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Antioxidant Activities of the Extracts and Components of *Teucrium orientale* L. var. *orientale*

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The effects of the developing stages of *Teucrium orientale* L. var. *orientale* on the antioxidant and DPPH radical scavenging activities of the extracts obtained with different organic solvents were investigated. The aerial parts of the plant samples were collected at the budding, flowering and vegetative stages and then the plant samples were extracted separately with petroleum ether, chloroform, acetone and methanol. The antioxidant activities of the extracts were evaluated using 2 different tests: the thiocyanate method and scavenging of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The acetone and methanol extracts of all of the harvesting stages and chloroform extracts of the budding and flowering stages had antioxidant activities. Nevertheless, the acetone extracts of the budding and flowering stages showed the highest antioxidant activity. Higher DPPH radical scavenging activities were found in only acetone and methanol extracts in all of the harvesting stages. The flavonoid contents of all of the extracts were also determined. Similar to antioxidant and DPPH radical scavenging activities, the amounts of total flavonoids were higher in acetone and methanol extracts of the flowering and budding stages. The acetone extract of the flowering stage, which contained the highest amount of total flavonoids, was subjected to chromatographic methods for the isolation of the active compounds. Thus, a new iridoid along with 4 known flavonoids and 1 known iridoid were isolated. The structures of isolated compounds were characterized by UV, IR, ^{1}H, ^{13}C NMR, and 2D-NMR spectroscopic methods as cirsilineol (**1**), luteolin-7-*O*-rutinoside (**2**), luteolin-7-*O*-glucoside (**3**), hesperetin-7-*O*-rutinoside (**4**), 8-*O*-acetyl harpagide (**5**) and 8-*O*-methyl harpagide (**6**). These isolated compounds were also tested for their antioxidant and DPPH radical scavenging activities. Flavonoids showed potent antioxidant and DPPH radical scavenging activities, but iridoids did not. The highest antioxidant activity was shown by luteolin-7-*O*-glucoside.

Key Words: *Teucrium orientale*, Labiatae, antioxidant, DPPH radical scavenging, Harvesting stage, Flavonoids, Iridoids.

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Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are various forms of activated oxygen and nitrogen that include free radicals such as superoxide anion ($O_2^{\cdot -}$), hydroxyl ($OH^{\cdot}$), and nitric oxide radicals ($NO^{\cdot}$) as well as non-free-radical species such as hydrogen peroxide (H_2O_2), and nitrous acid (HNO_2). In living organisms, various ROS and RNS can be formed by different mechanisms. Normal aerobic respiration, stimulated polymorph nuclear leukocytes and macrophages, and peroxisomes appear to be the main endogenous sources of most of the oxidants produced by the cells[1]. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides[2]. Free radicals can also cause lipid peroxidation in foods that leads to their deterioration. Oxidation does not only affect lipids. ROS and RNS may cause DNA damage that may lead to mutation[3].

All aerobic organisms have antioxidant defenses including antioxidant enzymes to remove or repair the damaged molecules[3,4]. However, these natural antioxidant mechanisms can be inefficient and hence dietary intake of antioxidant compounds is important[1]. Although some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are commonly used in processed foods, these compounds have been reported to have some side effects[5]. Therefore, research into the determination of the natural antioxidant source and antioxidant potentials of plants is important.

The genus *Teucrium* (Labiatae) comprises more than 300 species, most of which grow in Mediterranean countries. There are 27 species in the Turkish flora, 8 of which are endemic[6]. *Teucrium* species have been used as a stimulant, tonic, diaphoretic and appetizers, and against stomach pains and diabetes in Turkish folk medicine[7]. *T. orientale* L., named "Kirve otu" in Anatolia, is widespread in the dry and stony places of Turkey[6,7]. Recently, we reported the chemical composition and antioxidant properties of the essential oil isolated from *T. orientale*[8]. However, there has been no report on the antioxidant activities and pure components of *Teucrium orientale* L. Therefore, the aim of present study was to evaluate the variation of antioxidant and DPPH radical scavenging activities of petroleum ether, chloroform, acetone and methanol extracts of the aerial parts of *Teucrium orientale* L. var. *orientale* harvested during the budding, flowering and vegetative stages. Since acetone extract of the flowering stage of the plant sample was able to show the highest antioxidant and DPPH radical scavenging activities, it was subjected to further separation by chromatographic methods. Thus, 1 flavone, 2 flavone glycosides, 1 flavanone glycoside and 2 iridoid glycosides were isolated and their antioxidant and DPPH radical scavenging activities were evaluated.

Experimental

General procedures

NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer, operating at 400 MHz and 100 MHz for ^1H and ^{13}C, respectively, using $CDCl_3$ and DMSO-d_6. Chemical shifts are expressed in δ (ppm) downfield from TMS as an internal standard and coupling constants are reported in Hz. UV spectra were recorded on a JASCO V-530 spectrometer. The IR spectra were determined on a Shimadzu FT-IR 8000 spectrophotometer. CC was carried out using silica gel 60 (70-230 mesh). Silica gel 60 precoated plates, F-254 (Merck), were used for TLC and preparative TLC (Merck). The spots on TLC were visualized by spraying with 1% vanilin-H_2SO_4, followed by heating or exposing to NH_3vapor.

