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Antioxidant properties of volatile oils obtained from *Artemisia taurica* Willd. and *Salvia kronenburgii* Rech. Fil. plants and their effects on xanthine oxidase

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In this study, the antioxidant properties of volatile oils obtained from the earth parts of the *Artemisia taurica* Willd. and *Salvia kronenburgii* Rech. Fil. plants and their effects on xanthine oxidase enzyme were studied. The chemical contents of each volatile oil were determined by applying gas chromatograpghy-mass spectrometry (GC-MS) analysis. The total phenol and flavonoid amounts of each volatile oil and the total antioxidant capacities were determined and it was observed that there was a positive correlation among these values. It was found out that the volatile oils were effective on inhibiting the reactive oxygen kinds and that they scavenged the superoxide radical made up with xanthine-xanthine oxidase system and the hydroxyl radical made up with Fe^{+3} ascorbate EDTA-H_2O_2 system. Moreover, it was observed that both volatile oil samples reduced the 2,2-diphenyl-1-picrylhydrazyl (DPPH), a determined independent. It was also found out that *A. taurica* and *S. kronenburgii* volatile oils inhibited the xanthine oxidase enzyme and that *Artemisia* volatile oil was more effective on inhibiting this enzyme than *Salvia* volatile oil. When the inhibition kinetics were studied, it was observed that the inhibition kinds was successively competitive inhibition for *A. taurica* and uncompetitive inhibition for *S. kronenburgii.*

Key words: *Artemisia taurica* Willd, *Salvia kronenburgii* Rech.Fil, antioxidant activity, free radical, xanthine oxidase.

INTRODUCTION

Changing living conditions, environmental pollution, industrial wastes, solar rays, exhaust gases, heavy metals, cigarette, alcohol, ozone and miscellaneous chemicals are inescapable elements for today's humanity. These unfavourable conditions lie behind the nascence of free radicals (Dorman et al., 2000; Candan et al., 2003). Free radicals may damnify the basic cellular constituents such as lipid, protein, DNA, carbonhydrate and enzyme (Çakatay and Kayalı, 2006; Prakash et al., 2011). It is thought that the cellular injury associated with free radicals contributes to the complication of several chronic diseases. It is thought that the cellular injury resulting from free radicals conduces to the complications of cardiovascular diseases, and many chronic and neuro-degenerative diseases like diabet mellitus, cancer, and aging (Fu et al., 2011; Prasad et al., 2012).

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Abbreviations: XO, Xanthine oxidase; **DPPH,** 2,2-diphenyl-1-picrylhydrazyl; **TBA,** thiobarbituric acid; **NBT,** nitrobluetetrazolium; **EDTA,** ethylenediaminetetraacetate; **TCA,** trichloroacetic acid; BHT, butylated hydroxytoluene; **GC-MS,** Gas chromatography-mass spectrometry.

The enzyme xanthine oxidase (XO) (EC. 1.1.3.2) is a Mo-containing flavoprotein from the oxidoreductase group (Pei and Li, 2000; Çete et al., 2005). Xanthine oxidase is one of the vital sources of superoxide anion which is a crucial free oxygen radical. This enzyme is a metalo flavoprotein existing in all nucleate cells. It is known as a member of the enzyme group functioning in hydroxylation of molybdenum, iron and flavine in catalyzing the hydroxylation of purines. It functions as a rate limiting enzyme in purine nucleotide metabolism. Xanthine oxidoreductase is found in two forms convertible to each other. These are xanthine dehydro-genase and xanthine oxidase (Cai and Harrison, 2000). While xanthine dehydrogenase reduces both NAD^+ and oxygen, XO reduces only O_2 and, as a consequence, it forms O_2^-• and H_2O_2. Both enzymes catalyze the con-version of hypoxan-thine to xanthine and the conversion of xanthine to uric acid. Uric acid is the last product of purine destruction in humans (Champe and Harvey, 1997). In the meantime, molecular oxygen is converted to super-oxide anion by way of reduction (Li and Jackson, 2002).

