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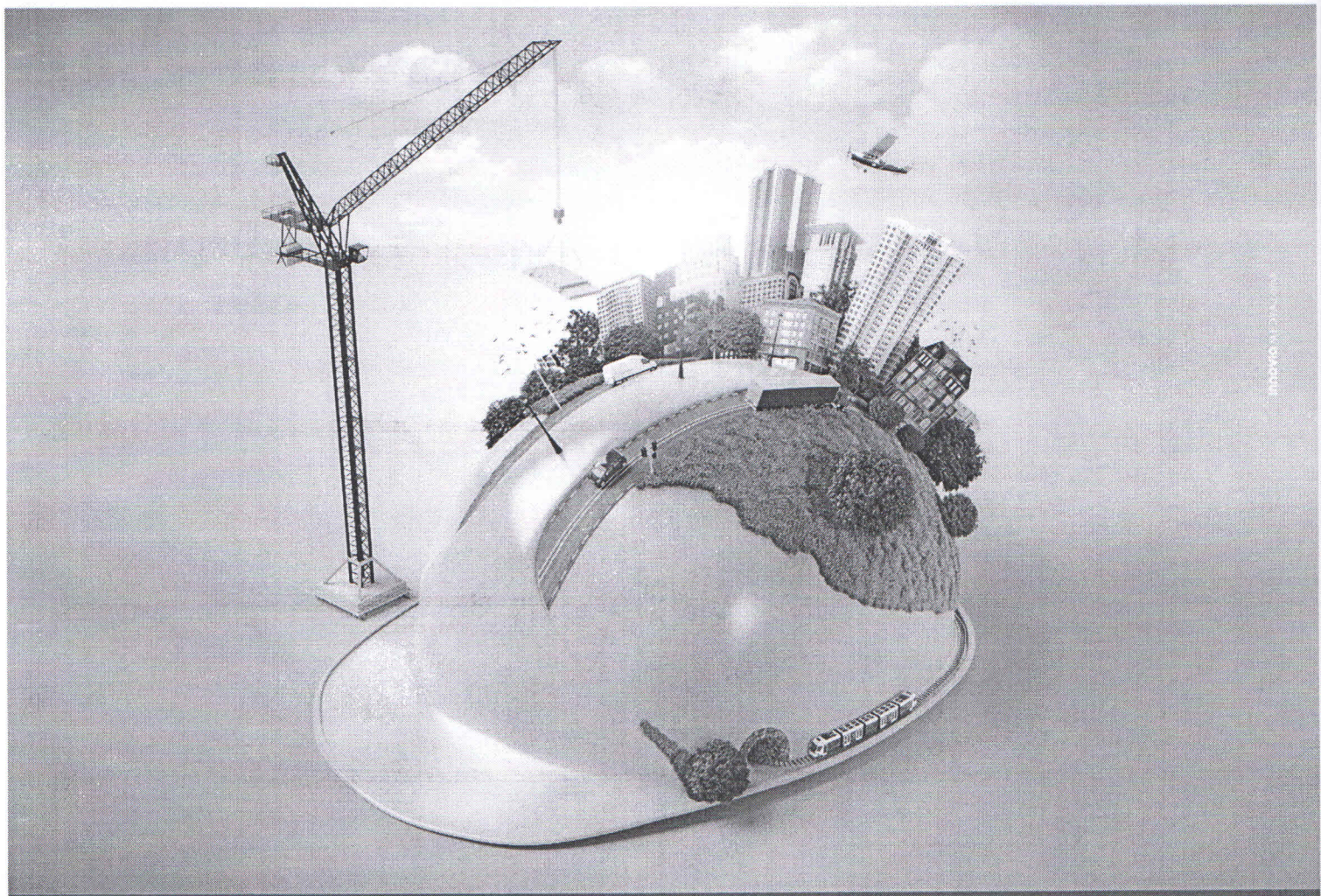

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Essential Oil Composition and Antioxidant Activity of *Saponaria prostrata* WILLD. subsp. *anatolica* HEDGE

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ABSTRACT

In this study, we investigated the chemical composition and antioxidant activity of the essential oil of *Saponaria prostrata* WILLD. subsp. *anatolica* HEDGE. The essential oil of plant was obtained by hydrodistillation method and analyzed by HS-SPME/GC-MS. Thirty-nine compounds were identified, which representing 91.77% of the oil. Among them Palmitic acid (36.53%), Pentanal (17.38%), 2-Pentadecanone, 6,10,14-trimethyl (9.68%), 2,6,10-Trimethyl,14-Ethylene-14-Pentadecne (5.96%) and 1,8-Diazacyclotetradecane-2,9-dion (4.64%) were the major compounds. The antioxidant capacity of *S. prostrata* subsp. *anatolica* was evaluated by different methods and results were compared with BHA, BHT, vit.E, vit. C, EDTA and trolox.

