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(RESEARCH ARTICLE)



Antioxidant activities of aqueous extracts from two *Thymus* species growing in Turkey

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Abstract

In this study, antioxidant activities of aqueous extracts from of *Thymus longicaulis* subsp. *chaubardii* (Rchb.f.) Jalas and *T. longicaulis* subsp. *longicaulis* C. presl were investigated. Infusion and decoction methods were used to prepare aqueous extracts. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, β -carotene linoleic acid and CUPRAC methods were used to determined antioxidant activities. Aqueous extracts of species exhibited good antioxidant activity. Especially, higher antioxidant capacity was found in decoction extracts compared to infusion extracts for all methods.

Keywords: *T. longicaulis* subsp. *chaubardii*; *T. longicaulis* subsp. *longicaulis*; Antioxidant activity; Decoction; Infusion

1. Introduction

Lamiaceae (Labiatae) family has 300–400 endemic species which widely distributed worldwide in particular in the region of Mediterranean. *Thymus* species are a large genus belonging to the Lamiaceae family. Turkey has 39 species with 64 taxa. Among them 27 taxa are endemic [1-4]. *Thymus* species have uses in the pharmaceutical and food industries due to their antioxidant, antimicrobial, antibacterial, cytotoxic, antifungal, insecticidal and other biological activities of their essential oils. The essential oils were found to be rich in phenolic compounds, especially carvacrol [5-8]. These species are native to region of Marmara, Aegean and Black sea in Turkey and used as traditional medicine because of their biological properties. In Turkey, species of the genus *Thymus* L. is called as "Kekik".

Thymus species are known as source of phenolic compounds which have strong antioxidant activities [9]. Antioxidant compounds are polyphenolic structure and present as of almost all plants, fruits, vegetables, microorganisms, fungi an animal tissues. Antioxidants neutralize active oxygen formation or keeping the active oxygen which can damage the body. Phenolic compounds act as antioxidants and inhibition various diseases such as cancer, cardiovascular and neurodegenerative [10, 11]. It is known that the different antioxidant compounds occur in plants. Natural antioxidants can occur in leaves, stem and seeds of plants [12-14].

There are few reports about chemical compounds and biological activities of various solvent extracts of *T. zygoides* Griseb [15], *T. longicaulis* subsp. *chaubardii* [16], *T. longicaulis* subsp. *chaubardii* var. *chaubardii* [17], *T. longicaulis* C. Presl. subsp. *longicaulis* var. *longicaulis* and *T. longicaulis* C. Presl. subsp. *longicaulis* var. *subisophyllus* [5]. *Thymus* species have rich in essential oil content, dominated usually by thymol [18-21]. Azaz *et al.* [18] reported that thymol was the main component in the oils of *T. longicaulis* subsp. *chaubardii* var. *chaubardii* and *T. zygoides* var. *lycaonicus*. Sarikurkcu *et al.* [19] reported that γ -terpinene, thymol and *p*-cymene were identified as the predominant compounds

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for *T. longicaulis* C. Presl subsp. *longicaulis* var. *longicaulis*. Also, Baser and Koyuncu [21] reported the main components of both varieties of *T. longicaulis* C. Presl. subsp. *chaubardii* was thymol.

Biological activities of diverse extracts and essential oils of *Thymus* species have previously been reported. However, to our best knowledge, there are no reports about the antioxidant capacity of aqueous extracts of *T. longicaulis* subsp. *chaubardii* (Rchb.f.) Jalas and *T. longicaulis* subsp. *longicaulis* C.presl (Figure 1).

In this study, we wish to report the antioxidant activities of aqueous extracts of *T. longicaulis* subsp. *chaubardii* (Rchb.f.) Jalas and *T. longicaulis* subsp. *longicaulis* C.presl. Antioxidant tests performed by three procedure systems.

