Phenolic composition and antioxidant activity of thedifferent extracts from *Thymus longicaulis C* Presl. subsp. *longicaulis* var. *longicaulis* and *T. longicaulis* C. Presl. subsp. *longicaulis* var. *subisophyllus* growing in Turkey

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Abstract: The objectives of the present study were to investigate the phenolic composition and *in vitro* antioxidant capacities of the infusions and different extracts of two *Thymus* taxa: *Thymus longicaulis* C. Presl. subsp. *longicaulis* var. *longicaulis* and *Thymus longicaulis* C. Presl. subsp. *longicaulis* var. *subisophyllus* (Lamiaceae). The quantitative estimation of total flavonoid, flavonoland phenolic contents of the studied extracts were performed by spectrophotometrical method; the aluminum chloride the aluminum chloride+Na acetate and Folin-Ciocalteau methods, respectively. The determination of phenolic acids in the studied species was achieved by using a modified reverse phase-High Pressure Liquid Chromatography method adopting an internal standard. Antioxidant activities of the extracts were determined by three test systems namely, radical scavenging on DPPH, β-carotene bleaching and Rancimat methods. The results were compared to those of BHT as synthetic antioxidant. Ethyl acetate extracts were found to be rich as a source of phenolics. In addition, the main phenolic acid of the extracts identified by HPLC-DAD was rosmarinic acid. Also, the infusions containing especially water-soluble compounds were observed to exhibit lower antioxidant capacities than those of the methanol and ethyl acetate extracts. Our results indicated that ethyl acetate fraction of studied *Thymus* taxa can be used as antioxidant in food and medicinal preparations. In addition, the present study revealed that the infusions of these plants exhibit lower activity in contrast to general believes in which aqueous extracts, as in the case of herbal teas, have high antioxidant activity.

Keywords: Lamiaceae, *Thymus longicaulis* subsp. *longicaulis* var. *longicaulis, T. longicaulis* subsp. *longicaulis* var. *subisophyllus,* antioxidant activity, phenolic compounds.

INTRODUCTION

Plant polyphenols, a specific group of secondary metabolites play an important role in protecting organisms against harmful effects of reactive oxygen species. That is, they have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated chronic diseases such as cardio and cerebrovascular ones, neurodegenerative and inflammatory diseases, Alzheimer's, diabetes or cancer. Thus recently, there is a global trend in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury and use of them as antioxidants in foods and drugs (Saxena, *et al,* 2012).

The family Lamiaceae has got 236 genera and 7172 species in the World (Harley *et al,* 2004). The genus *Thymus* belonging to the family Lamiaceae is the most important genera in point of the number of species included. This polymorphic genus is represented by 39 species and 64 taxa in Turkey flora and the ratio of endemism is 47% (Başer, 2002). *Thymus* species are known as ''kekik'' in Turkish and commonly used for spice, herbal tea and medicinal plant in Turkey (Baytop, 1999). The members of the family are very important due to their medicinal and aromatic properties leading to the production of herbal products and food supplements (Lawrence and Tucker, 2002). In addition, the use of the species in the form of herbal teas is a common habit and the most popular form of herb consumption in Turkey, as all the world (Baytop 1999).For this purpose, teas prepared from different parts of plants have been long used as complementary medicinal cures such as digestive problems, cold infections, diuretic, sudorific, sedative, etc. There are no nutritional values of them, but they can be an important antioxidant source (Speisky *et al,* 2006). A number of studies has been performed on Lamiaceae species, e.g. sage, oregone, thyme, mountain tea, which ensued in an improvement of natural antioxidant formulations for medicinal and cosmetic applications (Stahl-Biskup, 2002).

The chemistry of *Thymus* genus is fairly well known the two main classes of secondary metabolites, as the essential oils and polyphenols- especially flavonoids. Both essential oils and flavonoids are mainly responsible for the pharmacological activities of *Thymus* plants as carminative, spasmolytic, diuretic, antioxidant, antibacterial, urinary disinfectant and vermifuge (Stahl-

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Biskup, 2002; Villa, 2002). In addition, essential oils and extracts of many *Thymus* species are widely used in cosmetic and pharmaceutical industry and for flavouring and preservation of food products (Lawrence and Tucker, 2002). *T. longicaulis* C. Presl is an aromatic, evergreen, perennial and small sub shrub native to the Mediterranean, with dark green leaves and bright pink flowers (Davis 1982; Davis 1989). A great number of subspecies, varieties and forms were described within this polymorphic species; *T. longicaulis* subsp. *longicaulis* var. *longicaulis (TLL)*, *T. longicaulis* subsp. *longicaulis* var. *subisophyllus* (TLS) and *T. longicaulis* subsp. *chaubardii var. chaubardii* (Davis, 1989). Main components of the species are terpenes in essential oil and flavonoids (Chorianopoulos, *et al.,* 2004). Their pharmacological effects include antimicrobial, antibacterial, antiseptic, expectorant, antioxidant, and spasmolytic activities (Baytop 1999; Chorianopoulos *et al.,* 2004; Gortzi 2006).