Today, many plants found in their natural flora are both used as a folkremedy because of their contentand studied academically in medical researches. Especially essential (volatile) oils have been used in medication since antiquity. After it was discovered that they have lots of functional characteristics as well as antimicrobial, antioxidant and anticancer activities, studies on the bioactive principles of essential oils extracted from various plants and spices have gained popularity because adverse effects that are not or hardly seen in natural drugs are found with significantly higher quantities in synthetic drugs (Candan et al., 2003; Vardar et al., 2003).

Labiatae are a large family with nearly 200 genera and 3000 species, including some plants like sideritis, sage, mint and thyme. This family is important in terms of medicinal and aromatical plants (Satıl et al., 2007). Among the Labiatae family, Salvia L. is one of the most common genera with shrubs and herbaceous forms spreading naturally from the temperate zones to the hot zones across the world. It contains approximately 900 species all over the world. In Turkey, this genus is identified with 89 species and 94 taxons, and 50% of these is reported to be endemic (Başer, 2002). *Salvia kronenburgii* in the genus Salvia L. is an endemic grown in 1500-2500 metre-high regions peculiar to the Lake Van basin. The localities where *S. kronenburgii* is found are of brown and chestnut soil.

The plants were collected in steep, sloping, shallow stony and highly erosive soil. The genus has a cluster-like, shrubbier, and quite deep taproot. Its stem is puberulent, quadrangular, upright and ascendant. *Salvia* species are highly rich in essential oil. Since the ancient times, many plants peculiar to this species have been used for therapeutic purposes and as odorizer and flavour as well as ornamental plants in food industry as they have aromatic effects (Demirci et al., 2003). Diterpenoids obtained from the *Salvia* species are reported to have antioxidant, antibacterial or cytotoxic features (Perry et al., 2003).

Asteraceae is known to be the richest family of angiospermae with approximately 1000 genera and 20000 species (Bağcı and Kocak, 2008). Most of the plants are two or more years old, nonarboreal, in the form of shrub or tree. In this family, essential oil, inuline, and latex are the most common compounds. Owing to these compounds, most of the plants are used in pharmaceutics, food and other industrial fields (Seçmen et al., 1998; Tanker et al., 2004).

Artemisia taurica Willd. from the genus *Artemisia* is generally 900-1900 m high, and it spreads in prairies and steppes. It is a perennial species of Artemisia with an upright and ramified stem, ascendant up to 60 cm, with lignified leaves at the bottom, upper parts with dense white hairs, fragmental leaves with white hairs on both sides (Davis, 1975). Artemisia species have also been used for medicinal purposes since the time of ancient Egyptians. Nearly 20 Artemisia species are grown in Anatolia and some of these are used for medicinal purposes under different names (Baytop, 1999). Artemisia species are generally used in pharma-ceutics, perfumery, and flavouring industry (Scora and Kumamoto, 1984). They are used as appetizer, roborant and stimulant by the people. They have an antifebrile effect as well (Baytop, 1991).

Given these characteristics, this study aims to conduct a chemical analysis regarding the essential oils of *A. taurica* Willd. and *S. kronenburgii* Rech.Fil., and to examine whether they show antioxidant effects by scavenging free radicals (superoxide, hydroxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH)), and their effect on xanthine oxidase enzyme, thus to ensure the conscious consumption, and to light the way for the next pharmacological studies on those plants.

MATERIALS AND METHODS

Used chemicals

In this study, we procured gallic acid, quercetin, allopurinol, thiobarbituric acid (TBA), nitrobluetetrazolium (NBT), xanthine, xanthine oxidase from Sigma (USA) Company; ethylenediaminetetraacetate (EDTA), trichloroacetic acid (TCA), $FeCl_3$, K_2HPO_4, KH_2PO_4, NaH_2PO_4, $NaHPO_4$, $AlCl_3$, butylated hydroxytoluene (BHT), hydrogen peroxide, folin reagent, ascorbic acid, methanol, hexane, HCl, H_2SO_4 chemicals from Merck (Germany) Company, and deoxyribose from Aldrich-Chem.

Used plant materials

Supraterra parts of *S. kronenburgii* Rech.Fil. were collected from the dip slopes in the site of Kurubaş Geçidi (Kurubaş Strait) in northwest of Gürpınar/Van in May 2008; those of *A. taurica* Willd. were collected from the northern slopes of Zernek Dam in Gürpınar/Van in August 2007.

Table 1. Plants' essential oil yields.