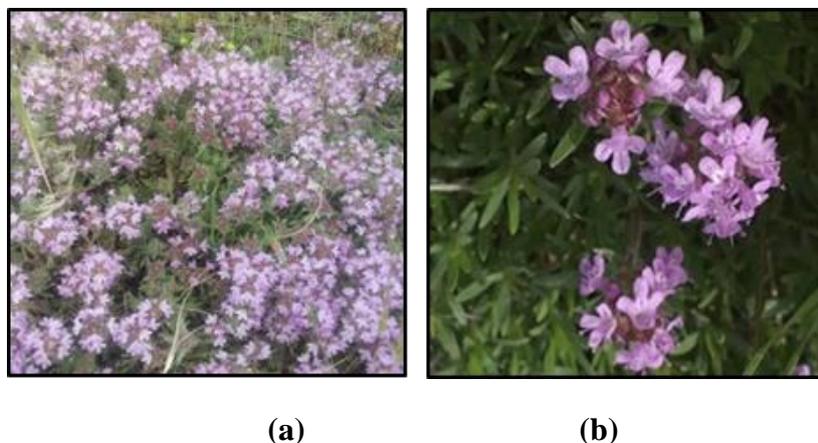


Figure 1 *T. longicaulis* subsp. *chaubardii* (a) and *T. longicaulis* subsp. *longicaulis* (b)

2. Material and methods

2.1. Plant material

Localities, altitude and collector numbers of the *Thymus* species are given in Table 1. The species were identified by Prof. Dr. Selami Selvi at Balikesir University. Voucher specimens were deposited at the Herbarium of Altinoluk Vocational School, Balikesir University, Balikesir, Turkey.

Table 1 List of the *Thymus* species.

Code	Collector Number	Species	Locality	Altitude (m)	Coordinates	Year
TC	SV 1154	<i>Thymus longicaulis</i> subsp. <i>chaubardii</i>	Balikesir: Karanlıkdere road edges and slopes	1220	N 39 19 521 E 28 49 145	2016
TL	SV 1150	<i>Thymus longicaulis</i> subsp. <i>longicaulis</i>	Balikesir: Bigadiç, Okçular village, road edges and slopes	500	N 39 19 22 E 28 10 46	2016

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.

Aqueous extracts were kept at -20 °C until they were used for antioxidant activity studies.

2.3. Antioxidant activity

2.3.1. β -carotene-linoleic acid

The β -carotene-linoleic acid method was used analyzed antioxidant activity [9, 22-24]. β -carotene (0.5 mg) in 1 mL of chloroform was added to 25 μ L of linoleic acid, and 200 mg of Tween 40 emulsifier mixture. After evaporation of chloroform under vacuum, 100 mL of distilled water saturated with oxygen, was through vigorous shaking. A mixture of four thousand microlitres was transferred into different test tubes containing different concentrations of the sample (10, 25, 50 and 100 μ g/mL). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as standard compounds. IC₅₀ values of all samples were estimated.

2.3.2. DPPH

The free radical scavenging activity of the extracts was determined by the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay [9, 22-25]. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 160 μ L of this solution was added to 40 μ L of sample solutions in methanol at different concentrations (10, 25, 50 and 100 μ g/mL). These tubes were left in the dark for 30 min. The measurements were made at 517 nm. BHA and BHT were used as standard compounds. The potentials of samples on DPPH were determined and compared to the standards. IC₅₀ values of all samples were calculated. The reduction in absorbance shows the DPPH free radical scavenging of samples capability.

2.3.3. CUPRAC

The reducing capacities of extracts were evaluated using CUPRAC method [24, 26, 27]. Briefly, 1 mM DMF, 10 mM CuCl₂, 7,5 mM Neocuproine, 1 M NH₄CH₃COO (pH 7.0) solution, and distilled water were mixed in volume ratio 1:1:1:0,6. After 180 μ L of the mixture was dispersed into the wells, 25 μ L diluted compounds (dilution ratio 1:20) in EtOH. The samples were kept for 30 min at 25 °C. The absorbance was measured at 450 nm against a reagent blank. Ethanol was used as a negative control. Curcumin was used as a positive control.

2.4. Statistical analysis

Antioxidant activity results evaluated by ANOVA test. (GraphPad, Software 8.3.0). P < 0.05 was considered to be significant.

3. Results and discussion

In the DPPH and β -carotene linoleic acid methods, the activities were identified at 10, 25, 50 and 100 μ g/mL concentrations. BHA and BHT were used as standard compounds. The results are given as 50% inhibition concentrations (IC₅₀) in Table 2. Also, Inhibition % of DPPH and β -carotene linoleic acid methods are given in Figure 2.

