Research Journal of Biological Sciences 5 (7): 492-498, 2010
ISSN: 1815-8846

Seasonal Variation in the Chemical Composition, Antioxidant Activity and Total Phenolic Content of *Teucrium persicum* Boiss. Essential Oils

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Abstract: Essential oils from the aerial parts of *Teucrium persicum* Boiss. (Labiatae), collected in 3 stages (pre-flowering, flowering and post-flowering) from plants grown in the Fars province of Iran were obtained by steam distillation. The chemical composition of the oils were analyzed by GC-MS. Analysis of the chemical profile of the isolated oils revealed the presence of >80 compounds, mainly sesquiterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes. They contained α-terpinyl acetate, α-cadinene, 1,4-cadinadiene, linalool and cadinol. Average yield was 1%. The studied essential oils showed good antioxidant activities as calculated by 3 *in vitro* assays: DPPH radical scavenging, Ferric Reducing Power Assay (FRAP) and Reducing Power (RP).

Key words: Chemical composition, distillation, oxygenated, hydrocarbons, monoterpenes, sesquiterpenes

INTRODUCTION

The genus of Teucrium (Labiatae) consists of about 300 species worldwide, chiefly in the Northern Hemisphere. This genus is scattered especially in Central and South America, Southeast Asia and around the Mediterranean region (Samec *et al*., 2010; Ulubelen *et al*., 2000). *Teucrium persicum* is an endemic plant of Iran and is distributed throughout the Fars province around the Lar region (Javidnia *et al*., 2007). Native people called this plant marv-e-talkh and use it for the treatment of headaches and abdominal pains such as colitis.

Teucrium species have edible value and therapeutic properties. They have been known as folk medicines for centuries in many parts of the world such as Iran, Iraq, Saudi Arabia and Egypt. These species have been used for the treatment of diabetes, obesity, hyperlipidemia, inflammation and rheumatoid. In addition, they have interesting antibacterial, antinociceptive, antioxidant, anticancer, diuretic, tonic, diaphoretic and analgesic properties. Some of Teucrium species are currently used in the preparation of flavored beer and wines, herbal teas, bitters and liqueurs.

This genus is important in food industries as natural preservative ingredients due to their antioxidant activities (El-Shazly and Hussein, 2004; Hachicha *et al*., 2007a; Menichini *et al*., 2009; Samec *et al*., 2010; Ulubelen *et al*., 2000; Yin *et al*., 2009). The plants of this genus are often aromatic shrubs. The essential oils of various Teucrium contain different productivity, ranging between 0.5 and 1.5% and the amounts and kinds of their chemical constituents, generally monoterpene hydrocarbons and oxygenated/non-oxygenated sesquiterpenes are different (Menichini *et al*., 2009).

Several studies about the essential oils of Teucrium were carried out to evaluate their antioxidant properties. Many of these revealed effective antioxidant activity (Bahramikia *et al*., 2009; Esmaeili *et al*., 2009; Javidnia *et al*., 2007; Mehrabani *et al*., 2009; Panovska and Kulevanova, 2005; Salah *et al*., 2006). Aging and many diseases such as autoimmune diseases, inflammation, cardiovascular disorders, arthritis, liver diseases, arthrosclerosis and cancer are attributed to oxidative agents (Pacifico *et al*., 2009; Yin *et al*., 2009).

Therefore, it is important to find sources of natural and effective antioxidants for the treatment of several diseases and the preservation of foods against oxidative damage. To the best of the knowledge, there are no published studies about the antioxidant properties of the *T. persicum*. This study was carried out to evaluate the seasonal variations in the chemical composition,

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antioxidant activity and yield of the essential oil of the *T. persicum* that to find the best productivity and antioxidant activity.

MATERIALS AND METHODS

Plant materials: Aerial parts of plant were collected in January 2010 (pre-flowering stage), March 2010 (flowering stage) and September 2009 (post flowering stage) on the Lar Mountain, near the Barak village at 1,200 m above sea level in Iran. Plants were dried in the shade at room temperature. Voucher specimens of this plant (No. 397) have been deposited at the Central Herbarium of Medicinal Plants (ACECR) in Iran.

Extraction of the essential oil: The air-dried aerial parts of plants were subjected to steam distillation for 3 h using a clevenger apparatus. The oil was dried over anhydrous sodium sulphate and stored at +4°C in a dark and sealed container.