Being traditional medicinal plants, they have an ethnobotanical usage. To the best of our knowledge, essential oil compositions of *T. longicaulis*, *T. longicaulis* subsp. *longicaulis* var. *subisophyllus* and *T. longicaulis* subsp. *longicaulis* var. *longicaulis* have been studied in previous studies (Başer *et al.,* 1992; Başer *et al.,* 1993; Başer and Koyuncu 1994; Grujic-Jovanovic *et al*., 2009; Sarikurkcu *et al*., 2010; Azaz, 2004). Different types of extracts (such as, with methanol, water, etc.) from *T. longicaulis* subsp. *longicaulis* var. *longicaulis* have been found to show the strongest antioxidant activity (Sarikurkcu *et al*., 2010). Although total phenolics and flavonoids of this plant have also been reported in the same study, there are no precise analyses for the chemical composition of phenolic acids in the plant. As a further step, the present study aimed to analyze compounds in the fraction of phenolics of the *T. longicaulis* subsp. *longicaulis* var. *longicaulis,* which seem to be related to antioxidant activity. In addition, the antioxidant activity of *T. longicaulis* subsp. *longicaulis* var. *subisophyllus* has not been reported previously. Furthermore, almost none of studies cited above seem to be comparative ones in terms of their antioxidant potency.

To find out new antioxidant resources, the present study was designed to evaluate the phenolic contents and the *in vitro* antioxidant potentials of the infusions, methanol and ethyl acetate extracts obtained from *T. longicaulis* subsp. *longicaulis* var. *longicaulis* and *T. longicaulis* subsp. *longicaulis* var. *subisophyllus.*

MATERIALS AND METHODS

Chemicals

The chemicals, gallic acid (GA), protocathechuic acid (protoCA), *p*-hydroxy benzoic acid (*p*-hydBA), caffeic acid (CA), chlorogenic acid (ChA), syringic acid (SA), *p*-coumaric acid (*p*-COU), ferulic acid (FA), *o*-coumaric acid (*o*-COU), rosmarinic acid (RA), *trans*-cinnamic acid (*tr*-CIN), propyl paraben,rutin and Folin-Ciocalteu phenol reagent were provided by Sigma Co. (St. Louis, MO), 2,2-diphenyl-1-picryhydrazyl radical (DPPH·), Aluminum chloride were from Aldrich Chemical Co. (Milwaukee, WI), while methanol, ethyl acetate, acetic and formic acids were purchased Merck GmbH (Darmstadt, Germany). They were all in analytical grade having high purity more than 99.9%. Crude olive oil was kindly provided by Üstün Co. (Balıkesir, Turkey).

Plant materials

T. longicaulis subsp. *longicaulis* var. *longicaulis* (TLL) and *T. longicaulis* subsp. *longicaulis* var. *subisophyllus* (TLS) herba were collected from Osmaneli (Bilecik), Avdan-Çiftlikköy, 573m (17.04.2010) and Osmaneli (Bilecik), Çiftlikköy-Berekete Village, 383m (17.04.2010), respectively and identified by Dr. Onur Koyuncu, according to Flora of Turkey and the East Aegean Islands (Davis, 1982; Davis, 1988) Voucher specimens of collected plant species were deposited at the Herbarium of the Eskişehir Osmangazi University, Faculty of Science (OUFE-TLL 14710, OUFE-TLS 12911).

Extraction

Extraction procedures were applied as described elsewhere in details (Öztürk *et al.,* 2009). Air-dried herbs were crushed and the apolar materials were extracted in Soxhlet extractor for 8 h with petroleum ether. Fat-free material was extracted with methanol (70%) at 40°C, 30 min (x4). The extracts were concentrated using rotary evaporator and aqueous solutions were lyophilized (ME).

Then, the aqueous phase was extracted with ethyl acetate at 25^{o}C. The ethyl acetate extract was concentrated using rotary evaporator (EA). For preparing tea infusions from studied species, 2g of the air-dried herb was infused into 100mL of boiling distilled water for 30 min, filtered and then lyophilized. The extracts were weighed to calculate the yields of the extractions.

Determination of total phenolic content

Total phenolic content was determined using a Folin-Ciocalteau colorimetric method and expressing the results as gallic acid equivalents (GAE) (Singleton & Rossi, 1965). This method is considered to be a qualitative procedure, as it is not completely specific for phenolic compounds and not all phenolic compounds exhibit the same level of activity in the assay.

Determination of total flavonoid content

Total flavonoid content was measured by the aluminum chloride colorimetric assay, using rutin as a standard (Miliauskas *et al.,* 2004). The studied extracts were dissolved in methanol (10 g/l). One mL of plant extract in methanol and one mL of aluminum chloride in ethanol

(20g/L) were dissolved in ethanol to 25mL. To 0.5mL of each sample or standard rutin solution, 0.5mL aluminum chloride solution was added and the absorption at 415 nm was read after 60 min. Blank samples were prepared from 1mL plant extract and 1 drop acetic acid, and diluted to 25mL. The flavonoid contents were found by comparing the absorbance values of the extracts with those of the standard rutin solutions, which were prepared as a stock solution of 0.05g rutin. All determinations were repeated in triplicate.