Örnekler	Percentage (v/w)
Artemisia Taurica Willd.	0.590 ± 0.065
Salvia Kronenburgii Rech.Fil.	0.716 ± 0.035

Obtaining essential oil from plant material

One hundred gram (100 g) herbal samples that were exsiccated in the shade and powderized were subjected to steam distillation with 500 mL water via Clevenger apparatus for 3 h. Obtained essential oils were preserved at + 4°C.

Gas chromatography-mass spectrometry (GC-MS) analysis of essential oils

Qualitative and quantitative analyses of the essential oils were conducted in Adana Çukurova University via Thermo Finnigan-Trace GC-MS using an autosampler. By using split method, a sample size of 1 µL was injected at the split rate of 50. Chromatographic separation was done with the split mode injection at the split rate of 50 via TR-MS capillary column (5%-phenyl 95%-dimethylpolysiloxane, length= 60 m, inner diameter=0.25 mm and film thickness 25 µm). The analysis was performed at a flow rate of 1 mL min^{-1}. by using helium carrier gas. The column temperature was programmed with 3°C min^{-1}. increases between 50-250°C. Temperatures of injection and ion source were 250 and 200°C, respectively. An ionization voltage of 70 eV and a mass range of 41-400 a.m.u. were applied. Separated components were determined by comparing the data of NIST and Wiley mass spectral library.

Determining the total phenolic content

The Folin-Ciocelteu reagent was used in determining the total phenolic content of the essential oil (Gamez-Meza et al., 1999). Phenolic content of the essential oils was given as gallic acid equivalent to mg g^{-1} oil.

Determining the total flavanoid content

In determining the flavonoid content of the essential oils, a methanolic $AlCl_3$ solution of 1 mL 2% was added to 1 mL essential oil dissolved in methanol. Absorbance of the samples was read against the check sample at the wavelength of 394 nm (Lamasion et al., 1990). Total flavanoid quantity of the essential oils was given as quercetin equivalent to mg g^{-1} oil.

Determining the total antioxidant capacity

This method is based on the formation of green phosphate/Mo(V) complex in acidic pH by reducing acidic Mo (VI) to Mo (V) (Prieto et al., 1999). Total antioxidant capacity of the essential oils was given as mM α-tocopherol acetate g^{-1} oil.

DPPH radical scavenging activity

In order to determine the obtained essential oils' feature of scavenging the DPPH radical, a DPPH solution of 5 mL (% 0.004) was added to the solutions in different concentrations of the essential oils which were diluted with methanol, and it was incubated for 30 min. Absorbance of the samples was read against the check sample at the wavelength of 517 nm (Cuendet et al., 1997; Al-Reza et al., 2010).

Hydroxyl radical scavenging activity

The obtained oils' feature of scavenging the hydroxyl radical was determined by measuring the thiobarbituric acid-reactive substances revealed with the deformation of deoxyribose by the hydroxyl radicals formed via the system of Fe^{+3}/ascorbate/EDTA/H_2O_2 (Kunchandy and Rao, 1990).

Superoxide radical scavenging activity

Scavenging of the superoxide radical by the essential oils of *Salvia* and *Artemisia* was determined with the reduction of NBT by the superoxide radical formed via xanthine/xanthine oxidase system (Robak and Gryglewski., 1988; Lee et al., 2002 Al-Reza et al., 2010).

Xanthine oxidase studies

Activity of xanthine oxidase (XO) (EC. 1.1.3.2.) was assessed according to the method of Prajda and Weber with spectrophotometric measurement of the absorbance increase at 293 nm during the formation of uric acid from xanthine (Prajda and Weber, 1975).

RESULTS

Essential oil products of plants

Plants' essential oil yield were given as dry plant of mL/100 g in Table 1.

GC-MS analysis of essential oil of *Salvia kronenburgii* Rech. Fill.

The chromatogram according to the GC-MS analysis of *Salvia* essential oil is presented in Figure 1, and the components, retention time and relative availibility precentages of the essential oil are given in Table 2.

GC-MS analysis of essential oil in *Artemisia taurica* Willd.

The chromatogram according to the GC-MS analysis of *Artemisia* essential oil is presented in Figure 2, and the components, retention time and relative availibility precentages of the essential oil are given in Table 3.