Gas chromatography-mass spectrometry analysis: GC-MS analyses were done using a Hewlett-Packard 5973-6890 system operating in EI mode (70 eV) equipped with a split injector (290°C; split ratio, 1: 10) and using a silica HP-5MS capillary column (30×0.25 mm i.d.; film thickness 0.25 μm). The column temperature program was 50°C (5 min) to 240°C at a rate of 3°C min^{-1}; the carrier gas was helium at a flow rate of 0.8 mL min^{-1}; the injection volume was 1 μL and the detector temperature was 290°C. Compounds were identified by comparison of mass spectra with the Wiley 7 n.l mass spectral database and those described by Adams (2001) as well as by comparison of their retention indices with literature data and the NIST database. The retention indices were determined in relation to a homologous series of n-alkanes (C8-24) under the similar operating conditions (Adams, 2001; Stein, 2010). Quantitative data was calculated electronically from the FID area data without the use of correction factors.

Antioxidant activity

DPPH assay: This assay is based on the spectrophotometric method. A test sample was added to a concentration of methanolic 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH). Then the mixture was incubated in the dark at room temperature for 30 min and the absorbance was measured at 517 nm. The difference between the initial DPPH radical adsorption and the adsorption of the sample after reaction was determined as antioxidant activity. IC_{50} values (concentration of the test samples providing 50% scavenging) were calculated from the graph-plotted scavenging percentage against the oil concentration. A lower IC_{50} value means a higher antioxidant power of the examined compound. Scavenging percentage of the DPPH stable radical was calculated in following way (Ramamoorthy and Bono, 2007; Rohman *et al*., 2010): DPPH scavenging activity (%) = 100 ×(1- (Abs at 517 test sample/Abs at 517 initial DPPH solution)).

The DPPH antioxidant activity was assessed by the method of Tofighi *et al*. (2009). Butyl Hydroxyanisole (BHA) and α-tocopherol were used as positive controls. All values are shown as the mean of 3 measurements (Huang *et al*., 2005; Tofighi *et al*., 2009).

Ferric Reducing Antioxidant Power (FRAP) assay: The FRAP assay was done according to the method of Benzie and Strain (1996). This assay is based on the ability of sample to reduce Fe^{3+} in the presence of a Tripyridyltriazine (TPTZ) solution. After forming the Fe^{2+} ion, the blue colored complex Fe^{2+}-tripyridyltriazine was produced. An increase above a complex concentration signals the reducing power of the sample. Solution absorbance was determined at 593 nm (Benzie and Strain, 1996; Hong *et al*., 2008; Huang *et al*., 2005; Khanavi *et al*., 2009).

Reducing Power (RP) assay: This assay was measured according to the method used by Hinneburg *et al*. (2006). The RP of the essential oil is based on the ability of the sample to reduce ferri cyanide to ferro cyanide. After the reduction reaction, a Prussian blue-colored complex $(Fe^{3+})_4[Fe^{2+}(CN^-)_6]_3$ formed. This complex is detectable. The increased absorbance of the reaction mixture was construed as an increase in the reducing activity of the sample. The positive control was BHA and the results were compared with it. The absorbance was measured at 700 nm (Hinneburg *et al*., 2006; Kumar *et al*., 2008; Rohman *et al*., 2010).

Total Phenolic Content (TPC): The TPC of essential oils was determined according to Ghafar *et al*. (2010). This method is established on the oxidation of the phenolic compounds by a molybdo-tungstate in the reagent to create a green-colored product. The Folin-Ciocalteu assay is not specific to phenols.

Therefore, this test gives a raw evaluation of the total phenolic compounds because of disturbed compounds such as sugars, ascorbic acid, organic acids and aromatic amines that may react with the reagent and give concentrations more than real TPC. Gallic acid was used as the standard (Ghafar *et al*., 2010; Khanavi *et al*., 2009; Velioglu *et al*., 1998).

Statistical analysis: All tests were repeated 3 times and data was expressed as mean±SD. Statistical analysis, plots and fittings were carried out by using Excel 2007.

RESULTS AND DISCUSSION

Extraction yield and physical properties: All oils were yellow and fragrant. The highest yield of the oil (1.1 g/100 g Dry Weight of plant, DW) was obtained from the flowering stage (EA). As the plant became more mature, post-flowering stage (ES), the essential oil yield decreased (1 g/100 g DW). A lower yield (0.98 g/100 g DW) was obtained from pre-flowering stage (EB). There are many reports about productivity of the essential oil of Teucrium for example, the oil yields of *T. montanum* and *T. marum* were calculated as 0.47% DW and 0.59% (v/w), respectively (Ricci *et al*., 2005; Vukovic *et al*., 2007). The productivity of *T. ramosissimum* Desf. was 0.14% (W/W) (Hachicha *et al*., 2007a). The essential oil of ES showed the lowest density (0.97 g cm^{-3}) while the oils of EA and EB had a similar density (0.98 g cm^{-3}). There is no earlier study in the literature regarding the seasonal variations in density with which to compare the results of the analysis.