Plant material

T. orientale L. var. *orientale* was collected at the budding (June, 2002), flowering (July, 2002) and vegetative stages (August, 2002) from Palandöken Mountain, Erzurum. A voucher specimen (ATA-1153) has been deposited in the herbarium of Atatürk University, Erzurum. The plant sample was identified by Dr. Ercan Kaya, Department of Biology, Kazım Karabekir Education Faculty, Atatürk University.

Extraction and isolation

Dried aerial parts of *T. orientale* were collected at the budding, flowering and vegetative stages and were chopped into small parts in a blender. Afterward, the samples (each one 25 g) were extracted individually 3 times with 100 mL portions of petroleum ether, chloroform, acetone and methanol by stirring for 24 h at room temperature. The extractions were followed by filtration and evaporation under reduced pressure and temperature. The yields and amounts of the extracts are listed in Table 1. In addition, 300 g of dried plant materials harvested at the flowering stage were extracted with acetone (4 x 1 L) at room temperature to isolate the antioxidant components. After solvent evaporation, 12.75 g of extract was obtained.

Table 1. The amounts of the extracts and yields obtained with different solvents from *Teucrium orientale*.

	Petroleum ether		Chloroform		Acetone		Methanol	
	Amount (g)	Yield (%)	Amount (g)	Yield (%)	Amount (g)	Yield (%)	Amount (g)	Yield (%)
Budding	0.31	1.24	0.77	3.08	1.12	4.48	5.40	21.60
Flowering	0.57	2.28	0.86	3.44	1.35	5.40	5.40	21.60
Vegetative	0.76	3.04	1.04	4.16	1.15	4.60	3.31	13.24

The concentrated acetone extract (12.50 g) was fractionated on silica gel CC (250 g, 70-230 mesh) using petroleum ether-EtOAc (5:5→0:10, v/v) and EtOAc-MeOH (9:1→3:7, v/v) to afford 7 fractions. Fraction 3 was subjected to CC (silica gel) using $CHCl_3$-EtOAc-MeOH (6:4:1, v/v/v) solvent system and compound **1** (43 mg) was isolated. Fraction 6 (2.2 g) was further portioned to 3 fractions (Fractions A-C) on silica gel CC (125 g), eluting with $CHCl_3$:MeOH (8:2→5:5, v/v). Compound **2** precipitated during the elution of fraction B. This precipitate was repeatedly washed with EtOAc for 5 times and compound **2** (45 mg) was crystallized on MeOH:$CHCl_3$ (1:1, v/v). Fraction B was also subjected to Sephadex LH-20 CC by eluting with MeOH to give compounds **3** (150 mg) and **4** (32 mg). Fraction 7 was chromatographed on a silica gel CC, where the column was eluted with EtOAc:MeOH (8:2, v/v) to give 3 fractions, coded fractions A'-C'. Fraction A' was submitted to silica gel CC using $CHCl_3$:MeOH:acetone (6:2:2, v/v/v) as eluent to give compound **2** (60 mg). Fraction B', which contained 2 compounds, was subjected to silica gel CC (140 g) using $CHCl_3$:MeOH (7.5:2.5, v/v) as eluent and 30 fractions were collected. While fractions 6-20 contained pure compound **5** (500 mg), fractions 21-30 contained compounds **5** and **6**. Compound **6** (90 mg) was further purified on preparative thin layer chromatography (TLC) using $CHCl_3$:MeOH solvent mixture (7.5:2.5, v/v).

Cirsilineol (1): Yellowish powder; UV (MeOH) λ_{max}: 274, 341 nm. IR: ν_{max}: 3445, 2930, 2861, 1730, 1653, 1607, 1453, 1369, 1284, 1138 cm^{-1}; ^1H and ^{13}C NMR (Table 2).

Table 2. ^{1}H and ^{13}C NMR data (δ, ppm) of flavonoids (**1-4**) (ppm from TMS, in DMSO-d_6).