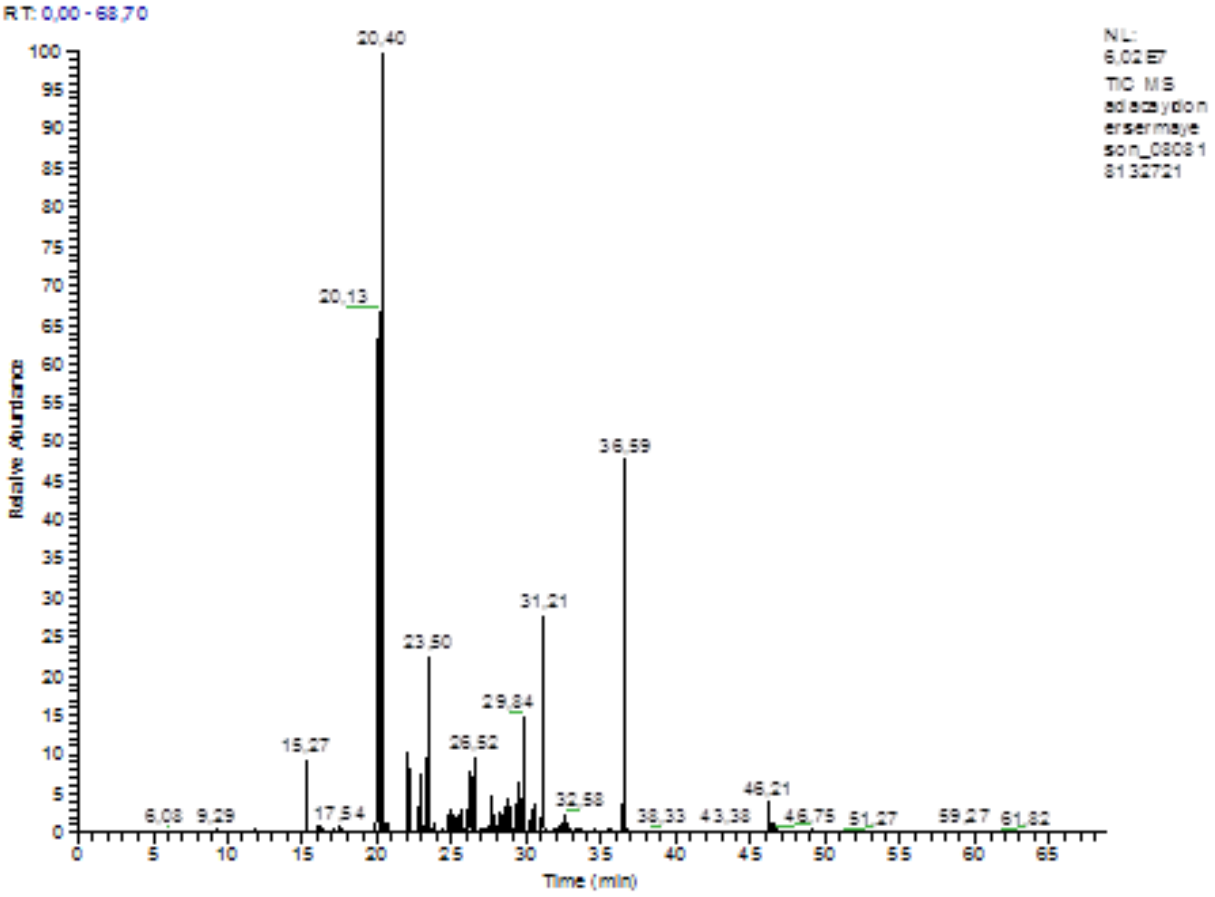

Figure 1. Essential oil chromatogram of *Salvia kronenburgii.*

Table 2. Chemical composition of the essential oil from *Salvia kronenburgii*.

