

The Total Phenol, Flavonol Amounts and Antiradical Activity of some *Oreganum* Species

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ABSTRACT: Total phenol contents of *O.vulgare* were found between 54.23 mg GAE/g (U4) and 124.55 mg GAE/g (U2), respectively. While total phenol contents of *O.minutiflorum* change between 49.50 mg GAE/g (U5) and 126.92 mg GAE/g (S3), total flavonol contents were found between 623.87 mg RE/g (U5) and 854.53 mg RE/g (U2). Antioxidant capacity ranged from 42.31 mg AAE/g (U5) to 103.04 mg AAE/g (S3). Total phenol contents of *O. majorana* changed between 51.86 mg GAE/g (S3) and 125,23 mg GAE/g (S2). Antiradical activity and antioxidant capacity values were found between 695.85 $IC_{50} =$ mg/mL (U5) and 1217.51 $IC_{50} =$ mg/mL S3 and 55.43 mg AAE/g (S3) to 110.90 mg AAE/g (U5), respectively.

KEYWORDS: Total phenol; Flavonol; Antioxidant capacity; Antiradical activity.

INTRODUCTION

Phenolics, denominated as phenolic acids and phenylpropanoids, are derived from two nonphenolic molecules, benzoic and cinnamic acids, respectively. The antioxidant capacity of some herbs used in dietology practice was determined by the DPPH free radical method, which was calibrated with ascorbic acid [1-3]. Proestos et al. [4] have identified 0.34 ve 0.22 mg /100g dry ferulik acid sample and catechin. With GC-MS, (+)-catechin, cafeic acid, hydroxycafeic acid, *p*-hydroxyphenyl propionic acid, and hydroxytyrosol are found respectively as % 88.9, %61.7, % 55.2, % 41.3 and % 36.8. Total antioxidant capacity of plant material depends not only on the content and composition of phenolics but also on the contents of other antioxidants,

for example ascorbic acid [2,3]. The aim of the present work was to study the total phenol, flavonol amounts, antioxidant capacity and antiradical activity of *Oreganum* species growing in Turkey.

EXPERIMENTAL SECTION

Materials

Plant materials [*Origanum vulgare* L. subsp. *hirtum* (Link) Ietsw. (İstanbul thyme), *Origanum minutiflorum* O. Schwarz et P.H. Davis (Sütçüler thyme), *Origanum majorana* L. (Alanya thym and White thyme) and *Origanum syriacum* var. *bevanii* (Holmes) Ietswaart (Suriye thyme and İsrail thyme)] are medicinal plants growing endemically in Turkey.

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Methods

Soxhlet and ultrasonic water bath extraction

About 10g of ground plant samples are weighed and solvent solutions and samples were extracted with Soxhlet apparatus for 5h and ultrasonic water bath device (2 h) and then obtained extracts are filtered by using filter paper. Removal of solvent and water was carried out with a rotary evaporator (40 °C+ Vacuum).

Determination of total phenolic and flavonol amounts antiradical activity and antioxidant capacity

Total phenolic amount of extracted spice and tea plant species were made according to Singleton and Rossi [5] by using the Folin-Ciocalteu Calorimetric method. Total flavonols were determined using the method proposed by Dai *et al.* [6]. Standard and samples were evaluated by measuring the absorbance at 410 nm. Antiradical activity is under the influence of holding free radicals and it has been determined by using 1, 1-diphenyl-2-picrylhydrazil (DPPH) method [7]. The absorbance of the standard and the samples were measured at 517 nm. Antioxidant capacity was determined by using Phosphomolybdenum complex method [8]. Results were given in mg ascorbic acid equivalent (AAE)/g by using a calibration curve prepared from solutions with ascorbic acid.

Statistical analysis

Obtained datas were statistically evaluated using the SPSS 10.0 statistical program, the importance of differences between groups was determined with variance analysis. Identificaton of differences between groups was determined with Duncan multiple comparison test [9].