Position	**1**		**2**		3		**4**	
	δ ^{13}C	δ ^{1}H (J in Hz)	δ ^{13}C	δ ^{1}H (J in Hz)	δ^{13} C	δ^{1} H (J in Hz)	δ^{13} C	δ^{1} H (J in Hz)
2	164.0	-	163.5	-	163.4	-	79.0	4.9 (1H, d, 16.9)
3	103.7	6.3 (1H, s)	103.6	6.8 (1H, *s*)	103.1	6.7 (1H, *s*)	42.6	2.7 (2H, dd, 16.9, 3.5)
4	182.5	-	182.6	-	182.4	-	198.0	-
5	152.8	-	161.6	-	161.5	-	163.2	-
6	130.9	-	100.2	6.1 (1H, d, 1.8)	100.1	6.4 (1H, d, 2.0)	97.1	6.2 (1H, s)
7	153.1	-	165.2	-	165.4	-	165.7	-
8	90.7	6.2 (1H, s)	95.6	6.5 (1H, d, 1.8)	95.4	6.8 (1H, d, 2.0)	96.1	7.0 (1H, s)
9	158.7	-	157.6	-	157.6	-	163.1	-
10	105.8	-	106.0	-	105.9	-	104.0	-
1′	123.8	-	121.7	-	120.5	-	131.4	-
2′	111.1	7.1 (1H, d, 2.0)	113.8	6.8 (1H, d, 2.6)	113.3	7.4 (1H, s)	114.7	6.9 (1H, d, 2.0)
3′	146.6	-	146.4	-	146.9	-	147.0	-
4′	150.5	-	150.9	-	152.6	-	148.6	-
5′	112.6	6.1 (1H, d, 8.4)	116.7	6.9 (1H, d, 9.7)	116.7	6.8 (1H, d, 8.1)	112.5	6.9 (1H, d, 8.4)
6′	118.7	7.1 (1H, dd, 8.4, 2.0)	120.0	7.5 (1H, dd, 9.7, 2.6)	120.1	7.4 (1H, d, 8.1)	118.7	7.4 (1H, dd, 8.5, 2.0)
OCH$_3$	60.3	3.6 (3H, s)	-	-	-	-	56.2	3.7 (3H, s)
OCH$_3$	56.1	3.6 (3H, s)	-	-	-	-	-	-
OCH$_3$	55.7	3.5 (3H, s)	-	-	-	-	-	-
β-D-Glucose								
1″	-	-	101.1	5.1 (1H, d, 6.9)	100.5	5.0 (1H, d, 7.3)	101.2	5.2 (1H, d, 7.3 Hz)
2″	-	-	73.6	3.2 (1H, t, 8.7)	73.6	3.2 (1H, t, 9.3)	72.7	3.2 (1H, t, 8.5)
3″	-	-	77.6	3.2-3.3 (1H, m)	77.6	3.2-3.3 (1H, m)	76.8	3.2-3.3 (1H, m)
4″	-	-	70.2	3.2 (1H, m)	70.1	3.2 (1H, m)	70.2	3.2 (1H, m)
5″	-	-	76.8	3.2-3.3 (1H, m)	76.9	3.2-3.3 (1H, m)	76.1	3.2-3.3 (1H, m)
6″a **6″b**	-	-	61.2	3.7 (1H, overlapped) 3.4 (1H, overlapped)	61.2	3.7 (1H, overlapped) 3.4 (1H, overlapped)	63.6	3.7 (1H, overlapped) 3.5 (1H, overlapped)
α-L-Ramnoz								
1‴	-	-	100.4	4.5 (1H, d, 1.4)	-	-	100.0	4.5 (1H, s)
2‴	-	-	71.3	3.2-3.3 (1H, overlapped)	-	-	71.3	3.2-3.3 (1H, overlapped)
3‴	-	-	70.8	3.2-3.3 (1H, overlapped)	-	-	70.9	3.2-3.3 (1H, overlapped)
4‴	-	-	76.7	3.2-3.3 (1H, overlapped)	-	-	73.6	3.2-3.3 (1H, overlapped)
5‴	-	-	68.9	3.2-3.3 (1H, overlapped)	-	-	69.0	3.2-3.3 (1H, overlapped)
6‴	-	-	18.9	1.1 (3H, d, 6.2)	-	-	18.4	1.1 (3H, d, 6.0)

Luteolin-7-*O*-rutinoside (2): Yellowish powder; UV (MeOH) λ_{max}: 256, 265, 343 nm. IR: ν_{max}: 3420, 1655, 1600, 1504, 1456, 1361, 1294, 1022, 1061, 1011 cm^{-1}. ^1H and ^{13}C NMR (Table 2).

Luteolin-7-*O*-glucoside (3): Yellowish powder; UV (MeOH) λ_{max}: 255, 265, 351 nm. IR: ν_{max}: 3389, 2917, 1655, 1606, 1495, 1445, 1260, 1174, 1070 cm^{-1}. ^1H and ^{13}C NMR (Table 2).

Hesperetin-7-*O*-rutinoside (4): Yellowish powder; UV (MeOH) λ_{max}: 230, 284, 336 nm. IR: ν_{max}: 3341, 2922, 1658, 1365, 1290, 1070, 1015 cm^{-1}. ^1H and ^{13}C NMR (Table 2).

8-*O*-Acetyl harpagide (5): Colorless powder; UV (MeOH) λ_{max}: 206, 279 nm. IR: ν_{max}: 3343, 2918, 1708, 1262, 1235, 1070 cm^{-1}. ^1H and ^{13}C NMR (Table 3).

8-*O*-Methyl harpagide (6): Colorless powder; UV (MeOH) λ_{max}: 208, 280 nm. IR: νmax: 3348, 2915, 1710, 1265, 1235, 1075. ^1H and ^{13}C NMR (Table 3).

Table 3. ^1H- and ^{13}C- NMR shifts (ppm) of iridoids **(5, 6)** (ppm from TMS, in DMSO-d_6).