Number	Component	RT	Area	Composition
1	α-Pinene	15.27	15909137	1.58
2	Camphane	16.17	1296833	0.13
3	β-Pinene	17.54	1327824	0.13
4	o-Cymene	19.86	1430258	0.14
5	DL-Limonene	20.13	207236972	20.62
6	Eucalyptol	20.4	248721241	24.75
7	(E)-β-ocimene	20.62	2181889	0.22
8	Linalool oxide trans	22.12	21507022	2.14
9	Linalool oxide cis	22.93	16966827	1.69
10	Linalool	23.5	58134949	5.78
11	6-Methyl-hepta-3,5-dien-2-one	23.9	2284962	0.23
12	α-Camphenolic aldehyde	25.17	3880727	0.39
13	2,6-dimethyl-1,3,5,7-octatetraene	25.63	6846047	0.68
14	trans- Pinocarveol	26.03	6963486	0.69
15	(E)-3-Caren-2-ol	26.21	21725354	2.16
16	β-Terpineol	26.29	2586624	0.26
17	L(-)-Camphor	26.52	22545951	2.24
18	Dihydrocarveol	27.21	2073294	0.21
19	α-Terpinenol	27.4	2106112	0.21
20	Borneol	27.64	8431329	0.84

Table 2. Contd.

21	cis-p-mentha-2,8-dien-1-ol	27.77	4510259	0.45
22	4-carvomenthenol	27.84	2084334	0.21
23	p-mentha-6,8-dien-2-ol	28.22	6065094	0.60
24	o-Acetyltoluene	28.36	4193256	0.42
25	α-Terpinenol	28.61	7963446	0.79
26	1,2-dimethyl-3-vinyl-1,4-cyclohexadiene	28.83	9375753	0.93
27	Terpinyl asetat	29.2	1793994	0.18
28	1-Verbonene	29.44	12813970	1.28
29	L-Carveol	29.84	48806273	4.86
30	Isobornyl formate	30.2	2739070	0.27
31	cis-Carveol	30.46	6631774	0.66
32	Geraniol formate	30.57	8710832	0.87
33	Cis-Geraniol	30.88	3210580	0.32
34	Carvone	31.21	83426017	8.30
35	p-Mentha-1,8-dien-3-on	32.34	1671112	0.17
36	cis-Carvon oxsit	32.58	4545397	0.45
37	p-mentha-1,8-dien-7-al	32.69	2775185	0.28
38	Geranyl Acetate	36.59	127360711	12.67
39	Dehydroaromadendrene	46.21	6966524	0.69
40	Ledene oxide	46.46	2327805	0.23
Total				99.37

