynağı olarak kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Lamiaceae, *Teucrium chamaedrys* subsp. *chamaedrys*, Antioksidan aktivite, Kaynatma, Demleme.

1. Introduction

The genus *Teucrium* belongs to the Lamiaceae (Labiatae) family which has 900 species and widely distributed worldwide in particular in the region of Mediterranean (Davis, 1982; Davis et. al., 1988; Guner et. al., 2000; Kaya et. al., 2009). *T. chamaedrys* have been used as herbal medicine for its various biological activities (Kaya et. al., 2009). *T. chamaedrys* has been traditionally used in decoctions or infusions as a tonic, appetizer, against haemorrhoids, for stomachache, to treat gastric ulcer (Yeşilada et. al., 1993). *T. chamaedrys* is distributed six subspecies in Turkey, among which *T. chamaedrys* subsp. *chamaedrys,* common name Kısamahmut grows in central Anatolia (Figure 1) (Davis et. al., 1988; Guner et. al., 2000).

Figure 1. *T. chamaedrys* subsp. *chamaedrys*

There are many studies in the literature on the chemicals and activities of essential oil of *T. chamaedrys* grown in di□erent parts of the world (Küçük et. al., 2006; Kazemizadeh et. al., 2008; Kaya et. al., 2009; Muselli et. al., 2009; Bagci et. al., 2010) Kazemizadeh et. al., reported that α-muurolene, *β*-caryophyllene and α-pinene were the major compounds of essential oil of *T. chamaedrys* L. subsp. *chamaedrys* from Iran (Kazemizadeh et. al., 2008). Germacrene D, *β*- caryophyllene and δ-cadinene were determined as major compounds of *T. chamaedrys* from Turkey (Bagci et. al., 2010). Also, *β*-caryophyllene, germacrene D were found to be as main compounds of essential oil of *T. chamaedrys* L. from Corsica and Sardinia (Muselli et. al., 2009).

In the literature, total phenolic and phenolic content were analyzed for *T. chamaedrys* extracts for using various methods (Papanov et. al., 1980; Özgen et. al., 2006; Gursoy and Tepe, 2009; Stankovic et. al., 2010a; Stankovic et. al., 2010b; Stanković et. al., 2012; Haziri et. al., 2017). Stanković et. al. reported that methanol, water, ethyl acetate, acetone and petroleum ether extracts of *T. chamaedrys* L. showed high concentration of total phenols, especially flavonoids. Investigation of antioxidant activity of methanol, water and acetone extracts, which have the highest concentration of phenolics, manifested high antioxidant capacity (Stanković et. al., 2010a). Also, Özgen et. al., reported that the antioxidant properties of *Thymus sipyleus* Boiss. subsp. *sipyleus* var. *sipyleus*, *Teucrium chamaedrys* L., *Mentha longifolia* (L.) Hudson subsp. *longifolia*, *Salvia limbata* C.A.

Meyer, and *Thymus fallax* Fisch. & Mey. The highest antioxidant activity was shown by *T. chamaedrys* (decoction, IC_{50}: 9.2 µg/ml), and the lowest one was *S. limbata* (decoction, IC_{50}: 619.59.2 µg/ml) (Özgen et. al., 2006).

Synthetic antioxidants were found to have cytotoxic effect. Hence investigation of biological activities and chemical components of plants are very important. Natural antioxidants from plants including phenolic acids, flavonoids, carotens, tannins, and essential oil are better than synthetic antioxidants because of their lower cytotoxicity and residue.

In this sense, the aim of our work is to determine the antioxidant activity of decoction and infusion of *T. chamaedrys* and compared to the synthetic antioxidants. As far as our knowledge, essential oil, phenolics and biological activities of various extracts of *Teucrium species* have previously been investigated. However, no report is available for the antioxidant activity of *T. chamaedrys* subsp. *chamaedrys* decoction and infusion.

2. Materials and Methods

2.1. Plant Material

The aerial parts of *Teucrium chamaedrys* subsp. *chamaedrys* were collected from Balıkesir: Ayvalık, Şeytan sofrası road, woodland edges, sloping slopes, (39°16'57.16"N, 26°38'41.16"E, altitude: 48 m), during the full-flowering season in July 2017, Turkey. The species were identified by Prof. 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, HIA 128). The plant samples were allowed to dry in the shade.

2.2. Preparation of Decoction and Infusion Samples

4 g of aerial parts of the plant, dried in the shade and chopped into small pieces. For infusion; 2 g of the plant were added to 98 mL of distilled boiling water and allowed to stay for 15 minute. For decoction; 2 g of the plant 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. The teas were filtered with an ashless filter paper. The filtrates were diluted with 25 mL of distilled water.