Determination of total flavonol content

Flavonols were determined as a species of rutin, as proposed previously (Miliauskas *et al.,* 2004). The calibration curve of rutin was constructed by mixing 2mL of rutin solution having concentrations in the range of 0.5-0.017mg/mL with 2mL (20g/L) aluminum chloride and 6 mL (50 g/l) sodium acetate. The absorption at 440nm was read after 2.5 h at room temperature. The same procedure was applied to plant extracts similar to rutin solution. The experiments were always triplicated.

Separation and analysis of phenolic acids by HPLC

An HPLC system consisting of a model of 600 E HPLC pump, 717 plus autosampler, 996 photodiode array detector (PAD), and data processor (Millennium 32) was used (Waters Corp., Massachusetts, USA). Ultrapure water (18.2 µS/cm) from a Millipore (Molsheim, France) water purification system and an octadecylsilane (ODS, C18) Ultrasphere column from Teknokroma (Barcelona, Spain) (100 x 4.6 mm inner diameter, particle size of 3 µm) were utilized in the HPLC analysis. Ultrapure deionized water was purified by a Millipore Synergy Water Purification System (Rotterdam, Netherland) to a specific resistance of 18mΩcm. Chromatographic analysis of the extracts was carried out by a gradient elution (A: methanol: water: formic acid (10:88:2 *v/v/v*); B: methanol: water: formic acid (90:8:2 *v/v/v*) as they were reported elsewhere (Öztürk *et al.,* 2007; Öztürk *et al.*, 2011). The separation was performed by using a linear gradient program. The flow-rate was 1 $mL.min^{-1}$ and the injection volume was 10mL. Signals were detected at 280 nm where phenolic acids absorb the monochromatic light maximum.

The relevant extracts were dissolved in a mixture of methanol and water (1:1; *v/v*) and injected through the column of HPLC. Internal standard (IS) technique was applied for the analysis of phenolic acids to increase the repeatability and propylparaben was employed for this purpose.

Radical scavenging activity

The measurement of the radical scavenging activity on DPPH was carried out according to the method described by Sanchez-Moreno *et al*. (1998). Plant extract (0.1-0.4 mL) was added to 3 mL of a 0.03% methanol solution of DPPH. The reaction mixture was left at ambient temperature for 30 min in dark; the changes in color (from violet to yellow) were read absorbance at 517 nm using a UV-VIS spectrophotometer. The results were compared to those of a synthetic antioxidant (BHT). The percentage inhibition was calculated from the following equation:

Activity [% of DPPH reduction] = $[(A_0-A) / A_0] \times 100$,

where A_0– absorbance of DPPH solution with methanol, A - absorbance of a DPPH solution with a tested extract solution (test) or BHT solution. Experiments were run in triplicate and the results were given to as average values with S.D. (standard deviation).

β-caroten bleaching method

The oxidative losses of *β*-carotene in a β-carotene/linoleic acid emulsion were used to assess the antioxidant ability of the studied *Thymus* extracts and infusions (Miura *et al.,* 2002). β-carotene (10mg) was dissolved in 3mL of chloroform. The β-carotene solution, dissolved in chloroform, was added to 40mg linoleic acid and 400 mg Tween 80. Chloroform was concentrated using a rotary evaporator at 50°C. 100mL of oxygenated distilled water was slowly added to the semisolid residue with vigorous agitation to form an emulsion. The extracts dissolved in methanol (0.2mL) were mixed with 3mL β-carotene emulsion and the absorbance was monitored spectrophotometrically at 470 nm. The tubes were placed in a incubator at 50°C and measurements were conducted at 15 min interval up to 180 min. The same procedure was repeated with the positive control BHT. Antioxidant activities of the extracts were determined by calculating the percentage inhibition (I%). All tests were repeated three times.

Rancimat method

Antioxidant activities of the studied extracts and infusions were also measured by the Rancimat method (A 743 Rancimat apparatus, Metrohm AG, SW) at the concentrations of 0.02-1% (Bozan *et al.,* 2002). The method is based on the conductometric determination of volatile degradation products and features automatic plotting of the conductivity against time. The evaluation is performed graphically after completion of the experiment. A flow of air (20 L/h) was bubbled through the oil heated at 110°C, and the volatile compounds were collected in cold water, increasing the water conductivity. The conductivity was monitored continuously until a sudden rise signified the end of the induction period. Each sample was dispersed in 3 g of olive oil rich in linoleic acid at the concentration of 1%. Olive oil without any antioxidant was used the positive control. The results were compared to those of a synthetic antioxidant (BHT). Induction index (II) was calculated by the following equation:

(II)=Induction time of sample/ Induction time of control.

The tests were applied in triplicate. As it was observed from the formula, a higher induction index indicates higher antioxidant activity (Esquivel *et al*. 1999).

STATISTICAL ANALYSIS

All data were the average of triplicate analyses. Mean values, standard deviations (SD), medians, and both minimum and maximum contents of all achieved results have also been determined. Correlation analysis of phenolic contents were carried out using the correlation and regression program in the Microsoft EXCEL program.