Keywords: Essential oil, Antioxidant activity, *Saponaria*, Caryophyllaceae, HS-SPME/GC-MS.

1 INTRODUCTION

Plant kingdom, including medicinal and dietary plants, offers many natural phytochemicals, e.g. phenolic diterpenes, flavonoids and phenolic acids. Antioxidant, anti-inflammatory and anticancer activities of these compounds might help prevention of oxidative damage [1-3]. There is a growing interest in determining the phytochemicals and valuable compounds of herbs [4]. The genus *Saponaria*, which belongs to Caryophyllaceae family, is annual, biennial or perennial herbs and divaricately branched, with decumbent prostrate or ascending branches and a dense indumentum of long, spreading, white hairs (rarely with viscid hairs). Leaves oblong-spathulate or ovate, obtuse, petiolate. Corymbs many-flowered, dense. Pedicels 1-3 mm. Calyx narrowly cylindrical, inflating in fruit, 9-14 mm, with ovate, acute teeth. Petals rose, 11-14 mm, with a distinct lamina which has two narrow coronal scales at the base, and a linear claw. *S. prostrata* subsp. *anatolica* is an endemic species and distributed in different regions of Turkey [5]. In this study we report the composition and antioxidant activity of the essential oil from the aerial parts of *S. prostrata* subsp. *anatolica* collected from the east region of Turkey.

2 MATERIALS AND METHODS

2.1 Plant Material

S. prostrata subsp. *anatolica* was collected during May 2014, from Kirişli village around the province of Bingol in Turkey (1300 m). The plant material was identified with volume 2 of Flora of Turkey and East Aegean Islands and was deposited in the Herbarium of the Department of Biology, University of Bingol, Turkey (Voucher No: BIN-HER-1391) [5].

2.2 Isolation of Essential Oil

Finely dry powdered aerial parts of *S. prostrata* subsp. *anatolica* (200 g) were subjected to hydrodistillation using Clevenger-type apparatus for 4 h. The obtained oil was collected, dried over anhydrous sodium sulphate and was stored in vials at low temperature prior to analysis.

2.3 GC-MS Analysis

Analysis of the essential oil was performed using a gas chromatography–mass spectrometry (GC–MS) Agilent 5975C series GC/MSD with Triple-Axis HED-EM detector (Agilent Technology Inc., Santa Clara, CA, USA), equipped with HP-5 MS capillary column packed with fused silica (30 m×0.25 mm i.d., 0.25 μm film thicknesses) and interfaced with a HP chemstation. Helium was carrying gas and flow rate of helium was 1 mL/min. Oven temperature was programmed, 60 °C (1 min, isothermal) to 246 °C (3 min, isothermal) at 3 °C/min. The temperature of injector was 250 °C and for detector it was 300 °C. The ionization energy was 70 eV. Identification of the constituents was based on computer matching against commercial Mass Finder 2.1 Library, Wiley and MS literature data [6]. All the analysis were performed in triplicate.

2.4 HS-SPME Procedure

The extraction of volatile compounds was carried out bya HS-SPME (headspace solid phase microextraction) method using a DVB/CAR/PDMS fiber, with 50/30 μm film thickness; before the analysis the fiber was preconditioned in the injection port of the GC as indicated by the manufacturer. 6.0 g of the grounded and homogenized sample was weighed into a 40 mL vial and suspended in 14.5 mL; the vial was equipped with a "mininert" valve (Supelco, Bellafonte, PA, USA). The vial was kept at 35 °C with continuous internal stirring and the sample was left to equilibrate for 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35 °C. After sampling the SPME fiber was introduced into the GC injector, and was left for 3 min to allow the analytes thermal desorption. In order to optimize the technique, the effects of various parameters, such as sample volume, sample headspace volume, sample heating temperature and extraction time, were studied on the extraction efficiency as previously reported by Verzera et al [7]. Each measurement was repeated in triplicate.

2.5 Metal Chelating Activity on Ferrous Ions

Metal-chelating abilities of essential oil of *S. prostrata* subsp. *anatolica* and standards (EDTA and vit. E) were estimated by the method of Dinis *et al.* [8]. Briefly, each extract at different concentrations (133.5, 200.25, 263.5, 327, 390, 453 μg/mL) was added to a solution of 2 mM $FeCl_2.4H_2O$ (25 μl) and this mixture incubated at room temperature for 60 min. After 5 mM ferrozine (100 μl) was added then, the final mixture was shaken vigorously and left stand at room temperature for 10 min until stable absorption values were obtained. The absorbance of the mixture was raed at 562 nm.