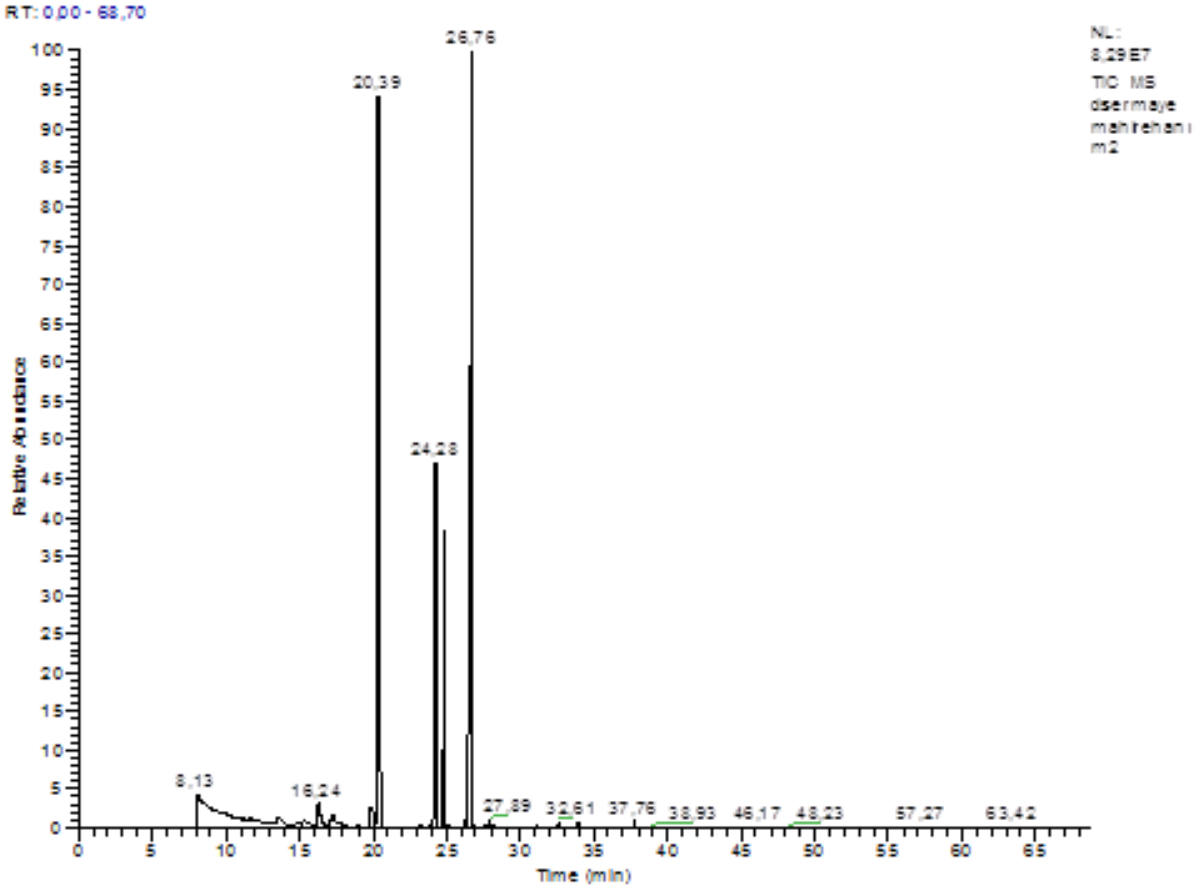

Figure 2. Essential oil chromatogram of *Artemisia taurica*.

Table 3. Chemical composition of essential oil from *Artemisia taurica*.

Number	Component	RT	Area	Composition
1	Santolina triene	13.58	13138504	0.58
2	α- Pinene	15.34	8123008	0.36
3	Camphene	16.24	39954195	1.75
4	Sabinene	17.23	11457823	0.50
5	o-Cymene	19.88	27318607	1.20
6	Eucalyptol	20.39	810254681	35.57
7	Trans-Caryophyllene	24.28	317842287	13.95
8	Thujone	24.84	178052243	7.82
9	Comphor	26.76	858268028	37.68
	Total			99.42

Table 4. Total phenol and total flavonoid value, total antioxidant capacity of essential oils *Artemisia taurica* and *Salvia kronenburgii*.

Sample	Total phenol (mg gallic acid g^{-1} oil)	Total Flavonoid (mg quercetin g^{-1})	Total antioxidant capacitiy (mM α-Tocopherol acetate g^{-1})
Artemisia essential oil	5.137 ± 0.050	7.984 ± 0.233	438.4 ± 4.198
Salvia essential oil	10.848 ± 0.077	12.600 ± 0.224	818.4 ± 7.563

Total phenol-flavonoid quantity and total antioxidant capacity of essential oils

Total phenol quantity, total flavonoid quantity and total antioxidant capacity of the obtained essential oils are shown in Table 4.

DPPH radical scavenging activity

As a result of our studies and calculations, the highest percentage in inhibiting the DPPH radical by herbal essential oils and positive controls, and their values of concentration values (IC_{50}) that inhibit the DPPH radical by 50% are shown in Table 5.

Hydroxyl radical scavenging activity

Table 5 demonstrates the highest inhibition percentage shown by essential oils and positive controls in inhibiting the formation of hydroxyl radical and their concentration that inhibits the formation of hydroxyl radical by 50%.

Superoxide radical scavenging activity

The highest inhibition percentage shown by essential oils and positive controls in inhibiting the superoxide formation and their concentration that inhibits the formation of superoxide radical by 50% are given in Table 5.

Xanthine oxidase studies

The highest percentage shown by essential oils in xanthine oxidase inhibition and IC_{50} values are demonstrated in Table 5. Maximum velocity (V_{max}) and substrate concentration at half of the maximum velocity (K_m) calculated via the Lineweaver-Burk plot which was drawn to determine the inhibition type are shown in Table 6.

DISCUSSION

Plants are the main sources of natural antioxidant compounds. Antioxidant compounds existing in fruits, vegetables, spices, vegetable oils and seeds have been discussed in lots of researches, and it has been shown that their antioxidant effects stem from phenolic compounds, particularly from the flavonoid structure (Dapkevicius et al., 1998; Merken et al., 2001). Antioxidant effect of phenolic compounds originates from their characteristics such as taking electron or hydrogen atom from free radicals, scavenging free radicals by ending the chain reactions, solvating with transition metals, and preventing or reducing the singlet oxygen atom (Cotelle, 2001).