RESULTS

Extraction Yields and phenolic contents

Solvent extraction is the most common method used to extraction of active ingredients from plant materials. The extraction yield depends on solvent, time and temperature of extraction as well as on the active compounds of the sample. The aerial parts of the studied *Thymus* taxa was extracted with methanol and ethyl acetate. The extraction yields of *Thymus* samples are given in table 1. Infusions of the studied TLL and TLS showed a higher yield was obtained compared to the other extracts. The contents of total phenols, flavonoids and flavonols in the extracts were determined as stated in the experimental section. Calibration equation for total phenol determination was found as y=0.0593x + 0.1123 (r^2=0.9921). Otherwise, it was calculated as y=0.0075x + 0.0487 (r^2=0.9891) for total flavonoid content and as y=0.0044x + 0.0345 (r^2 = 0.9864) for total flavonol content. The results of total phenols, flavonoids and flavonols of the extracts were given in table 1.

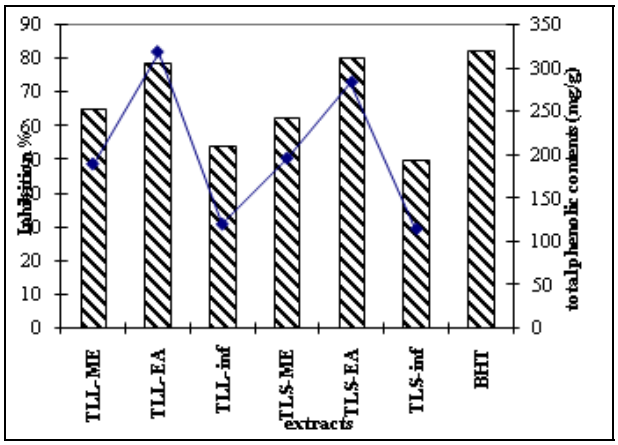

Fig. 1: Comparisons of Radical Scavenging Activities (Inhibition %) and Total Phenolic Contents of studied *Thymus e*xtracts

Extracts and infusions investigated in the present study appeared to be richer in total phenol, where as their total flavonoid and flavonol contents were quite low by to the total phenol amounts. According to the data presented in table 1, as the ethyl acetate extracts of studied *Thymus* species contained the highest amount of total phenol content, infusions contain the lowest.

HPLC analysis

In the *Thymus* taxa studied, eleven phenolic acids (gallic, protocathechuic, p-hydroxy benzoic, vanillic, caffeic, chlorogenic, syringic, *p*-coumaric, ferulic, *o*-coumaric, rosmarinic acids) were determined by an HPLC gradient system using modified methods, which was described elsewhere (Öztürk *et al.* 2007;Öztürk *et al.* 2011). All of the phenolic acids were resolved entirely from each other. It may be seen that no additional clean-up step to purify the extracts is necessary and all phenolic acids could be quantified. The integrated peak areas and their retention times were computed to obtain the rate of peak normalization of the relevant phenolic acids, and their amounts were calculated in the related extracts via their calibration curves. To increase the repeatability, the IS technique was applied to the analysis to propylparaben was the suitable IS. The quantitative data are given in table 2.

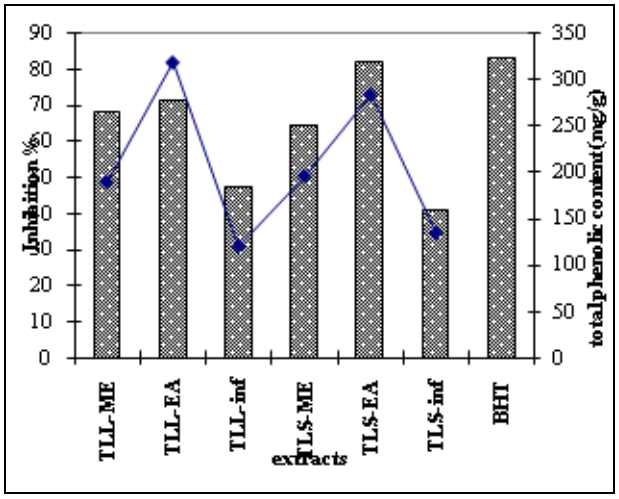

Fig. 2: Comparisons of inhibition % Data in the β -carotene-L in oleic acid bleaching assay and total Phenolic contents of studied *Thymus* extracts.

TLL-EA and TLS-EA extracts were found to be the richest for phenolic acids as determined by both UV spectrophotometry and HPLC. The results showed that rosmarinic acid (RA) is one of the major phenolic compounds in all the extracts and the concentration varied from one to another in the extracts and infusions. In addition, proto CA, CA, ChA, SA, FA, *o*- and *p*-COU acids were detected in nearly all extracts.

Literature data show the presence of different phenolic compounds such as rosmarinic acid, caffeic acid, ferulic acid, carnosic acid, apigenin and luteolin in the *Thymus* species (Vila 2004; Jordan *et al.,* 2009; Zheng and Wang, 2001; Wang *et al.* 2004; Boros *et al.,* 2010). The presence of these compounds in the infusions and the extracts obtained from the investigated *Thymus* taxa may also be the main cause of its high radical-scavenging activity and high total phenolic contents.