2.3. Antioxidant Activity

The antioxidant activities were determined based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (Ozer, 2018; Köksal et. al., 2017; Ertaş et. al., 2015; Özer et. al., 2018; Miller, 1971; Blois, 1958). *β*-carotene linoleic acid assays (Miller, 1971; Ozer, 2018; Özer et. al., 2018) and cupric (Cu^{2+}) ion reducing power assay (CUPRAC) (Apak et. al., 2004; Bener et. al., 2016).

3. Findings and Discussion

The antioxidant effects of *T. chamaedrys* subsp. *chamaedrys* decoction and infusion are shown in Figure 2, Figure 3, Table 1 and Table 2. BHA, BHT, and curcumin were used as standard compounds. Inhibition of *T. chamaedrys* subsp. *chamaedrys* tea extracts ranged in 42.9-86.5% scavenging effect on DPPH radical and 28.0-58.4% scavenging effect on linoleic acid peroxidation at the all concentrations (10, 25, 50 and 100 µg/mL). In general, the antioxidant activity of linoleic acid and free radical scavenging were markedly inhibited by *T. chamaedrys* subsp. *chamaedrys* decoction extract compared with the standart campounds (BHA and BHT).

In DPPH assay, at the concentrations of 10, 25, 50 and 100 µg/mL, decoction extract showed the best activity (58.3, 69.9, 71.8 and 84.1%, respectively), while the standards had 43.5, 62.1, 75.4, 81.1%; 54.9, 69.2, 72.7, 78.8% inhibition of BHA and BHT, respectively. The decoction extract at concentrations of 10, 25 and 100 µg/mL showed higher antioxidant activities than standards. The highest activity was shown by infusion extract (86.5%) at concentration of 100 µg/mL and the lowest one was infusion at 10 µg/mL concentration (42.9%).

In linoleic acid peroxidation, among the infusion and decoction extracts, the highest antioxidant activity was determined in decoction of *T. chamaedrys* subsp. *chamaedrys*, which exhibited 58.4% inhibition at the concentration of 100 µg/mL. At the concentrations of 10, 25, 50 and 100 µg/mL, decoction extracts showed more activity than infusion extracts. Decoction extracts showed moderate inhibition activity of 55.4, 55.9, 57.2 and 58.4% at the concentrations of 10, 25, 50 and 100 µg/mL, respectively.

In CUPRAC assay, the tea samples showed good activity. The decoction and infusion of *T. chamaedrys* subsp. *chamaedrys* was identified to be 2.4 and 1.5 mmol TR g^{-1}. Curcumin was used as a standard compound (0.9 mmol TR g^{-1}). The results are given in the Table 2 and Figure 3.

Our results indicate clearly, decoction extracts showed the higher antioxidant activity than infusion extracts in *β*-carotene linoleic acid and CUPRAC antioxidant activity assays. These results indicated for the first time that the decoction and infusion of *T. chamaedrys* subsp. *chamaedrys* aerial

parts have high antioxidant activity. The high antioxidant activity of *T. chamaedrys* subsp. *chamaedrys* tea might be due to the phenolics and high phenolic capacity. In the literature, There is some information about the essential oil and chemical compounds of *T. chamaedrys* subsp. *chamaedrys*. However, there is no information about the antioxidant activities of the teas (infusion and decoction) of *T. chamaedrys* subsp. *chamaedrys* used in traditional medicine.

According to recent reports, germacrene D, α-pinene, *β*-caryophyllene, *β*-pinene and *β*-myrcene were the major compounds of the essential oil of *T. chamaedrys* subsp. *chamaedrys* (Küçük et. al., 2006). Uluben et. al., have identified steroidal compounds from acetone extract of *T. chamaedrys* subsp. *chamaedrys* from Turkey (Uluben et. al., 1993). Also, sitosterol, naringenin, ursolic acid and *β*-caryophyllene demonstrated quite strong antioxidant activity (Weng and Wang, 2000; Somova et. al., 2003; Zbarsky et. al., 2005; Lien et. al., 2008; Dahham et. al., 2015). Gursoy and Tepe have examined the in vitro antimicrobial and antioxidant activities and the amount of total phenolics of the methanol extracts of *T. chamaedrys* C. Koch (Gursoy and Tepe, 2009). Haziri et. al., reported to investigate the antibacterial activity of di□erent solvent extracts of *Teucrium chamaedrys* (L.) growing wild in Kosovo (Haziri et. al., 2017). Additionally, the total phenolic content, flavonoid concentrations and antimicrobial activity were manifested in methanol, acetone and ethyl acetate extracts of *T. chamaedrys* L. from Serbia (Stankovic et al., 2012). Also, Stankovic et. al., reported that the phenol and flavonoid contents and antioxidant activity of twenty different extracts obtained from the whole plant and plant parts of *Teucrium chamaedrys* L. var. *glanduliferum* Haussk (Stankovic et. al., 2010b). Previous studies have been showed that, the high phenolic content and significant corelation indicated that these compounds contribute to the strong antioxidant activity (Stankovic et. al., 2010a; Stankovic et. al., 2010b; Stankovic et al., 2012).