Generally phenolic compounds are represented by flavonoids, tannins and phenolic acids (Giampieri et al., 2012). Flavonoids are one of the functional nutrients and important components having antioxidant features (Cotelle, 2001). Flavonoids show their antioxidative activity by way of inhibiting such enzymes as xanthine

Table 5. The highest percentage in inhibiting the DPPH, hydroxyl, superoxide anion radical and xanthine oxidase by *Artemisia* and *Salvia* essential oils and positive controls, and their concentration values (IC_{50}) which inhibit the DPPH, hydroxyl, superoxide anion radical and xanthine oxidase by 50%.

Parameter	Inhibition (%)	IC_{50} (µL mL^{-1})
DPPH		
Artemisia essential oil	82.175 ± 0.366	14.238 ± 0.129
Salvia essential oil	83.712 ± 0.408	7.438 ± 0.100
BHT	52.420 ± 0.990	24.210 ± 0.926
Ascorbic acid	60.000 ± 1.699	16.752 ± 0.804
Hydroxyl radical		
Artemisia essential oil	76.206 ± 0.344	0.404 ± 0.016
Salvia essential oil	78.275 ± 1.503	0.192 ± 0.001
BHT	58.212 ± 0.276	29.984 ± 1.149
Superoxide radical		
Artemisia essential oil	60.000 ± 4.810	0.205 ± 0.047
Salvia essential oil	57.142 ± 3.092	0.335 ± 0.045
BHT	52.891 ± 0.858	55.242 ± 1.196
Ascorbic acid	50.217 ± 0.716	82.512 ± 3.854
Xanthine Oxidase		
Artemisia essential oil	75.29 ± 0.1	0.321 ± 0.3
Salvia essential oil	41.03 ± 0.7	-

Table 6. The Vmax and Km values obtained from the interaction of Xanthine Oxidase with the different concentrations of *Artemisia taurica* and *Salvia kronenburgii* essential oils.

Essential oil	Concentration (µL mL^{-1})	Vmax (µmol dk^{-1})	K_m (µM $^{-1}$)
Artemisia taurica	Control	0.0309	90.91
	0.64	0.0309	161.29
	0.32	0.0309	133.33
	0.156	0.0308	107.52
Salvia kronenburgii	Control	0.0329	90.91
	0.3	0.0284	80.00
	0.8	0.0213	60.61
	1.0	0.0149	47.85

oxidase, lipoxygenase, cyclooxygenase, forming chelation with metal ions, interacting with other antioxidants, grabbing such free radicals as superoxide anions, lipid peroxyl and hydroxyl. Flavonoids have antiviral, anti-allergic and antitumor features as well.

We examined the total antioxidant capacity, total phenol and flavonoid content of the both essential oils we used in our study. It is seen that there is a direct proportion between the total antioxidant capacity and the total phenol-flavonoid quantities of those essential oils.

GC-MS analysis was carried out in order to determine the chemical composition of the essential oils obtained from the plants. As a result of the analysis, camphor, 1.8-cineole and trans caryophyllene were detected as the first three main compounds in the essential oil of *A. taurica*. As a result of the GC-MS analysis of the essential oil of *S.*

kronenburgii which is endemic to the vicinity of Van, 1.8-cineole, limonene and geranyl acetate were detected as the first three main compounds. Given the first three main compounds of the two essential oils in consequence of their GC-MS analyses, we see that 1.8-cineole is the shared compound for the essential oils of *Artemisia* and *Salvia*. 1.8-cineole has antibacterial, sedative, antilaryngiticand hypotensive features. Furthermore, it is a monoterpene used in treating such diseases as asthma and bronchitis due to its antiinflammatory effect (Juergens et al., 2003; Faleiro et al., 2003).