Table 1: Extract yields, contents of total phenols, flavonoids and flavonols in the studied *Thymus* extracts (TLL and TLS)

Extracts	Extraction Yields (%)[1]	Total phenolic compounds (mg/g±SD) (in GAE)[2]	Total flavonoids (mg/g±SD) (in RE)[3]	Total flavonols (mg/g±SD) (in RE)[4]
TLL-ME[6]	11.01	188.98±1.35[5]	21.43±0.94	4.12±0.12
TLL-EA[7]	3.4	318.43±2.65	59.42±0.63	9.00±0.03
TLL-inf[8]	10.89	119.43± 2.65	11.43±0.88	1.54±0.11
TLS-ME	17.38	195.63±1.05	28.13±0.87	3.92±0.22
TLS-EA	2.59	283.60±2.84	32.32±1.02	6.67±0.19
TLS-inf	17.33	113.70±1.66	7.63±0.61	0.88±0.09

[1]%, w/w on dry weight basis; [2]Data expressed in mg equivalent of gallic acid to 1 g of extract; [3,4]Data expressed in mg equivalent of rutin to 1g of extract; [5]The results are represented as means ± standard deviation of three measurements.[6] ME; Methanol extract, [7]EA; Ethyl acetate extract, [8]inf; Infusion

Table 2: The contents of phenolic acids (mg/100g extract) in the studied *Thymus* extracts (TLL and TLS)

Extracts	GA	Proto CA	*p*-OHBA	VA	CA	ChA	SA	*p*-COU	FA	*o*-COU	RA
TLL-ME[1]	-	2.13	1.93	5.05	0.84	5.99	0.81	1.68	18.1	7.60	515.0
TLL-EA[2]	-	3.21	1.75	7.02	0.86	8.13	0.89	1.81	24.03	10.44	698.0
TLL-inf[3]	0.26	1.48	2.07	5.31	0.56	7.18	0.72	1.73	6.84	2.85	390.0
TLS-ME	0.50	0.98	-	-	1.26	3.35	0.52	1.53	15.4	11.2	491.0
TLS-EA	0.45	1.73	-	-	1.85	4.38	0.94	1.69	19.86	13.98	652.0
TLS-inf	1.22	1.50	-	1.54	0.87	2.48	1.22	1.33	8.56	5.67	226.0

[1] ME; Methanol extract, [2]EA; Ethylacetate extract, [3]inf; Infusion

Table 3: Radical scavenging activity (Inhibition %), β-carotene linoleic acid bleaching assay (Inhibition %) and Rancimat method (Induction Index) of the extracts of *Thymus* taxa (TLL and TLS)

Extracts	Radical Scavenging Activity			β-carotene linoleic acid bleaching assay	Rancimat Method	
	Inhibition %			Inhibition %	Induction Index	
	$9.6x10^{-4}$ g/ml	$1.8x10^{-3}$ mg/ml	$3.6x10^{-3}$ mg/ml		0.02%	1%
TLL-ME[2]	20.32±1.23[1]	27.01±1.54	65.28±2.01	67.65±4.45	1.12±0.09	1.89±0.35
TLL-EA[3]	25.62±1.54	31.21±1.76	78.84±2.34	71.32±3.21	1.21±0.43	1.99±0.53
TLL-inf[4]	16.35±1.74	18.60±1.36	54.37±1.94	46.92±2.56	1.01±0.04	1.56±0.59
TLS-ME	23.77±1.82	27.19±1.87	62.63±2.43	64.37±3.78	1.09±0.05	1.96±0.21
TLS-EA	21.75±1.18	36.47 ±1.53	80.20±1.51	81.54±3.78	1.19±0.08	2.11±0.76
TLS-inf	15.39±1.11	18.27±1.49	50.23±2.20	40.63±2.36	1.03±0.07	1.49±0.31
BHT	27.30±1.68	40.46±1.57	82.40±2.98	82.89±3.56	1.26±0.13	2.24±0.54

[1]Results are expressed as mean ± standard deviation (n = 3), [2]ME; Methanol extract, [3]EA; Ethylacetate extract, [4]inf; Infusion.

Determination of the antioxidant activity

Natural antioxidant compounds present in plant materials are responsible for inhibiting or preventing the unhealthy consequences of oxidative stress, preventing first chain initiation by scavenging initiating radicals, metal chelating, decreasing localized oxygen concentration. It is thus important that for evaluating the antioxidant activity compounds and extracts, using several analytical methods and different substrates (Mensor *et al.,* 2001). The methods selected here are commonly used for the determination of antioxidant activities of plant extracts.

Radical scavenging activity

The $DPPH^{\bullet}$ is a stable radical and gives maximum absorbance at 517 nm. When reduced to the hydrazine derivative by an antioxidant via electron or hydrogen atom transfer reactions, this absorption maximum decreases (Zheng and Wang, 2001). Percent inhibition values estimated for all the extracts are presented in table 3. The higher percent inhibition value means the higher antioxidant activity (Brand-Williams *et al*, 1995).