Chemical composition of essential oils: In total, 86 components were identified in 3 collection stages. Table 1 shows the components and their properties (the retention indices and percentage composition) where the

Table1: Seasonal variation in the chemical composition of the aerial parts of *Teucrium persicum* essential oils

		Area (%)		
Compounds	RIa	EA_b	EB_c	Es_d
α-Thujene	922	0.14	0.08	0.09
α-Pinene	928	0.42	0.17	0.25
Sabinene	969	0.50	0.18	0.13
ß-Pinene	971	0.92	0.32	0.42
β-Myrcene	990	1.80	1.57	1.61
Herboxide second isomer	1005	0.17	0.17	0.19
α-Terpinene	1013	0.14	0.10	0.11
γ-Cymene	1023	t[e]	0.11	0.22
1,8-Cineole	1032	4.85	2.97	5.74
β-Ocimene	1037	0.29	0.14	0.28
cis-Ocimene	1048	0.73	0.37	0.55
γ-Terpenine	1056	0.17	0.09	0.11
(Z)-β-Terpinole	1067	-	0.04	-
p-Mentha-3,8-diene	1071	-	0.07	-
trans-Linalool oxide(furanoid)	1073	0.51	0.48	1.21
α-Terpinolene	1086	0.41	0.16	0.26
Linalool	1109	7.72	5.48	7.63
Oct-1-en-3-yl acetate	1112	0.88	0.21	0.65
cis-2-Menthenol	1123	0.25	0.17	0.10
1,3,8-p-Menthatriene	1130	-	0.06	0.18
cis-Sabinol	1136	t	0.06	0.06
trans-Pinocarveol	1139	0.17	0.14	0.04
Nerol oxide	1156	0.23	0.17	0.41
Isomenthone	1162	0.03	-	-
Borneol	1169	0.12	0.35	0.64
Terpinene-4-ol	1178	0.36	0.28	0.30