RESULTS AND DISCUSSION

Origanum vulgare

Total flavonol contents of *O.vulgare* were found between 716.86 mg RE/g (U4) and 1291.69 mg RE/g (S3). In addition, the total phenol content of this plant ranged from 54.23 mg GAE/g (U4) to 124.55 mg GAE/g (U2) (Table 1). Depending on total phenol and total flavonol contents, the antiradical activity of extracts was found higher. The total amount of phenolic substance was determined between 54.23 and 124.55mg GAE / g and application of ultrasonic water bath device (methanol: water: acetic acid solvent mixture) is determined as the most successful extraction(P<0.05). Total phenolic compounds of

Istanbul thyme samples, Exarchou *et al.* [10] in ethanol and acetone extracts 97.00-174.99 mg CAE / g extract, Dorman *et al.* [7] in water extracts 149.00 mg GAE / g extract, Sahin *et al.* [11] in water extracts 220.00 mg GAE / g extract, Capecka *et al.* [12] in methanol: water (80:20) 22:21 extracts mg GAE / g extract, Chun *et al.* [13] in ethanol: water (60:40) extracts 55.35 mg GAE / g extract had found. Total phenolic contents reported by the above researchers seemed to support our findings. The total amount of flavonols ranged from 716.86 to 1291.69 mg RE/g. The most successful extraction application extracting the total amount of flavonols as routine equivalent is Soxhlet device application, acetone: water: acetic acid solvent mixture, respectively. In order to determine the antioxidant properties, antiradical activity, and antioxidant capacity were determined as 982.57µg/mL with C50 = 721.69 and, 127.23 mg AAE / g with 73.67, respectively. The antiradical activity was determined as follows, Dorman *et al.* [7] in Istanbul thyme water extract IC50 = 335.0 Ig / mL, Sahin *et al.* [11] in methanol extract IC50 = 9.9 Ig / mL, Capecka, Mareczek, Leja (2005) 84% in methanol: water (80:20) Chun *et al.* [13] 80-82% in 60% ethanol extract. In addition, Koşar *et al.* [14] reported rosmarinic acid as one of the best radical scavenging. However, the results of the study conducted on other species support our findings. Mensore *et al.* [15] and Refaei *et al.* [16] reported antioxidant capacity to differ according to solvents used.

Origanum minutiflorum

Total phenol contents of *O.minutiflorum* changed between 49.50 mg GAE/g (U5) and 126.92 mg GAE/g (S3) (Table 2). Total flavonol contents were found between 623.87 mg RE/g (U5) and 854.53 mg RE/g (U2). The antiradical activity was found high and changed between 796.18 IC₅₀=mg/mL (U1) and 1266.60 IC₅₀=mL (U3). In addition, antioxidant capacity ranged from 42.31 mg AAE/g (U5) to 103.04 mg AAE/g (S3). The lowest amount of total phenolic substances (49.50 mg GAE / g), with U5 application, the highest amount was found (126.92 mg GAE / g) extracts obtained with S3 applications. The total amount of flavonoid was found between 622.66 and 854.53.69µg RE/g (P <0.05). Exarchou *et al.* [10] reported that the amounts of ethanol and acetone extract were found between 97.00 and 174.99 mg CAE/g, Dorman *et al.* [7] water extract

Table 1: Total phenolic extracts, flavonol amounts, antioxidant capacity and values related to the antiradical activity of *O.vulgare* (n:3).

	Total phenol (mg GAE/g)	Total flavonol ($\mu\text{g RE/g}$)	Antiradical activity ($\text{IC}_{50}=\mu\text{g/mL}$)	Antioxidant capacity (mg AAE/g)
S1**	108.33 \pm 0.00 c	856.94 \pm 1.46 g	727.41 \pm 0.74 h	109.60 \pm 1.34 d
S2	114.75 \pm 1.17 b	893.17 \pm 1.27 f	721.69 \pm 1.04 j	116.62 \pm 1.27 c
S3	101.90 \pm 1.55 d	1291.69 \pm 1.67 a	794.37 \pm 1.06 c	126.39 \pm 1.71 a
S4	91.76 \pm 1.55 e	995.82 \pm 1.71 d	786.42 \pm 0.72 d	110.75 \pm 1.40 d
U1	92.43 \pm 1.17 e	1027.22 \pm 0.91 c	760.91 \pm 3.57 f	121.20 \pm 2.39 b
U2	124.55 \pm 1.01 a	884.72 \pm 1.31 f	738.47 \pm 1.04 g	127.23 \pm 0.48 a
U3	89.73 \pm 0.59 f	1215.61 \pm 1.11 b	862.41 \pm 2.45 b	82.67 \pm 0.13 e
U4	54.23 \pm 0.59 h	716.86 \pm 1.17 h	982.57 \pm 4.54 a	73.67 \pm 1.49 g
U5	56.60 \pm 1.01 g	936.65 \pm 0.84 e	767.74 \pm 4.54 e	77.48 \pm 0.13 f