According to table 3, almost all the extracts showed significantly radical scavenging activity. Highest levels of antiradical activities were by detected the ethyl acetate extracts, which nearly as active as the synthetic antioxidant, BHT, whereas the infusions showed lower activities. The hierarchy of radical scavenging activity of the extracts was in order of: BHT ≅TLS-EA≅TLL-EA> TLS-ME > TLL-ME > TLL-inf> TLS-inf.

The results demonstrated that there is a correlation between higher radical scavenging activity and larger amount of total phenolics in the extracts (fig. 1). This data is supported by previous reports, which showed that phenolic compounds generally correlate with antioxidant capacities measured by DPPH assay (Miura *et al.,* 2002; Exarchou *et al.,* 2002; Esquivel *et al*., 1999). According to Sarıkürkçü *et al.* (2010), although the methanol extracts of TLL exhibit the highest radical scavenging activity, water extracts show significant antiradical activities.

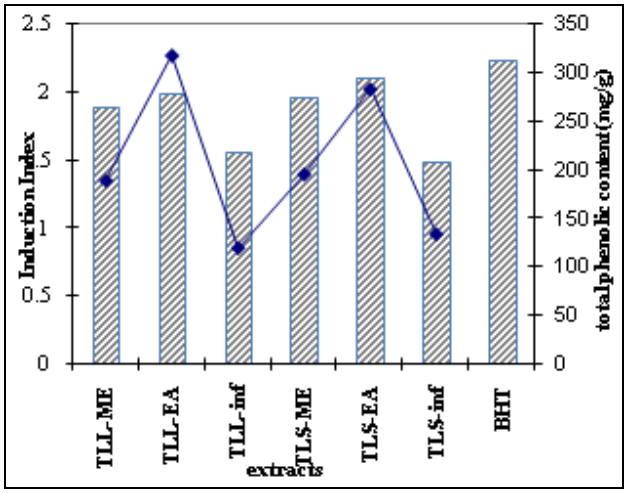

Fig. 3: Comparisons of Induction Index Values in Rancimat Method and Total Phenolic Contents of studied *Thymus* extracts.

The β-carotene bleaching assay

This method is widely used to determine the oxidation of unsaturated fatty acids, in foods and pharmaceuticals. The inhibition percentages of the extracts of studied *Thymus* taxa are given in table 3. As can be seen from the table, the ethyl acetate extracts (TLS-EA and TLL-EA) showed higher inhibition capacities were found to be as active as the positive control, BHT. On the other hand, the other extracts and infusions inhibited the oxidation of linoleic acid at a statistically same degree and they exerted lesser activities than positive control. Hierarchy of the extracts was BHT≅TLS-EA>TLL-EA>TLL-ME>TLS-ME>TLL-inf>TLS-inf .

With β-carotene bleaching method used in a study, essential oil and hexane, ethyl acetate, methanol and water extracts of TLL exhibited different patterns of antioxidant activities and the most active one was the essential oil among them. In this study, the methanol and water extracts showed lesser antioxidant activities than synthetic antioxidants (Sarikurkcu *et al*., 2010).

The high antioxidant activity of ethyl acetate extract tested using β-carotene model could be due to the content and composition of major phenolic compounds (fig. 2).

Rancimat method

The Rancimat method was utilized to monitor the change of electrical conductivity of water. In this method, lipid oxidation gives rise to a formation of volatile secondary oxidation products under elevated temperature and accelerated aeration (Tabart *et al.,* 2009).

As shown in table 3, the extracts obtained from *Thymus* taxa exhibited different effects on retarding olive oil oxidation. The longer induction time indicated the higher antioxidant activity. The highest induction index data were found in ethyl acetate extracts, although none of the extracts was as active as the positive controls, BHT being in turn, BHT ≅TLS-EA≅TLS-EA>TLS-ME>TLL-ME >TLL-inf> TLS-inf. In contrast, infusions of *Thymus taxa* did not inhibit sufficiently the oxidation of olive oil at the studied concentrations. These results and their comparisons are demonstrated in fig. 3.

DISCUSSION

Essential oils and the organic solvent extracts from aromatic plants have been extensively investigated for their antioxidant activity in different systems. However, there is very little information about the antioxidant activities of the teas (infusions) used in traditional medicine, in spite of the fact that they have been consumed as popular beverages in our country.

In this study, antioxidant and radical scavenging capacities of the infusions and some extracts of *Thymus longicaulis* subsp. *longicaulis* var. *longicaulis* (TLL) and *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* (TLS) were evaluated to investigate a relationship between phenolic contents and antioxidant activity. The results obtained from the present study indicated that the ethyl acetate extracts of TLL and TLS have the highest total phenolic content, DPPH scavenging ability and antioxidant activities (in *β*-carotene bleaching assay and Rancimat method) among the extracts. That is, the antioxidant activity of the ethyl acetate extracts were well correlated with the content of their phenolic compounds. In contrast, infusions with water-soluble substances were showed the lowest antioxidant and antiradical activities.