Table1: Continue

		Area (%)		
Compounds	RIa	EA_b	EB_c	Es_d
γ-Terpineol	1198.00	6.91	3.81	4.35
Myrtenol	1200.00	-	0.09	0.08
3,5,7-Octatriene-2-ol,2,6-dimetyl-	1213.00	-	-	0.09
trans-Carveol	1215.00	0.02	-	0.20
cis-Carveol	1225.00	0.14	0.24	0.37
Nerol	1235.00	0.85	0.49	0.61
Linalyl acetate	1262.00	5.59	4.42	7.67
Dihydrolinalool acetate	1276.00	1.46	-	-
Caprinic alcohol	1278.00	0.13	0.38	0.55
Carvacrol	1327.00	0.09	0.14	0.33
γ-Elemene	1337.00	0.95	1.00	0.90
Piperitenone	1345.00	-	-	0.10
α-Terpinyl acetate	1356.00	10.78	5.84	7.89
Eugenol	1361.00	-	0.04	-
cis-Carvyl acetate	1367.00	1.30	0.73	1.09
α-Copaene	1377.00	0.05	0.08	0.13
Geranyl acetate	1388.00	2.39	1.38	2.42
α-Elemene	1394.00	0.83	1.17	1.16
α-Gurjunene	1410.00	0.45	0.53	0.52
β-Caryophyllene	1420.00	0.26	0.45	0.47
Aromadendrene	1442.00	0.25	0.77	1.41
cis-4(14),5-Muuroladiene	1447.00	0.11	0.07	0.21
α-Humulene	1453.00	0.12	0.13	0.23
γ-Gurjunene (5,11-Guaiadiene)	1459.00	0.09	0.26	0.63
trans-Cadina-1(6),4-diene	1473.00	0.12	t	0.23
Germacrene-D	1481.00	0.30	0.48	0.72
β-Selinene	1487.00	0.39	0.80	0.73
d-Cadinene	1492.00	0.16	t	0.30
Bcyclogermacrene	1499.00	2.23	3.35	2.31
α-Murrolene	1502.00	0.83	0.20	1.14
γ-Cadinene	1517.00	0.38	0.30	1.17
Phenol, 2,4-bis(1,1-dimethylethyl)-	1519.00	0.41	-	0.42
1,4-Cadinadiene	1532.00	8.37	11.85	9.24
α-Cadinene	1535.00	9.73	13.01	9.73
Calamenene	1541.00	-	-	0.24
α-Calacorene	1545.00	-	-	0.06
Elemol	1552.00	0.07	-	-
Nerolidol	1562.00	0.15	-	-
Palustrol	1570.00	0.18	0.20	0.12
Viridiflorol	1592.00	0.45	0.55	0.42
Caryophyllene oxide	1595.00	-	-	0.08
(-)-Globulol	1603.00	-	0.67	-
Ledol	1607.00	0.25	0.54	0.18
β-Oplopenone	1611.00	0.11	0.22	0.10
1,10-di-epi-Cubenol	1619.00	0.19	0.35	0.16
γ-Eudesmol	1623.00	0.73	1.24	0.67
α-Cadinol	1650.00	4.86	5.28	2.95
β-Eudesmol	1661.00	-	-	2.09
Cadinol	1669.00	7.60	9.74	6.21
α-Bisabolol	1684.00	0.37	0.91	0.31
Acorenone B	1703.00	5.08	8.48	2.53
Aromadendrene oxide	1748.00	0.05	0.08	0.06
Spathulenol	1784.00	0.36	0.76	0.31
n-Hexadecanol	1882.00	0.17	0.26	0.15
(Z)-Phytol	1951.00	0.11	0.22	0.14
Manoyl oxide	2011.00	0.12	0.36	0.16
Octadecanal	2038.00	0.03	0.11	t
Geranyl 3-phenylpropanoate	2135.00	0.10	0.05	t
Geranyl linalool	2192.00	0.04	0.08	t
Schareol	2228.00	-	0.10	-
Monoterpene hydrocarbons	5.11	3.19	3.95	-
Oxygen-containing monoterpenes	45.36	28.31	42.89	-
Sesquiterpene hydrocarbons	25.62	34.45	31.63	-
Oxygen-containing sesquiterpenes	20.45	29.02	16.19	-
Miscellaneous	0.98	1.18	0.87	-
Total identified compounds	97.52	96.15	95.53	-

[a]RI, Retention Indices relative to C8–C24 n-alkanes on the HP-5MS column. Components listed in order of elution from a HP-5MS column; [b]Flowering stage; [c]Pre-flowering stage; [d]Post-flowering stage; [e]Trace ($p<0.05\%$)

components are listed in order of elution from a HP-5MS column. Most of these compounds have been reported in the essential oils of Teucrium species (Antunes *et al*., 2004; Cavaleiro *et al*., 2004; Cozzani *et al*., 2005; Hachicha *et al*., 2007b; Menichini *et al*., 2009; Ricci *et al*., 2005; Saroglou *et al*., 2007; Vukovic *et al*., 2008). In total 74, 73 and 76 compounds were identified in the essential oils of EB, EA and ES, representing 96.15, 97.52 and 95.53% of total oils, respectively. As shown in Table 1, the major constituents in the EA were α-terpinyl acetate (10.78%), α-cadinene (9.73%), 1,4-cadinadiene (8.37%), linalool (7.72%) and cadinol (7.6%). The main components of EB were α-cadinene (13.01%), 1,4-cadinadiene (11.85%), cadinol (9.74%), acorenone B (8.48%) and α-Terpinyl acetate (5.84 %) and the principal components of ES were α-cadinene (9.73%), 1,4-cadinadiene (9.24%), α-terpinyl acetate (7.89%), linalyl acetate (7.67%) and linalool (7.36%).

In EB, sesquiterpenes formed the most abundant portion of the oil (63.4%) with a predominance of oxygenated sesquiterpenes (34.4%). On the other hand, oxygen-containing monoterpenes accounted for 29.0% of the total oil with α-terpinyl acetate (5.84%) and linalool (5.48%) as the main compounds while monoterpene hydrocarbons were almost 3%. In EA, the main components were monoterpenes (50.4% of the total oil) and among these, the major components were oxygen-monoterpenes (45.3%). Overall, 46.0% of the oil consisted of sesquiterpenes. Of these, sesquiterpene hydrocarbons (25.6%) and oxygenated sesquiterpenes (20.4%) were present in similar amounts.