*means in the same raw with the same letters are not significantly different ($p<0.05$). **S1- methanol:aceton:water:acetic acid (55:40:4.5:0.5) with soxhlet apparatus;S2- methanol:water:acetic acid (95:4.5:0.5) with soxhlet apparatus;S3- aceton:water:acetic acid (95:40:4.5:0.5) with soxhlet apparatus;S4- ethanol:water:acetic acid (95:40:4.5:0.5) with soxhlet apparatus; U1- methanol:aceton:water:acetic acid (55:40:4.5:0.5) with ultrasonic water bath;U2- methanol:water:acetic acid (95:4.5:0.5) with ultrasonic water bath;U3- aceton:water:acetic acid (95:40:4.5:0.5) with ultrasonic water bath;U4- ethanol: water: acetic acid (95:40:4.5:0.5) with ultrasonic water bath;U5-water:acetic acid (95:40:4.5:0.5) with ultrasonic water bath

Table 2: Total phenolic extracts, flavonol amounts, antioxidant capacity and values related to antiradical activity, Duncan multiple comparison test results* (n:3) for *Sütçüler Thyme* extract.

	Total phenol (mg GAE/g)	Total flavonol ($\mu\text{g RE/g}$)	Antiradical activity ($\text{IC}_{50}=\mu\text{g/mL}$)	Antioxidant capacity (mg AAE/g)
S1**	107.99 \pm 2.11 d	738.59 \pm 0.42 b	975.27 \pm 0.67 c	81.68 \pm 1.62 e
S2	103.93 \pm 2.11 ef	622.66 \pm 0.91 e	870.08 \pm 0.62 d	89.77 \pm 1.03 d
S3	126.92 \pm 0.59 a	843.66 \pm 2.93 a	827.22 \pm 1.25 e	103.04 \pm 1.06 a
S4	119.14 \pm 2.55 b	662.51 \pm 2.55 d	973.99 \pm 1.23 c	89.69 \pm 2.34 d
U1	115.43 \pm 1.01 c	727.73 \pm 1.71 bc	796.18 \pm 4.84 f	97.09 \pm 0.53 b
U2	105.96 \pm 1.17 de	854.53 \pm 1.3a	839.37 \pm 2.47 e	94.57 \pm 1.08 c
U3	91.08 \pm 0.00 g	703.57 \pm 1.58 c	1266.60 \pm 0.44 a	69.78 \pm 1.18 g
U4	102.24 \pm 1.01 f	722.89 \pm 0.75 bc	968.25 \pm 1.98 c	78.55 \pm 1.38 f
U5	49.50 \pm 1.76 h	623.87 \pm 1.26 e	1200.16 \pm 1.23 b	42.31 \pm 1.03 h

*means in the same raw with the same letters are not significantly different ($p<0.05$). **S1- methanol:aceton:water:acetic acid (55:40:4.5:0.5) with soxhlet apparatus;S2- methanol:water:acetic acid (95:4.5:0.5) with soxhlet apparatus;S3- aceton:water:acetic acid (95:40:4.5:0.5) with soxhlet apparatus;S4- ethanol:water:acetic acid (95:40:4.5:0.5) with soxhlet apparatus; U1- methanol:aceton:water:acetic acid (55:40:4.5:0.5) with ultrasonic water bath;U2- methanol:water:acetic acid (95:4.5:0.5) with ultrasonic water bath;U3- aceton:water:acetic acid (95:40:4.5:0.5) with ultrasonic water bath;U4- ethanol:water:acetic acid (95:40:4.5:0.5) with ultrasonic water bath;U5-water:acetic acid (95:40:4.5:0.5) with ultrasonic water bath

Table 3: Total phenolic extracts, flavonol amounts, antioxidant capacity and values related to antiradical activity, Duncan multiple comparison test results* (n:3) for Syria thyme extract.