	5; 8-*O*-Acetyl harpagide		**6**; 8-*O*-Methyl harpagide	
Position	δ ^{13}C	δ ^1H (J in Hz)	δ ^{13}C	δ ^1H (J in Hz)
1	93.1	5.9 (1H, d, 1.8)	92.1	5.5 (1H, *s*)
3	141.9	6.3 (1H, d, 6.2)	140.6	6.2 (1H, d, 6.2)
4	108.0	4.9 (1H, dd, 6.6, 1.5)	109.2	4.9 (1H, dd, 6.2,1.1)
5	71.8	-	70.3	-
6	76.3	3.5 (1H, m)	76.6	3.5 (1H, m)
7a	45.1	1.8 (1H, dd, 14.8, 4.2)	47.5	1.6 (1H, dd, 12.7, 5.1)
7b		2.0 (1H, d, 15.4)		1.7 (1H, dd, 13.2, 5.1)
8	87.0	-	76.0	-
9	54.9	2.6 (1H, broad s)	58.9	2.6 (1H, broad s)
10	22.7	1.3 (3H, s)	25.4	1.1 (3H, s)
OCH_3	-	-	49.3	3.1 (3H, s)
C=O	170.8	-	-	-
$COCH_3$	22.7	1.9 (3H, s)	-	-
***β*-D-Glucose**				
1′	98.0	4.4 (1H, d, 7.7)	98.1	4.4 (1H, d, 8.1)
2′	73.7	3.0 (1H, t, 8.4)	73.6	3.0 (1H, t, 8.5)
3′	77.8	3.1-3.2 (1H, m)	77.8	3.1-3.2 (1H, m)
4′	70.8	3.1 (1H, m)	70.7	3.1 (1H, m)
5′	76.8	3.1-3.2 (1H, m)	76.8	3.1-3.2 (1H, m)
6′a	61.8	3.7 (1H, dd, 11.7, 6.1)	61.6	3.7 (1H, dd, 11.8, 6.1)
6′b		3.4 (1H, dd, 11.7, 6.1)		3.4 (1H, dd, 11.8, 6.1)

Antioxidant activity

Antioxidant activity was determined according to the thiocyanate method[9]. Briefly, 2.5 mL of phosphate buffered saline (0.02 M, pH 7.4) containing the required amount of sample was mixed with 2.5 mL of 0.02 M linoleic acid (Fluka) emulsion (contains equal weight of Tween-20 in pH 7.4 phosphate buffered saline (Sigma)) in a test tube and incubated in darkness at 40 °C. The amounts of peroxides were determined by reading absorbance at 500 nm after coloring with $FeCl_2$ and thiocyanate at intervals during incubation. To eliminate the solvent effect, the antioxidant activity of the control test sample, containing the same amount of solvent in the linoleic acid emulsion, was measured.

DPPH radical scavenging activity

This was carried out as described previously[9]. Briefly, 2 mM DPPH (Fluka) radical solution in methanol was prepared and then 0.5 mL of this solution was mixed with 3.5 mL of sample solutions in ethanol containing the required amount of test sample. After 30 min incubation in darkness, absorbance was measured at 517 nm. Decreasing DPPH solution absorbance indicates increasing DPPH radical scavenging activity. This activity is given as % DPPH radical scavenging, calculated by the equation

% DPPH Radical Scavenging = ((Control Absorbance - Sample Absorbance)/(Control Absorbance)) x 100.

The control is the DPPH solution without extracts or our pure compounds.

Determination of amounts of flavonoids

The flavonoid content was determined according to the $AlCl_3$ method[10]. Briefly, a final volume of extract solution was adjusted to 4 mL with methanol. Subsequently, 1 mL of 2% $AlCl_3$ was added and then the absorbance of sample solution was measured at 430 nm. Data were given as rutin equivalent in Figures 3a-c.

Statistical analysis

In the activity measurements, 3 measurements were made and the results were mean of these measurements. Statistical calculations were carried out using SPSS 9.0 software. To determine whether there were any differences between the activities of extracts and pure compounds, variance analysis was applied to the results and values of $P < 0.05$ were considered significantly different ($\alpha = 0.05$).

Results and Discussion

In order to determine the effect of harvesting periods of *Teucrium orientale* L. on the variation of antioxidant and DPPH radical scavenging activities, the aerial parts of this plant were collected during the budding, flowering and vegetative stages and then were extracted individually with petroleum ether, chloroform, acetone and methanol. The amounts and yields of the extracts are given in Table 1. Antioxidant activities of these extracts were evaluated using the thiocyanate method. The results are shown in Figure 1a and Figure 1b. As seen from these figures, all extracts, except the petroleum ether extracts of all stages, retarded the oxidation of linoleic acid emulsions in comparison with the control ($P < 0.05$). The acetone extracts of the budding and flowering stages had the highest antioxidant activity among the extracts tested. On the other hand, there was no significant effect of harvesting stages on the antioxidant activities ($P > 0.05$). Nevertheless, the extracts of the budding and flowering stages showed slightly higher antioxidant activity than those acetone and methanol vegetatives. The activity of the extracts that there is a obtained by polar organic solvents (acetone and methanol) was also greater than those obtained by unpolar organic solvents (chloroform and petroleum ether). Hence, it can be suggested that the polar compounds present in the herb are mainly responsible for its antioxidant activity. Similarly, the methanol and acetone extracts of the plant sample harvested at all 3 stages exhibited the highest DPPH radical scavenging activity (Figure 2b), whereas the DPPH radical scavenging activity was very low for the petroleum ether and chloroform extracts (Figure 2a). DPPH radical scavenging activity increased markedly with increasing concentration of acetone and methanol extracts. The present results suggest that there is a relationship between the antioxidant

and DPPH radical scavenging activity of the extracts of *T. orientale*. For instance, methanol and acetone extracts of the plant sample showed both the highest antioxidant activity and DPPH radical scavenging activity.