The results of HPLC analysis of the studied extracts showed that rosmarinic acid (RA) dominated in all extracts (table 2). In addition, proto CA, CA, ChA, SA, FA, *o*- and *p*-COU acids were detected in nearly all extracts.

As our literature survey, the antioxidant activities of the essential oil and hexane, ethyl acetate, methanol and water extracts of *T. longicaulis* subsp. *longicaulis* var. *longicaulis* herb originated Muğla, Turkey have been published previously elsewhere. According to Sarıkürkcü *et al*. 2010, *T. Longicaulis* subsp. *longicaulis* var.

Longicaulis exhibited highly strong antioxidant activity profiles in four different test systems namely β-carotene/linoleic acid, DPPH, reducing power and chelating effect. In addition, other studies have shown the presence of different phenolic compounds such as apigenin, luteolin, rosmarinic acid, caffeic acid, ferulic acid, carnosic acid in the *Thymus* species (Vila 2004; Jordan *et al.,* 2009; Zheng and Wang, 2001; Wang *et al*., 2004; Boros *et al.,* 2010).

These results showed for the first time that the extracts of *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus* aerial parts, possesses significant antioxidant activity being related to the presence of polyphenolic compounds.

The ethyl acetate extracts were introduced with highest amount of total phenol, total flavonoid and total flavonol compounds as a good solvent in extracting with highest antioxidant activity and positive correlation existed between antioxidant activity and total phenolic. The potency of these extracts could provide a chemical basis. However, by and large, infusions had lower antioxidant values than methanol and ethyl acetate extracts. Data from present results revealed that *T. longicaulis* subsp. *longicaulis* var. *subisophyllus* and *T. longicaulis* subsp. *longicaulis* var. *longicaulis* act as an antioxidant agent due to its antiradical and antioxidant activity.

CONCLUSION

In the present study, significant antioxidant activities were revealed in the investigated species. The extracts examined here exhibited high antiradical, which was found to be in correlation to the content of mainly polyphenols. In addition, a very strong protective activity of the ethyl acetate extract in lipid per oxidation processes was determined. Results also showed that *T. longicaulis* subsp. *longicaulis* var. *subisophyllus* and *T. longicaulis* subsp. *longicaulis* var. *longicaulis* can be a source of polyphenols confirm their antioxidants activities and emphasize their potential uses as natural preservatives in foods, cosmetics and pharmaceutical preparations. In addition, the present study revealed that the infusions of these plants exhibit lower activity in contrast to general believes in which aqueous extracts, as in the case of herbal teas, have high antioxidant activity. The present results may encourage additional and more detailed studies on the phenolic composition of these plant extracts.

REFERENCES

Azaz AD, Irtem HA, Kurkcuoglu M and Baser KHC (2004). Composition and the *in vitro* anti-microbial activities of the essential oils of some *Thymus* species. *Z Naturforsch C.,* **59**: 75-80.

Başer KHC, Tumen G, Ozek T and Kurkcuoglu M (1992). Composition of the essential oil of *Thymus longicaulis* C. Presl. var. *subisophyllus* (Borbas) Jalas from Turkey. *J. Essent. Oil Res.,* **4**: 311-312.

Başer KHC, Ozek T, Kirimer N and Tumen G (1993). The occurrence of three chemotypes of *Thymus longicaulis* C Presl subsp *longicaulis* in the same population. *J. Essent. Oil Res.,* **5**: 291-295.

Başer KHC and Koyuncu M (1994). Composition of the essential oils of two varieties of *Thymus longicaulis* C Presl subsp *chaubardii* (Boiss. et Heldr. ex Reichb. fil.) Jalas. *J. Essent Oil Res.,* **6**: 207-209.

Başer KHC (2002). Aromatic biodiversity among the flowering plant taxa of Turkey. *Pure Appl. Chem.,* **74**: 527-545.

Baytop T (1999). Therapy with medicinal plants in Turkey past and present, 2nd ed. Nobel Tıp Kitabevi, Istanbul, p.283.

Boros B, Jakabova S, Dörnyei A, Horvath G, Pluhar Z, Kilar F and Felinger A (2010). Determination of polyphenolic compounds by liquid chromatography-mass spectrometry in Thymus species. *J. Chromatog. A.,* **1217**: 7972-7980.

Brand-Williams W, Cuvelier ME and Berset C (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Tech.,* **28**: 25-30.

Bozan B, Öztürk N, Koşar M, TunalıerZ and Başer KHC (2002). Antioxidant and free radical scavenging activities of eight *Salvia* species. *Chem. Nat. Compd.,* **38**: 198-200.

Chorianopoulos N, Kalpoutzakis E, Aligiannis N, Mitaku S, Nychas GJ and Haroutounian SA (2004). Essential oils of *Satureja, Origanum* and *Thymus* species: Chemical composition and antibacterial activities against Foodborne Pathogens. *J. Agric. Food Chem.,* **52**: 8261-8267.

Davis PH (1982). Flora of Turkey and the east aegean islands (Vol. 7). University Press, Edinburgh, pp.349-381.