DPPH method was used to evaluate the free radical scavenging efficacy of decoction and infusion of these species. The decoction of these species have good antioxidant activity for all tested methods. As seen in Table 2, among the studied species, decoction of *T. longicaulis* subsp. *longicaulis* determined a remarkable activity for β -carotene and DPPH assays, contrasted to BHA and BHT. Especially, *T. longicaulis* subsp. *longicaulis* decoction showed a salient DPPH activity. IC₅₀ value for the radical scavenging activity of *T. longicaulis* subsp. *longicaulis* decoction was found to be 9.51 μ g/mL. Also, free radical scavenging activity of *T. longicaulis* subsp. *longicaulis* decoction was compared to those of BHA and BHT. In addition, IC₅₀ values for BHA and BHT were found to be 13.40 μ g/mL and 12.84 μ g/mL, respectively. These results showed that the free radical scavenging effect of *T. longicaulis* subsp. *longicaulis* decoction was higher than both of BHA and BHT. Lower IC₅₀ value exhibited higher radical scavenging effectiveness. IC₅₀ values for DPPH free radical scavenging activities for infusion of *T. longicaulis* subsp. *longicaulis* and *T. longicaulis* subsp. *chaubardii* and decoction of *T. longicaulis* subsp. *chaubardii* were found to be 12.01 μ g/mL, 17.98 μ g/mL and 14.09 μ g/mL, respectively.

Decoction of *T. longicaulis* subsp. *longicaulis* showed great lipid peroxidation inhibition in the β -carotene-linoleic acid system (IC₅₀, 12.13 μ g/mL). IC₅₀ values of BHA and BHT were found to be 12.23 μ g/mL; 8.57 μ g/mL and 11.89 μ g/mL; 10.04 μ g/mL, respectively. None of the tested extracts exhibited high antioxidant activity than standards for the β -carotene-linoleic acid method. The lower inhibition value was found in infusion of *T. longicaulis* subsp. *chaubardii*

(IC₅₀ 28.85 µg/mL). To bring about as a result, the weak activity estimated in these species is connect to a low amount of phenolics [9, 19, 22, 24, 28].

Cu²⁺ reducing ability is commonly used to identified the reducing powers of curcumin and decoction and infusion of *Thymus* species (Figure 3). In CUPRAC method, same as other methods, decoction of *Thymus* species have better activity than the infusion as well as curcumin, which was used as a standard compound. Cu²⁺ reducing powers of the decoction of *Thymus* species decreased as follows: *T. longicaulis* subsp. *chaubardii* (1.42 mmol TR g⁻¹) and *T. longicaulis* subsp. *longicaulis* (1.40 mmol TR g⁻¹).

As far as our literature survey could as certain, antioxidant activity of *T. longicaulis* subsp. *longicaulis* and *T. longicaulis* subsp. *chaubardii* decoctions and infusions have not previously been reported. In the literature, biological activities of various extracts of *Thymus* species have been reported. Kaska *et al.* [15] reported the phenolic content, antioxidant capacities, anthelmintic and cytotoxic activities of ethanol, methanol, acetone and water extracts of *T. zygioides* Griseb. On the other hand, antialzheimer, antidiabetic, antioxidant and antiobesity activities of ethanol extracts of *T. zygioides* var. *lycaonius* has previously been reported [29]. Also, antioxidant activity of hexane, ethyl acetate, methanol and water extracts of *T. longicaulis* subsp. *longicaulis* var. *longicaulis* were investigated [19]. Öztürk [5] reported total phenolic content and antioxidant activities of infusions, methanol and ethyl acetate extracts of *T. longicaulis* subsp. *longicaulis* var. *longicaulis* and *T. longicaulis* subsp. *longicaulis* var. *subisophyllus*. Additionally, Galasso *et al.* [30] reported the antioxidant, anti-inflammatory activities and phenolic compounds of *T. longicaulis* C. Presl hydroalcoholic extracts. According to this report, rosmarinic acid and methylapigenin rich extract, exhibited a strong antioxidant and anti-inflammatory effectiveness.

Table 2 Antioxidant activity of the *Thymus* species, BHA and BHT (IC₅₀ µg/mL)

		TC*	TL*	BHA**	BHT**
DPPH	Infusion	17.98±3.01	12.01±0.52	10.56±0.63	11.57±4.02
	Decoction	14.09±2.05	9.51±1.24	13.40±1.05	12.84±1.62
β-carotene linoleic acid	Infusion	28.85±10.18	22.12±3.53	12.23±1.44	11.89±1.38
	Decoction	14.90±3.16	12.13±0.96	8.57±0.58	10.04±0.78

IC₅₀ values are mean ± SD (n=3).