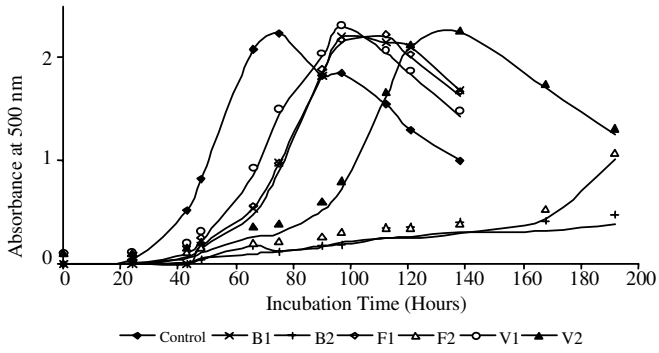


Figure 1a. Comparison of antioxidant activities of *T. orientale* extracts harvested at the budding, flowering and vegetative stages. The results are means of 3 experiments in each of which 3 measurements were obtained. B1: Budding-petroleum ether extract, B2: Budding-chloroform extract, F1: Flowering-petroleum ether extract, F2: Flowering-chloroform extract, V1: Vegetative-petroleum ether extract, V2: Vegetative-chloroform extract.

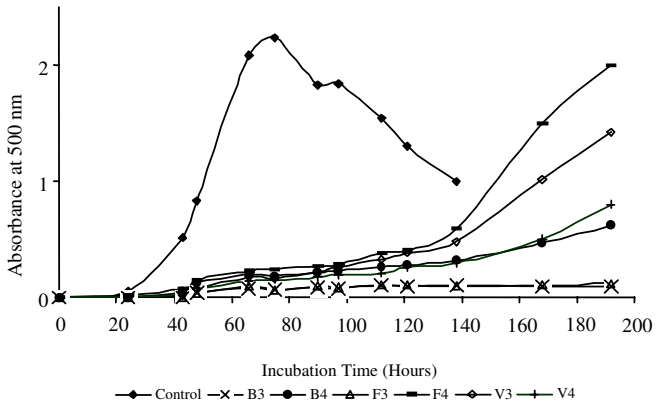


Figure 1b. Comparison of antioxidant activities of *T. orientale* extracts harvested at the budding, flowering and vegetative stages. The results are means of 3 experiments in each of which 3 measurements were obtained. B3: Budding-acetone extract, B4: Budding-methanol extract, F3: Flowering-acetone extract, F4: Flowering-methanol extract, V3: Vegetative-acetone extract, V4: Vegetative-methanol extract.

It is thought that the phenolic and/or polyphenolic compounds biosynthesized in the plant sample might be responsible for antioxidant activity[9]. It has been reported that the genus *Teucrium* contains various clerodane diterpenoids and flavonoids (mainly flavones)[11,12]. Therefore, these compounds were probably responsible for the antioxidant activities detected. Hence, total flavonoid contents of all of the extracts were determined. The amount of total flavonoid of all extracts is shown in Figure 3a-c as rutin equivalents. These figures showed that acetone extracts of the plant sample harvested at the flowering and budding stages contained higher amounts of flavonoids compared to the other extracts. Based on these results, the antioxidant and DPPH radical scavenging activities of *T. orientale* may be attributed to the high content of flavonoids.

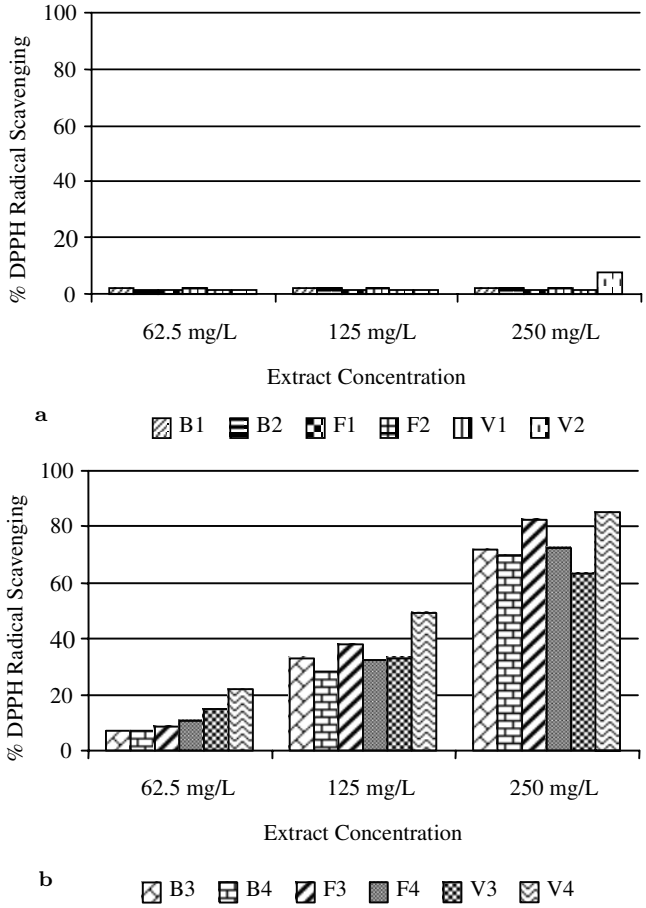


Figure 2. Comparison of DPPH radical scavenging activities of *T. orientale* extracts harvested at the budding, flowering and vegetative stages. The results are means of 3 experiments in each of which 3 measurements were obtained. B1: Budding-petroleum ether extract, B2: Budding-chloroform extract, B3: Budding-acetone extract, B4: Budding-methanol extract, F1: Flowering-petroleum ether extract, F2: Flowering-chloroform extract, F3: Flowering-acetone extract, F4: Flowering-methanol extract, V1: Vegetative-petroleum ether extract, V2: Vegetative-chloroform extract, V3: Vegetative-acetone extract, V4: Vegetative-methanol extract.