2.6 Reducing power assay

The reducing power of essential oil was determined by the method of Oyaizu, with minor modification [9]. The essential oil at different concentrations (125-437.5 μg/mL) were mixed with sodium phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1 mL, 1%). The mixture was incubated at 50 °C for 20 min. After the incubation, trichloroacetic acid (1 mL, 10%) was added to the mixture, followed by centrifuging at 3000 rpm for 10 min. The upper layer of solution (1 mL) was mixed with distilled water (1 mL) and ferric chloride (0.2 mL, 0.1%), and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated increased reductive power. BHT and vit. C were used for comparison. Analysis was repeated in triplicate and the mean value was reported.

2.7 ABTS Radical Scavenging Activity

Radical scavenging capacity of SEO was determined via the ABTS (2,2%-azino-bis-3-ethyl-benzthiazoine-6-sulphuric acid) radical, generated by a metmyoglobin/hydrogen peroxide system, as described previously [4]. Plant essential oil (10 µl) was added to a 1cm pathlength spectrometer cuvette (1 ml capacity) containing 20 mM phosphate buffered saline pH 7.4, 2.5 mM metmyoglobin and 150 mM ABTS. The reaction was initiated by addition of 75 mM hydrogen peroxide, and the absorbance change at 734 nm monitored at 30°C. BHA and Trolox were used for comparison.

3 RESULTS AND DISCUSSION

Thirty nine compounds representing 91.77% of the oil were identified and compounds, their retention times and percentages are summarized in Table 1.

Table 1 Essential oil composition of *Saponaria prostrata* subsp. *anatolica*

No	Retation Time	Compound	Percentage
1	6.982	Cyclododecanone-2,12,12-D4 Trimethylamine	0.87
2	7.869	Pentanal	**17.38**
4	8.830	Butanal, 3-Methyl	1.19
5	10.306	Hexanal	1.76
6	12.360	1,6-Octadiene, 5,7-dimethyl	0.77
7	15.033	Furan, 2-pentyl-2-n-Pentylfuran	0.71
8	16.154	Octanal	0.42
9	16.612	Benzaldehyde	1.17
10	19.181	Phenylacetaldehyde	0.45
11	19.313	Nonanal	1.96
12	19.885	Cyclohexasiloxane. dodecamethyl	0.15
13	20.486	1,6-Octadiene, 5-Methyl-, (Z)	0.18
14	20.932	1-Nonanol	0.14
15	21.990	Camphor	0.14
16	22.385	n-decanal	0.95
17	24.159	Cycloheptasiloxane, Tetradecamethyl	0.11
18	25.332	1,2-Cyclohexanedimethanamine, cis	0.14
19	29.961	4-Dodecene, (Z)	0.14
20	30.127	1-Tetradecanol	0.16
21	31.632	1,3-Tetradecenal	0.16
22	32.216	Hexadecane	0.31
23	34.659	Tetradecanal	0.15
24	35.077	Heptadecane	0.39
25	36.175	1,2-Benzenedicarboxylic Acid, Diethyl Ester	0.33
26	36.479	Cyclododecane	0.35
27	37.468	Lauric aldehyde	0.40
28	37.823	36.74 Heptadecane	0.23
29	40.341	Nonadecane	0.13
30	41.365	1-Tetradecene	0.10
31	42.183	Methyl Palmitate	0.11
32	42.681	Eicosane	0.03
33	47.728	2-Undecene, (Z)	0.06
34	55.733	Hexanedioic acid, bis(2-ethylhexyl) ester	1.70
35	62.931	1,8-Diazacyclotetradecane-2,9-dion	**4.64**
36	32.111	Tetradecane	1.72
37	48.871	2-Pentadecanone, 6,10,14-trimethyl	**9.68**
38	52.596	Palmitic acid	**36.53**
39	55.875	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	**5.96**
	Total		**91.77**

The major compounds of SEO were Palmitic acid (36.53%), Pentanal (17.38%), 2-Pentadecanone, 6,10,14-trimethyl (9.68%), 2,6,10-Trimethyl,14-Ethylene-14-Pentadecne (5.96%) and 1,8-Diazacyclotetradecane-2,9-dion (4.64%). Other components (<2.0 and > 1.0 %) identified in the oil were Nonanal (1.96%), Hexanal (1.76%), Tetradecane (1.72%), Hexanedioic acid, bis(2-ethylhexyl) ester (1.70%), Butanal, 3-Methyl (1.19%) and Benzaldehyde (1.17%). Also many minor compounds (<1.0 %) were identified in the oil.