*TC: *T. longicaulis* subsp. *chaubardii*, *TL: *T. longicaulis* subsp. *longicaulis*

**Standard compounds.

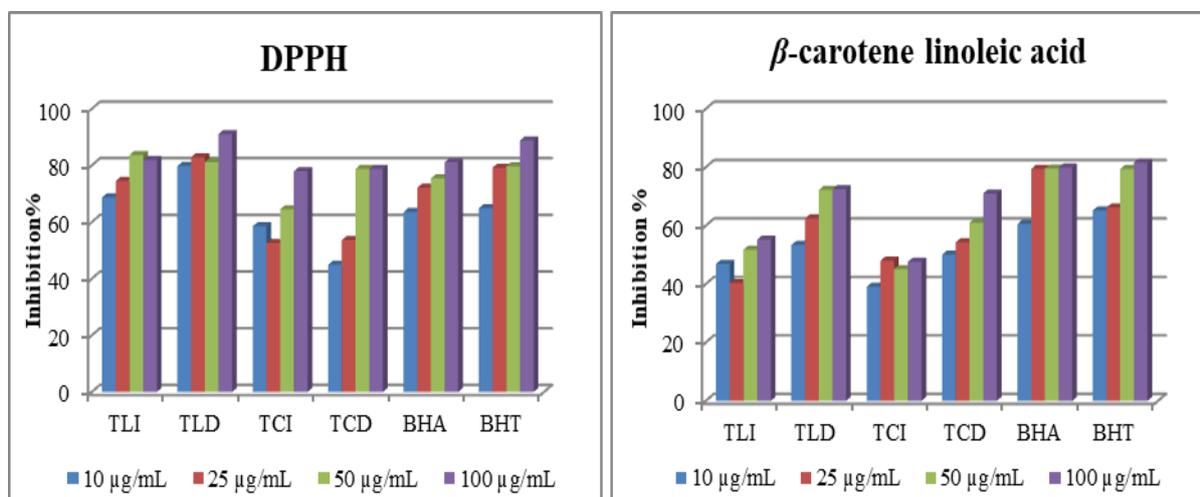


Figure 2 Inhibition (%) of DPPH and lipid peroxidation assays of the *T. longicaulis* subsp. *longicaulis* (decoction, TLD; infusion, TLI), *T. longicaulis* subsp. *chaubardii* (decoction, TCD; infusion, TCI), BHA and BHT.

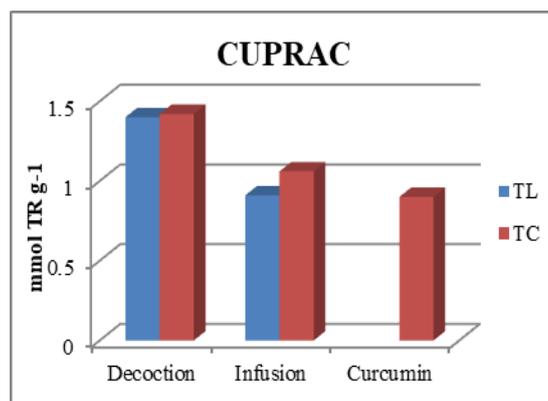


Figure 3 CUPRAC assay of the *T. longicaulis* subsp. *chaubardii* (TC), *T. longicaulis* subsp. *longicaulis* (TL) and curcumin.

4. Conclusion

In conclusion, we examined and reported the antioxidant activities of aqueous extracts of *T. longicaulis* subsp. *chaubardii* and *T. longicaulis* subsp. *longicaulis* in Turkey. Our results indicate clearly that aqueous extracts show strong antioxidant activity. Therefore decoction and infusion samples of these plants can be considered as a source of natural antioxidants. The results compared with the literature, the content of phenolic of decoction and infusion of the samples are an important factor for the antioxidant capacities. Antioxidant reduces physiological stress in organs and cells and prevents the formation and development of many diseases so it is important for nutrition. It was concluded that the decoction and infusion of these *Thymus* species can be used as an effective natural antioxidant in food and pharmaceutical industries.

Compliance with ethical standards

Acknowledgments

This study was presented at the “EurasianBioChem 2019” congress under the name “Antioxidant activities of decoction and infusion of *Thymus longicaulis* subsp. *chaubardii* and *T. longicaulis* subsp. *longicaulis*” and abstract published.

Disclosure of conflict of interest

There was no conflict of interest in this study.

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