The acetone extract of *T. orientale* harvested at the flowering stages had the highest antioxidant and DPPH radical scavenging activities and amount of flavonoid. Hence, it was subjected to further separation by chromatographic methods. In the following process, 1 flavone (cirsilineol, compound **1**), 2 flavone glycosides consisting of luteolin 7-*O*-rutinoside (compound **2**) and luteolin 7-*O*-glucoside (compound **3**), 1 flavanone glycoside (hesperetin-7-*O*-rutinoside, compound **4**) and 2 iridoid glycosides, namely 8-*O*-acetyl harpagide (compound **5**) and 8-*O*-methyl harpagide (compound **6**), were isolated. The structures of the isolated compounds were characterized by UV, IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopic methods. The ^{1}H and ^{13}C NMR shifts of these compounds are presented in Tables 2, 3 and 4. The structures of the compounds were also confirmed by 2D-NMR methods (DEPT, APT, ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, HMBC and NOE tests) and by comparing with the previously reported spectral data[13−16]. Many flavonoids isolated from the species of *Teucrium* have been previously reported[11,12]. However, hesperetin-7-*O*-rutinoside was isolated from *Teucrium* species for the first time in the present study. Likewise, harpagide and 8-*O*-acetyl harpagide were isolated and characterized from the other *Teucrium* species[17,18]. Furthermore, compound **6** (8-*O*-methyl harpagide) was isolated and characterized for the first time in the present study.