Davis PH (1988). Flora of Turkey and the east aegean Islands, Supplement 1. University Press, Edinburgh, p.538.

Esquivel MM, Ribeiro MA and Bernardo-Gil MG (1999). Supercritical extraction of Savory oil: Study of antioxidant activity and extract characterization. *J. Supercrit. Fluid.*, **14**: 129-135.

Exarchou V, Nenadis N, Tsimidou M, Gerothanasis IP, Troganis A and Boskou D (2002). Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage and summer savory. *J. Agric. Food Chem.,* **50**: 5294-5299.

Gortzi O, Lalas S, Chinou I and Tsakinis J (2006). Reevaluation of anti-microbial and anti-oxidant activity of *Thymus* spp. extracts before and after encapsulation in liposomes. *J. Food Protect,* **69**: 2998-3005.

Grujic-Jovanovic S, Marin PD, Dzamic A and Ristic M (2009). Essential oil composition of *Thymus longicaulis* from Serbia. *Chem Nat Comp*., **45**: 265-266.

Harley RM, Atkins S, Budantsev AL, Cantino PD, Conn BJ, Grayer R, Harley MM, de Kok R, Krestovskaja T, Morales R, Paton AJ, Ryding O and Upson T (2004). *Labiatae*, *In*: Kadereit JW (Ed.). The families and genera of vascular plants. Vol.VII, *Lamiales*. Berlin: Springer. pp.167-282.

Jordan MJ, Martinez RM, Martinez C, Monino I and Sotomayor JA (2009). Polyphenolic extract and essential oil quality of *Thymus zygis* ssp. *gracilis* shrubs cultivated under different watering levels. *Ind. Crops Products,* **29**: 145-153.

Lawrence BM and Tucker AO (2002). The genus *Thymus* as a source of commercial products: *In*: Thyme The genus *Thymus* (eds; Stahl- Biskup E & Saez F), Taylor & Francis Group, pp.252-262.

Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS and Leitao SG (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res*., **15**: 127-130.

Miliauskas G, Venskutonis PR and Van Beek TA (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem*., **85**: 231-237.

Miura K, Kikuzaki H and Nakatani N (2002). Antioxidant activity of chemical components from Sage (*Salvia officinalis* L.) and Thyme (*Thymus vulgaris* L) measured by the oil stability index method. *J. Agric. Food Chem.,* **50**: 1845-1851.

Öztürk N, Tunçel M and Tunçel NB (2007). Determination of phenolic acids by a modified HPLC: Its application to various plant materials. *J. Liq. Chromatog. R T.,* **30**: 587-596.

Öztürk N, Tunçel M and Potoğlu-Erkara İ (2009). Phenolic compounds and antioxidant activities of some *Hypericum*ssp.: A comparative study with *H. perforatum*. *Pharm. Biol.,* **47**(2): 120-127.

Öztürk N, Tunçel M, Uysal ÜD, Öncü EM and Koyuncu O (2011). Determination of rosmarinic acid by high-performance liquid chromatography and its application to some *Salvia* species and rosemary. *Food Anal. Meth.,* **4**: 300-306.

Sanchez-Moreno C, Larrauri JA and Saura-Calixto F (1998). A procedure to measure the anti-radical efficiency of phenols. *J. Sci. Food Agric.*, **76**: 270-276.

Sarikurkcu C, Ozer MS, Eskici M, TepeB, Can Ş and Mete E (2010). Essential oil composition and antioxidant activity of *Thymus longicaulis* C. Presl subsp. *longicaulis* var. *longicaulis*. *Food Chem. Toxicol.,* **48**: 1801-1805.

Saxena M, Saxena J and Pradhan A (2012). Flavonoids and phenolic acids as antioxidants in plants and human health. *Int. J. Pharm. Sci. Rev. Res.,* **16**: 130-134.

Singleton V and Rossi J (1965). Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *Amer. J. Enol. Viticult.,* **16**: 144-158.

Speisky H, Rocco C, Carrasco C, Lissi EA and Lopez-Alarcon C (2006). Anti-oxidant screening of medicinal herbal teas. *Phytother. Res.,* **20**: 462-467.

Stahl-Biskup E (2002). Essential oil chemistry of the genus *Thymus*- A global view: *In*: Thyme the genus *Thymus* (eds; Stahl- Biskup E & Saez F) Taylor & Francis Group, pp.75-124.

Tabart J, Kevers C, Pincemail J, Defraigne JO and Dommes J (2009). Comparative anti-oxidant capacities of phenolic compounds measured by various tests. *Food Chem.,* **113**: 1226-1233.

Venskutonis PR (2004). Food additives: The dilemma of synthetic or natural. *Acta Aliment*., **33**: 1-5.

Vila R (2002). Flavonoids and further polyphenols in the genus *Thymus*: *In*: Thyme the genus *Thymus* (eds; Stahl-Biskup E & Saez F), Taylor & Francis Group, pp.144-176.

Wang H, Provan GJ and Helliwell K (2004). Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. *Food Chem.,* **87**: 307-311.

Zheng W and Wang SY (2001). Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.,* **49**: 5165-5170.