	Total phenol (mg GAE/g)	Total flavonol (μ g RE/g)	Antiradical activity (IC ₅₀ = μ g/mL)	Antioxidant capacity (mg AAE/g)
S1**	98.86 \pm 2.55 b	718.06 \pm 2.54 f	522.02 \pm 0.93 g	102.20 \pm 1.73 a
S2	50.51 \pm 3.04 e	517.60 \pm 1.05 g	818.44 \pm 2.51 b	63.06 \pm 0.79 f
S3	65.73 \pm 2.03 d	1236.14 \pm 2.20 b	982.73 \pm 1.04 a	62.91 \pm 0.53 f
S4	65.73 \pm 2.68 d	889.55 \pm 1.27 e	744.39 \pm 0.42 c	78.70 \pm 1.26 e
U1	86.01 \pm 2.03 c	1128.66 \pm 2.18 c	673.23 \pm 1.48 e	90.60 \pm 1.95 b
U2	105.96 \pm 3.10 a	1443.86 \pm 1.27 a	737.03 \pm 0.74 c	84.12 \pm 3.67 d
U3	88.72 \pm 2.11 c	917.32 \pm 2.02 e	611.30 \pm 0.86 f	100.45 \pm 2.00 a
U4	97.17 \pm 3.66 b	1086.39 \pm 1.11 d	692.14 \pm 1.25 d	87.17 \pm 0.13 c
U5	67.08 \pm 2.56 d	718.06 \pm 2.38 f	610.65 \pm 1.09 f	82.44 \pm 0.73 d

*means in the same raw with the same letters are not significantly different ($p < 0.05$). **S1- methanol:acetone:water:acetic acid (55:40:4.5:0.5) with soxhlet apparatus; S2- methanol:water:acetic acid (95:4.5:0.5) with soxhlet apparatus; S3- acetone:water:acetic acid (95:40:4.5:0.5) with soxhlet apparatus; S4- ethanol:water:acetic acid (95:40:4.5:0.5) with soxhlet apparatus; U1- methanol:acetone:water:acetic acid (55:40:4.5:0.5) with ultrasonic water bath; U2- methanol:water:acetic acid (95:4.5:0.5) with ultrasonic water bath; U3- acetone:water:acetic acid (95:40:4.5:0.5) with ultrasonic water bath; U4- ethanol:water:acetic acid (95:40:4.5:0.5) with ultrasonic water bath; U5-water:acetic acid (95:40:4.5:0.5) with ultrasonic water bath

149.00 mg GAE / g, Şahin et al. (2004) water extract 220.00 mg GAE / g, Skerget et al. [17] methanol extract 186.00 mg GAE / g, Capecka et al. [12] methanol: water (80:20) extract 22:21 mg GAE / g, Chun et al. [13] ethanol: water (60:40) extract 55.35 mg GAE / g found in extracts. Antiradical activity and antioxidant capacity were found respectively between IC₅₀ = 796.18 and 1266.60, μ g / mL and between 42.31 AAE 103.04 mg / g. Dorman et al. [7] in İstanbul thyme water extract IC₅₀ = 335.0 μ g / mL. The highest amounts of extracts were obtained with U1 for antiradical activity and S3 for antioxidant capacity. Exarchou et al. [10] studied using ethanol and acetone and two different solvents in extraction, extracts antiradical activity compared using DPPH method. Our findings were compatible with the results of other researchers.

Origanum syriacum

The total flavonol and antiradical activity values of *O. syriacum* extracts were found higher compared with total phenol and antioxidant capacity values (Table 3). While total phenol contents of extracts ranged from 517.60 mg RE/g (S1) to 1443.86 mg RE/g (U2), antiradical activity values were found between 522.02 IC₅₀= mg/mL (S1) and 982.73 IC₅₀= mg/mL (S3). Total

phenolic substance and flavonoid amounts were found between 50.51 and 105.96 / g and 517.60 and 1443.86 μ g RE GAE / g, respectively. U2 was determined as the most successful application for both phenolic substance and flavonoid amounts. Kosar et al. [14] determined rosmarinic acid, as we found as a major component, as one of the best radical scavengers. Antioxidant capacity was found between 62.91-102.20 mg AAE / g. The highest antiradical activity was established in extract obtained with the application of S1. When variance analysis results scanned, for Syria thyme extracts' total phenolic substance, flavonol amounts, antioxidant capacity, and antiradical activity values were found statistically important at $p < 0.05$ level was determined to be important.