Table 1. Inhibition (%) of DPPH and lipid peroxidation of *T. chamaedrys* subsp. *chamaedrys*, BHA and BHT.

		10 **µg/mL**	25 **µg/mL**	50 **µg/mL**	100 **µg/mL**
DPPH	**Decoction**	58.3±2.3	69.9±5.0	71.8±10.7	84.1±17.0
	Infusion	42.9±9.9	58.1±2.1	75.3±3.2	86.5±8.1
	BHA	43.5±2.1	62.1±4.1	75.4±3.9	81.1±2.9
	BHT	54.9±2.6	69.2±0.7	72.7±0.8	78.8±1.6
***β*-carotene linoleic acid**	**Decoction**	55.4±4.4	55.9±5.23	57.2±5.8	58.4±4.4
	Infusion	29.8±7.11	28.0±3.6	44.0±9.8	52.0±9.9
	BHA	60.6±4.3	79.4±15.3	79.5±13.1	79.9±6.9
	BHT	55.2±14.7	56.2±10.5	59.4±15.0	81.6±16.8

Table 2. Antioxidant activity of *T. chamaedrys* subsp. *chamaedrys* extracts and curcumin (CUPRAC).

CUPRAC (mmol TR g^{-1})	*T. chamaedrys* subsp. *chamaedrys*
Decoction	2.4±0.7
Infusion	1.5±0.2
Curcumin	**0.9**

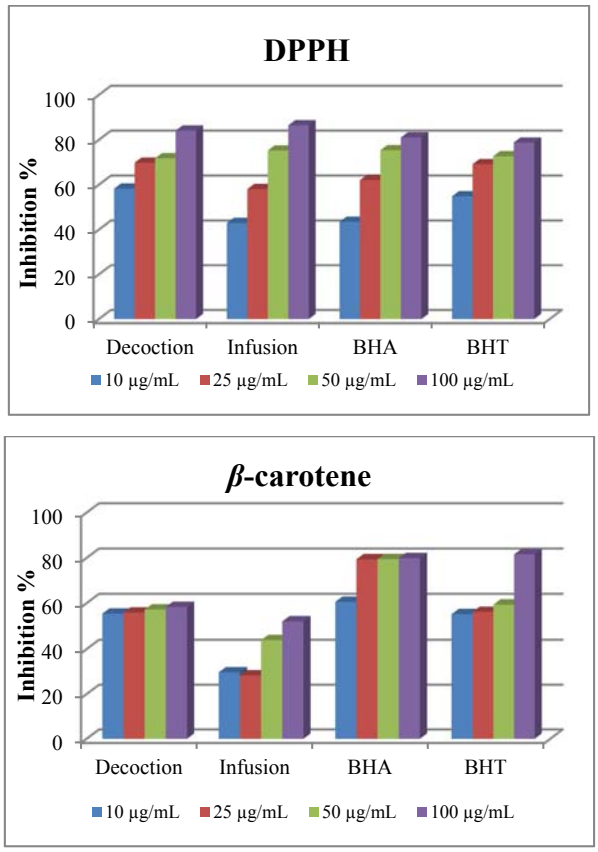

Figure 2. Antioxidant activities of decoction and infusion of *T. chamaedrys* subsp. *chamaedrys*, BHA and BHT (DPPH and *β*-carotene linoleic acid assays).

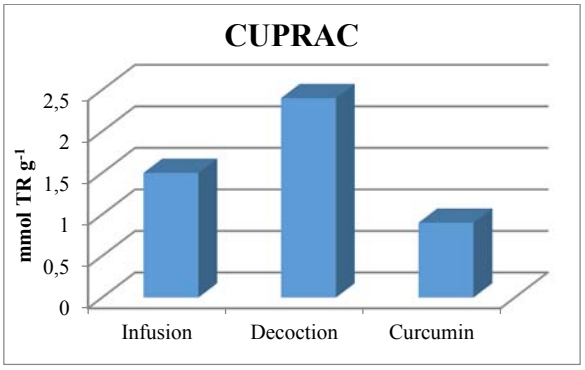

Figure 3. Cu^{2+} reducing power (CUPRAC) assay of the extracts and curcumin.

4. Conclusions and Recommendations

Our results revealed that infusion and decoction of *T. chamaedrys* subsp. *chamaedrys* were an effective antioxidant in different in vitro antioxidant assays such as DPPH, *β*-carotene linoleic acid, and CUPRAC methods, when they are compared to synthetic antioxidant compounds (BHA and BHT). Hence, This study supports that *T. chamaedrys* subsp. *chamaedrys,* used in tea, food, pharmaceutical and cosmetic industry, are a source of natural antioxidant.

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