Sesquiterpenes (47.8%) were the major part of ES. In this fraction, sesquiterpene hydrocarbons (31.6%) were prevailing. Monoterpenes were 46.8% of the oil with a prevalence of oxygen-containing monoterpenes (42.8%). As shown in Table 1, the main compounds identified from different seasonal phases were almost the same but the amounts of the corresponding components were different. In all samples, the highest content was oxygen monoterpenes (38.8±9.2%) while the lower was hydrocarbon monoterpenes (4.0±0.9%).

According to the data, the main groups of components in these essential oils are cadinane-type sesquiterpenes. These sesquiterpenes constituted >200 compounds and they show various biological activities including as a wood preservative, fungicide, anti-malaria and anti-HIV (Ferreira *et al*., 2005; Liu *et al*., 2007; Wu *et al*., 2005). In all of the oils, there are small amounts of diterpenoid compounds such as geranyl linalool, manoyl oxide and phytol. Differences in the amounts and types of compounds might be attributed to the environmental status and phenological conditions. The results are in agreement with those of Yildirim *et al*. (2004) who out that there were significant quantitative changes among the essential oils of *T. orientale* L. var. *orientale* with respect to harvesting stages in terms of chemical composition (Yildirim *et al*., 2004).

When the chemical profiles of the essential oils studied were compared with previously reported ones, they were somewhat different. In contrast to the present study, Masoudi *et al*. (2009) reported epi-α-cadinol (23.2%) as the main component of the plant. They identified 31 constituents corresponding to 95.9% of total essential oil (Masoudi *et al*., 2009).

In addition, Javidnia *et al*. (2007) detected nearly 81 compounds, representing 93.5% of the total oil of which the caryophyllene oxide (10.6%) was the major compound followed by α-pinene (9.4%), geranyl linalool (7.8%), γ-cadinene (7.4%), elemol (6.9%) and α-cadinol (5.5%).

Cultivar variations, geographical differences, times of plant growing and preparation procedures may have influenced oil compounds either at the qualitative or quantitative level (Javidnia *et al*., 2007; Masoudi *et al*., 2009; Vukovic *et al*., 2007). Previous studies indicated that sesquiterpenes were the main compounds of the essential oil of Teucrium. For example, Hachicha *et al*. (2007a, b) identified 57 components in the oil of *T. alopecurus*. Predominant compounds were mainly sesquiterpenes hydrocarbons (61.3%) and oxygenated sesquiterpenes (26.9%) (Hachicha *et al*., 2007a). Vukovic *et al*. (2007) investigated the essential oil of *T. montanum* and recognized 45 compounds, representing 97.95% of the total.

The main constituents of the oil were mono and sesquiterpene hydrocarbons. They reported δ-cadinene (17.19%) and β-selinene (8.16%) as the main constituents of the oil (Vukovic *et al*., 2007). Saroglou *et al*. (2007) studied the chemical profile of the essential oil of *T. royleanum*. They reported that sesquiterpene hydrocarbons formed the main portion (42.2%) of the oil among which β-santalene and cis-α-bisabolene were the predominant compounds (Saroglou *et al*., 2007).

Antioxidant activity

FRAP assay: The reducing capacities of essential oils of *T. persicum* were calculated according to the FRAP assay. An aqueous solution of ferrous sulphate (50-500 μmol mL^{-1}, $y = 0.002x - 0.025$, $R^2 = 0.993$) was prepared as acalibration curve. The results were expressed as μmol Fe^{2+} equivalents per gr DW and are shown in Table 2. FRAP values point to a considerably higher reducing power of ES (220±7.2 μmol Fe^{2+} g^{-1} DW) compared with EB (73±3.4 μmol Fe^{2+} g^{-1} DW) and EA (52±3.9 μmol $Fe^{2+}g^{-1}$ DW). The results of the study compared with the

Table 2: Antioxidant activities of essential oils of aerial parts of *Teucrium persicum*

Stages	DPPH[a] mg mL^{-1}	FRAPb µmol Fe^{2+} g^{-1} Dwg	RPAc µg BHA[h] g^{-1} DW
Es[d]	0.29	220±7.2	51.7±4.30
EB[e]	13.52	73±3.4	13.0±2.40
EA[f]	14.20	52±3.9	10.3±2.90

Data presented is mean±S.D from 3 different experiments; [a]DPPH radical scavenging assay; [b]Ferric reducing power assay; [c]Reducing power assay; [d]Post-flowering stage; [e]Pre-flowering stage; [f]Flowering stage; [g]Dry weight; [h]Butyl hydroxyanisole

research of Samec *et al.* (2010) indicate that the reducing power of essential oils of *T. persicum* are similar to the average FRAP value for leaf (75.81±34.99 µmol Fe^{2+} g^{-1} DW) and flower (97.65±54.38 µmol Fe^{2+} g^{-1} DW) infusions of *T. arduini* (Samec *et al*., 2010).