Metal chelating power of SEO, vit. E and EDTA is presented in Fig 1. Significant differences in the chelating power were observed amongst the concentrations. At a concentration of 390 µg/mL, the chelating power of ferrous ions was following order absorbance; EDTA (0.113), vit. E (0.842) and SEO (1.050).

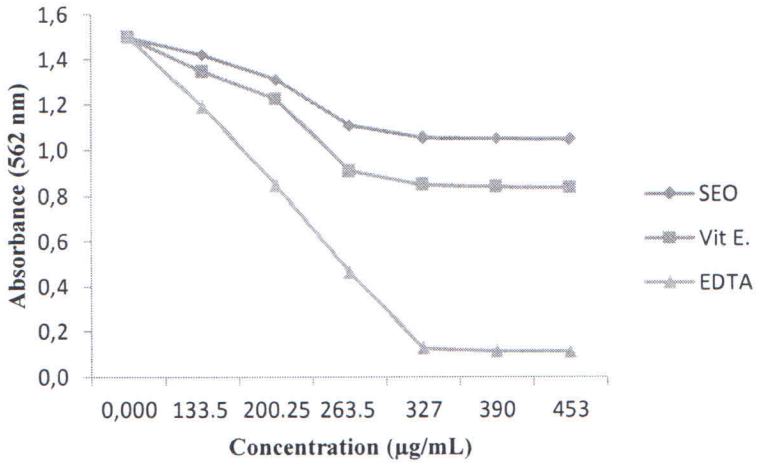

Figure 1: Metal Chelating capacity of SEO and standarts.

The reducing power increased with the concentration of essential oil. The absorbance of Fe^{3+}-Fe^{2+} transformation of 375 µg/mL concentration were found as 1.91; 1.64; 0.96, respectively. The sequence for reducing power was BHT > vit. C > SEO (Fig 2).

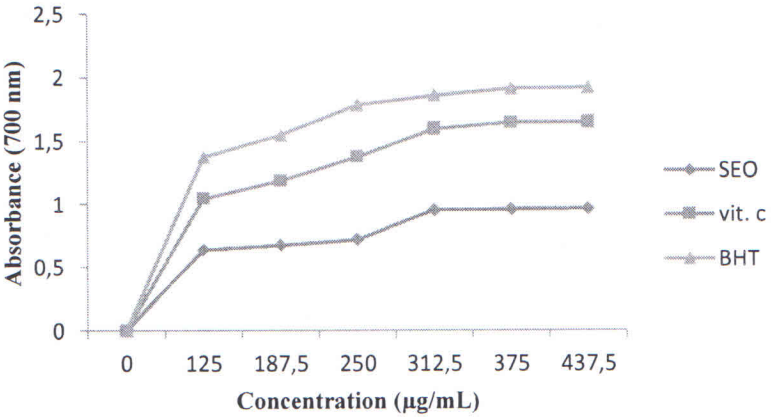

Figure 2: Reducing power of SEO and standarts.

The results of ability to scavenge the ABTS radical following the order Trolox > BHA > SEO which presented values at 133 μg/mL of 0.059; 0.091; 0.210, respectively (Fig 3).

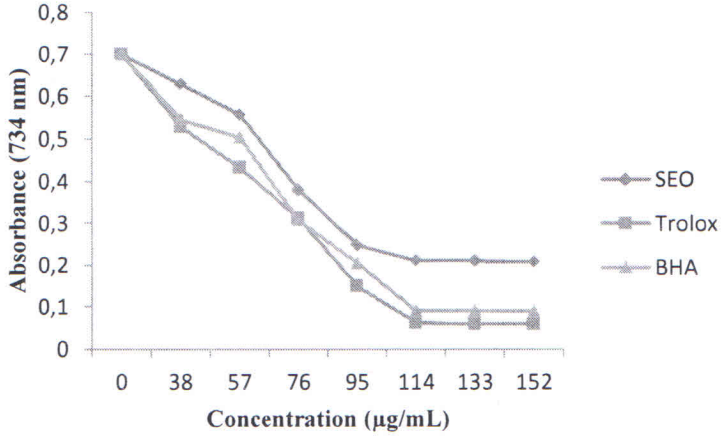

Figure 3: ABTS of SEO and standarts.

4 CONCLUSION

This is the first study of chemical composition and antioxidant activity of *S. prostrata* subsp. *anatolica*. Caryophyllaceae family have remarkable rate of endemism and medical importance. The results clearly indicate that *S. prostrata* subsp. *anatolica* present effective antioxidant activities in different experiment systems and it has a lot of chemical compounds. The obtained results suggesting that this herb may be useful for human health.

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