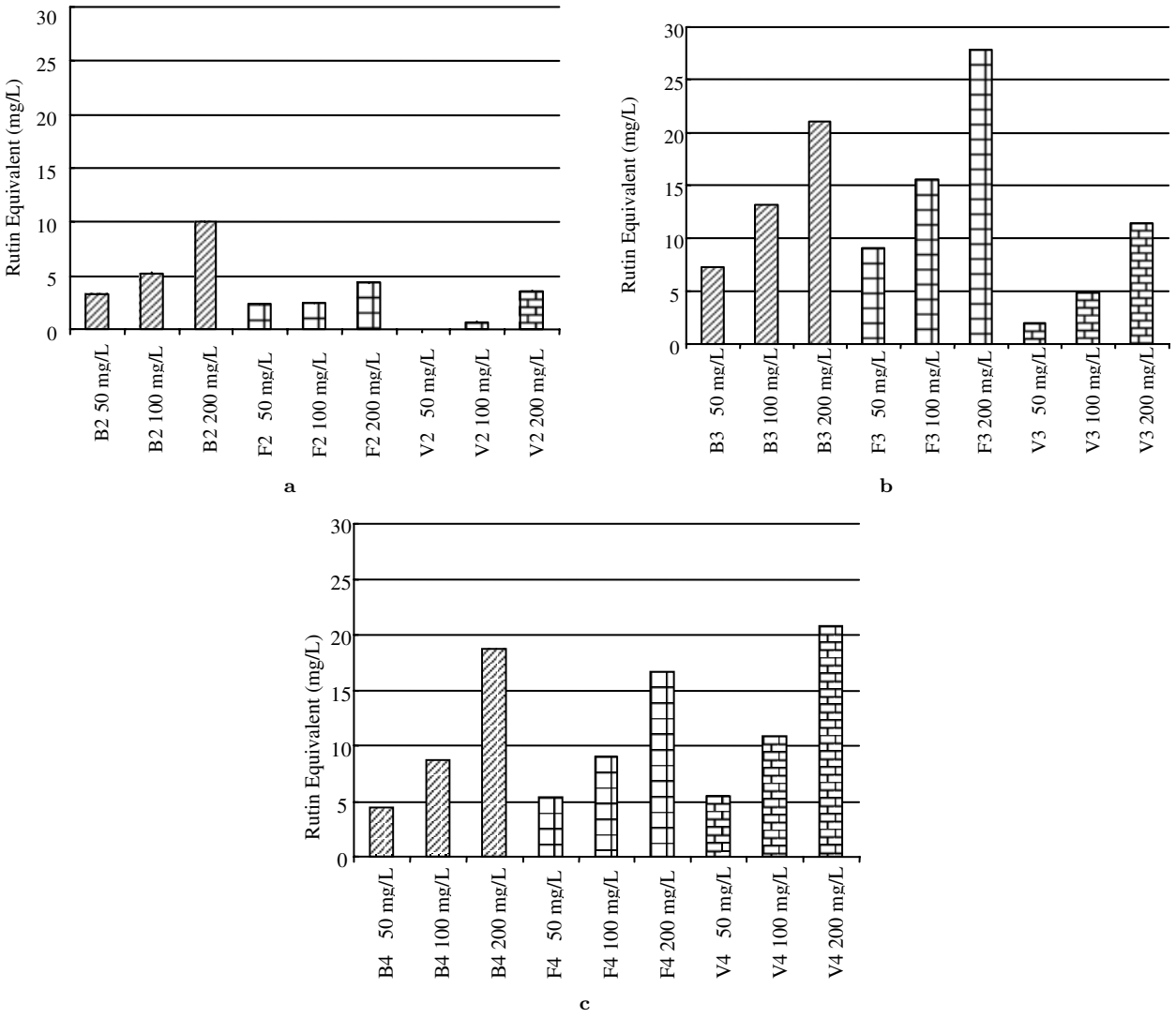


Figure 3. Comparison of total flavonoid content of *T. orientale* extracts harvested at the budding, flowering and vegetative stages. The results are means of 3 experiments in each of which 3 measurements were obtained. B2: Budding-chloroform extract, B3: Budding-acetone extract, B4: Budding-methanol extract, F2: Flowering-chloroform extract, F3: Flowering-acetone extract, F4: Flowering-methanol extract, V2: Vegetative-chloroform extract, V3: Vegetative-acetone extract, V4: Vegetative-methanol extract.

Antioxidant and DPPH radical scavenging activities of these isolated compounds were also determined. Their antioxidant potentials are shown in Figures 4 and 5. As expected, the flavonoids exhibited antioxidant and DPPH radical scavenging activities but iridoids did not. However, the antioxidant potentials of the isolated compounds were lower than that of a commercial antioxidant, BHT. The highest antioxidant and DPPH radical scavenging activities were shown by luteolin 7-*O*-glucoside and luteolin 7-*O*-rutinoside. Cirsilineol contains mainly methoxy groups and has lower antioxidant and DPPH radical scavenging activities in comparison to those flavonoids that have more free-hydroxyl groups. This supports the idea that the higher antioxidant potentials of luteolin 7-*O*-glucoside and luteolin 7-*O*-rutinoside can be attributed to their free-hydroxyl groups. It has also been reported that phenolic compounds are responsible for the antioxidant activity[9,19]. Thus, it can be concluded that the phenolic compounds containing more free-hydroxyl groups have higher antioxidant potential than their methoxy and glycoside derivatives.

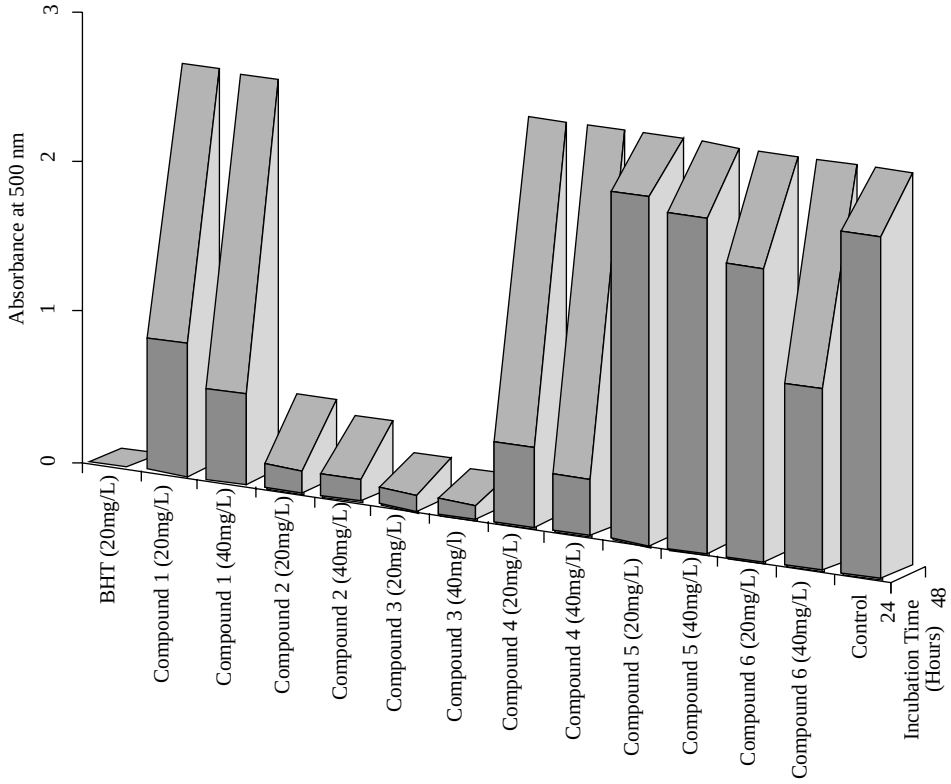


Figure 4. Comparison of antioxidant activities of the pure compounds isolated from the acetone extract of *T. orientale*. The results are means of 3 experiments in each of which 3 measurements were obtained.