DPPH radical scavenging activity: In the DPPH assay, the ability of the examined essential oils to perform as a giver of the hydrogen atom or electron in transforming the purple-colored radical DPPH● into the yellow-colored DPPH-H with a reduced shape was studied. All samples possessed inhibitory activity. ES exhibited the highest radical scavenging potential (IC_{50} = 0.29 mg mL^{-1}) followed by EA (IC_{50} = 13.52 mg mL^{-1}) and EB (IC_{50} = 14.20 mg mL^{-1}). The greatest effect was obtained by ES (IC_{50} = 0.29 mg mL^{-1}) though it was less effective than BHA (IC_{50} = 0.016 mg mL^{-1}) and α-tocopherol (IC_{50} = 0.015 mg mL^{-1}). The results are in agreement with Kadifkova-Panovska *et al.* (2005) who explained that the extract of *T. polium*, *T. chamaedrys* and *T. montanum* possessed inhibitory activity with IC_{50} of 10, 11 and 10 mg mL^{-1}, respectively. These results were less effective than standard compounds, silymarin, quercetin and luteolin with IC_{50}: 1.96, 0.06 and 0.08 mg mL^{-1}, respectively (Kadifkova-Panovska *et al*., 2005).

Reducing power: BHA was used to produce the standard calibration curve (5-60 µgmL^{-1}, y = 0.006x + 0.058, R^2 = 0.997). The reducing power of oil was expressed as µg BHA equivalent per g DW.

Among the essential oils evaluated, ES (51.7±4.3 µg BHA g^{-1} DW) has the highest reducing power followed by EB (13±2.4 µg BHA g^{-1} DW) and EA (10.3±2.9 µg BHA g^{-1} DW).

Total phenolic content: Phenolic components are secondary metabolites in plants and many of them accounted for the antioxidant components, mainly due to their redox properties. Folin-Ciocalteu assay is not specific to polyphenols (Ghafar *et al*., 2010; Rohman *et al*., 2010). As shown in Table 3, the ES revealed the highest content of total phenolics (1.71±0.12 mg GAE g^{-1} DW), approximately 4-fold more than EB and 8-fold more than EA (0.39±0.04 and 0.20±0.03 mg GAE g^{-1} DW, respectively). The difference might be due to growing times, various environmental conditions and changes of seasons.

Table 3: Total Phenolic Content (TPC) of essential oils of aerial parts of *Teucrium persicum*

Stages	TPC (mg GAE g^{-1} DW)[d]
ES[a]	1.71±0.12
EB[b]	0.39±0.04
EA[c]	0.20±0.03

Data presented is mean±S.D from three different experiments; [a]Post-flowering stage; [b]Pre- flowering stage; cFlowering stage; [d]Gallic acid equivalents per gram dry weight

The phenolic content of some Teucrium has already been determined. The phenolic content of essential oils that we analyzed were less than the average values found for *T. arduini* flower (30.49±1.00 mg GAE g^{-1} DW) and leaf (23.39±3.60 mg GAE g^{-1} DW) (Samec *et al*., 2010). Also, Gursoy and Tepe (2009) reported that the non-polar extract of *T. chamaedrys* had 97.12±1.28 µg GAE mg^{-1} of total phenolic amount while the polar extract had 69.75±2.62 µg GAE mg^{-1} (Gursoy and Tepe, 2009). Gallic acid was used as a standard phenolic compound for the calibration curve (20-200 mg L^{-1}, y = 0.003x - 0.027, R^2 = 0.991). Total phenolic content was expressed as mg Gallic Acid Equivalents (GAE) per gram DW.

CONCLUSION

The results of antioxidant activities and chemical composition of the oils are compatible with TPC. The relations between antioxidant activities and TPC obtained from various essential oils suggest close correlations (FRAP and TPC: y = 3.658x - 73.19, R^2 = 0.982; DPPH and TPC: y = -10.46x + 174.3, R^2 = 0.994 and RP and TPC: y = 32.02x+4.068, R^2 = 0.994).

ACKNOWLEDGEMENT

This research has been supported by Tehran University of Medical Sciences and Health Services grant.

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