Origanum majorana

While total phenol and total flavonol contents of *O. majorana* ranged from 51.86 mg GAE/g (S3) to 125.23 mg GAE/g (S2) and 389.59 mg RE/g (S3) to 1093.64 mg RE/g (U5), antiradical activity and antioxidant capacity values were found between 695.85 IC₅₀= mg/mL (u5) to 1217.51 IC₅₀= mg/mL S3 and 55.43 mg AAE/g (S3) and 110.90 mg AAE/g (U5) (Table 4). Alanya thyme extracts' total phenolic substance and flavonoid

Table 4: Total phenolic extracts, flavonol amounts, antioxidant capacity and values related to antiradical activity, Duncan multiple comparison test results* (n:3) for Alanya thyme (*Origanum majorana*) extract.

	Total phenol (mg GAE/g)	Total flavonol (μ g RE/g)	Antiradical activity (IC ₅₀ = μ g/mL)	Antioxidant capacity (mg AAE/g)
S1**	76.21 \pm 0.59 f	625.08 \pm 3.48 de	781.76 \pm 1.76 f	80.84 \pm 0.35 f
S2	125.23 \pm 0.59 a	834.00 \pm 1.31 b	670.75 \pm 3.01 h	107.85 \pm 0.35 a
S3	51.86 \pm 2.11 g	389.59 \pm 1.51 f	1217.51 \pm 0.61 a	55.43 \pm 1.18 g
S4	92.43 \pm 1.17 c	695.12 \pm 4.73 c	773.90 \pm 0.88 f	92.05 \pm 2.87 d
U1	76.88 \pm 0.00 f	570.73 \pm 1.00 e	853.00 \pm 0.00 c	84.20 \pm 0.00 e
U2	85.33 \pm 1.76 d	668.55 \pm 5.69 cd	782.01 \pm 0.97 e	98.31 \pm 3.43 c
U3	85.33 \pm 0.59 d	670.97 \pm 4.12 cd	875.22 \pm 0.43 b	104.41 \pm 0.61 b
U4	82.29 \pm 0.59 e	657.68 \pm 4.39 cd	828.88 \pm 0.14 d	98.01 \pm 0.48 c
U5	98.18 \pm 2.55 b	1093.64 \pm 1.67 a	695.85 \pm 0.31 g	110.90 \pm 2.74 a

*means in the same raw with the same letters are not significantly different ($p < 0.05$). **S1- methanol:acetone:water:acetic acid (55:40:4.5:0.5) with soxhlet apparatus; S2- methanol:water:acetic acid (95:4.5:0.5) with soxhlet apparatus; S3- acetone:water:acetic acid (95:40:4.5:0.5) with soxhlet apparatus; S4- ethanol:water:acetic acid (95:40:4.5:0.5) with soxhlet apparatus; U1- methanol:acetone:water:acetic acid (55:40:4.5:0.5) with ultrasonic water bath; U2- methanol:water:acetic acid (95:4.5:0.5) with ultrasonic water bath; U3- acetone:water:acetic acid (95:40:4.5:0.5) with ultrasonic water bath; U4- ethanol:water:acetic acid (95:40:4.5:0.5) with ultrasonic water bath; U5- water:acetic acid (95:40:4.5:0.5) with ultrasonic water bath

amounts were determined between 51.86-125.23 mg GAE / g and 389.59-1093.64 μ g RE / g, respectively. Exarchou et al. [10] in ethanol and acetone extracts 97.00-174.99 mg CAE / g extract, Dorman et al. [7] in water extract 49.00 mg GAE / g extract, Sahin et al. [11] in water extract 220.00 mg GAE / g extract, Skerget et al. [17] in methanol extract 186.00 mg GAE / g extract, Capecka et al. [12] in methanol: water (80:20) extract 22:21 mg GAE / g extract, Chun et al. [13] in ethanol: water (60:40) extract 55.35 mg GAE/g extract had found. Antioxidant capacity and antiradical activity values, respectively, IC₅₀ = 670.75-1217.51 μ g/mL and 55.43-110.90 mg AAE / g were determined. To determine antioxidant features, for antiradical activity the lowest amount capturing 50% of free radicals determined with S2 application. When variance analyse results scanned, for Alanya thyme extracts' total phenolic substance, flavonol amounts, antioxidant capacity, and antiradical activity values, statistical difference between the extracts at $p < 0.05$ level was determined to be important.

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