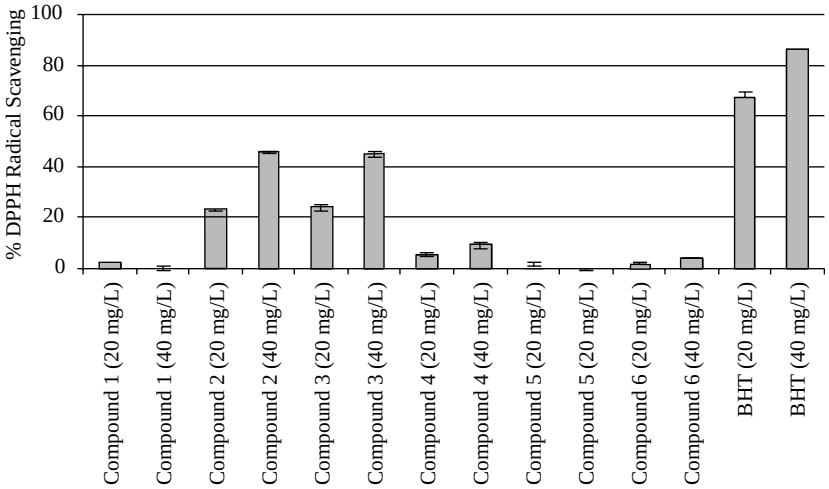
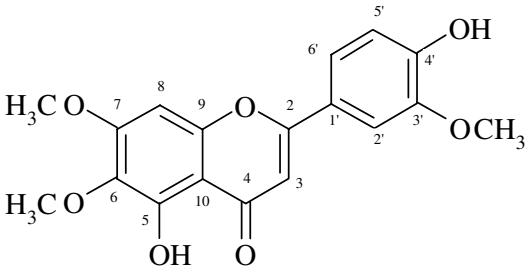
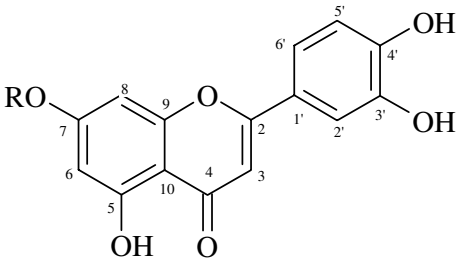


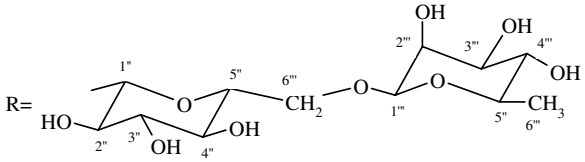
Figure 5. The DPPH radical scavenging activities of the pure compounds isolated from the acetone extract of *T. orientale*. The results are means of 3 experiments in each of which 3 measurements were obtained.

In conclusion, acetone and methanol extracts of *T. orientale* harvested at the budding, flowering, and vegetative stages and luteolin 7-*O*-glucoside and luteolin 7-*O*-rutinoside, which were isolated from the acetone extract of the flowering stage, had potent antioxidant and DPPH radical scavenging activities. These results suggest that these compounds as well as these extracts can be used as a potential antioxidant.

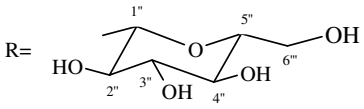


Compound **1**: Cirsilineol

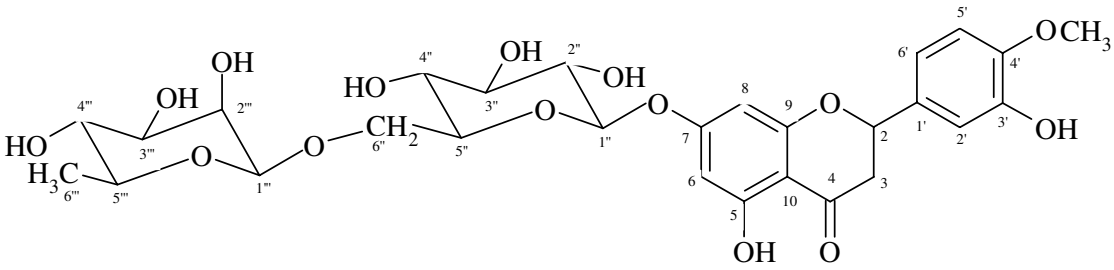




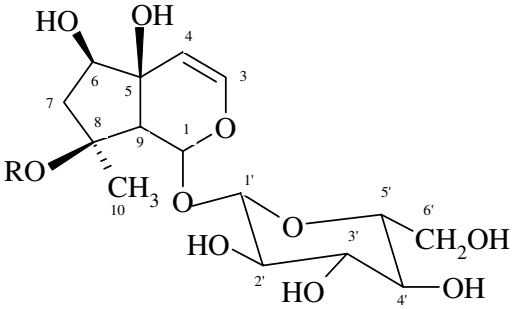
Compound **2**: Luteolin 7-*O*-rutinoside



Compound **3**: Luteolin 7-*O*-glucoside



Compound **4**: Hesperetin 7-*O*-rutinoside



Compound **5**: 8-*O*-Acetyl harpagide, R= $COCH_3$

Compound **6**: 8-*O*-Methyl harpagide, R= CH_3

Figure 6. Structures of the compounds isolated from *Teucrium orientale* L.

However, *Teucrium* species have recently been associated with hepatotoxic effects[20,21]. Therefore, further investigations are required to assess the safety and efficacy of *Teucrium orientale*.

Acknowledgment

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