Being the second main compound in the essential oil of *Salvia*, L-limonen is a monoterpene commonly found in citrus and several other plant species. Due to its fragrance, it is used in cosmetic industry and cleaning products. Studies proved that limonen has antiinflammatory, anticancer and antimicrobial effects; according to some experiments on animals, it has also an antitumor effect. In addition to these, it is effective in cardioatherosclerosis (Mazzanti et al., 1998; Sever and Özbek, 2005; Özbek et al., 2007). The third main compound of the *Salvia* essential oil, geranyl acetate is described to have sedative, antibacterial, antivirus and antidepressant effects (Lima et al., 1996; Duarte et al., 2006).

Camphor, the first main compound of the *Artemisia* essential oil, is a bicyclic monoterpene found in many plantal structures in nature. It is antibacterial and has some features to invigorate blood circulation, to clear up cardiac dysfunction and pneumonopathy (Baricevic et al., 2001; Mirva et al., 2001; Capek et al., 2003). Caryophyllene, the third main compound constituing 19.95 percent of the *Artemisia* essential oil, is reported to show an antiinflammatory feature (Kamatou et al., 2005; Duarte et al., 2006).

Since antiquity, it is known that plants, plant extracts and essential oils have antimicrobial, antioxidant, antifungal among others effects to different extents. Extracts of medicinal plants are used as a preserver in foods and as raw materials in a great deal of sectors such as medicine, pharmaceutics, perfumery and cosmetics. Therefore, they have been scrutinized in several studies and there are significant results related to their antioxidant effects (Nielson and Rios, 2000; Karanika et al., 2001). Given the DPPH radical-inhibition percentages of the essential oils, the highest DPPH radical-scavenging activity was observed in the essential oil of *Salvia*. When we looked at the essential oil concentrations which scavenged 50 percent of the DPPH radical, we observed a high radical-scavenging effect in Salvia parallel to its percental inhibition value.

Hydroxyl is the most harmful free radical for molecules in living cells such as DNA base, lipids, aminoacids and carbonhydrates. It was found that the destruction of deoxyribose by the hydroxyl radical generated via the system of Fe^{+3}/ascorbate/EDTA/H_2O_2 is inhibited by the studied oil samples.

In our study, it was seen that the essential oils of *Artemisia* and *Salvia* are more effective than the positive controls in scavenging the hydroxyl radical. As it is known, superoxide radical is a reactive oxygen species which is generated primarily and most easily in enzymatic and non-enzymatic reactions in both environmental agents and organisms. Due to its long half-life, it can be moved to further regions from its birthplace. So it is a significant radical in biological systems. When we compared the superoxide radical-inhibition percentages of the essential oils, we found that the essential oil of *Artemisia* is more effective than that of *Salvia*.

In the last stage of our experiment, we examined the effect of essential oils on the enzyme xanthine oxidase. As it is known, xanthine oxidase is one of the crucial sources of superoxide anion. It catalyzes the reactions which convert hypoxanthine to xanthine and xanthine to uric acid. Mean while, molecular oxygen is reduced and then converted to superoxide anion. There are a great deal of studies about plant extracts' inhibition of xanthine oxidase and their inhibition kinetics (Sweeney et al., 2001; Candan, 2003; Filha et al., 2006; Lin et al., 2008). It has been found out that some flavonoids like chrysene, luteolin, flavone and quercetin cause xantine oxidase inhibition as well (Nagao et al., 1999). When inhibitions of essential oils were assessed according to IC_{50}values, it was seen that *Artemisia* is the most effective essential oil in the inhibition of xanthine oxidase with a 75 percent inhibition value (IC_{50}= 0.321 ± 0.3 µL mL^{-1}). It was seen that *Salvia* essential oil has a 41 percent inhibition value; its IC_{50} value was not observed because it did not show an activity to inhibit 50 percent of xanthine oxidase.

As it was seen that the essential oils contain inhibitory for the enzyme xanthine oxidase, their inhibition kinetics was examined to determine the inhibition type of the reaction. For this purpose, a Lineweaver-Burk plot was drawn and values of V_{max} and K_m were determined for the reactions. While V_{max} did not change with the increasing concentrations of the *Artemisia* essential oil, K_m increased, so it was put forth that the inhibition type is competitive. On the other hand, despite the increasing concentrations of the *S. kronenburgii* essential oil, both V_{max} and K_m decreased, so it was put forth that the inhibition type is uncompetitive.

In consequence of the all in-vitro studies, it was observed that the essential oils of *A. taurica* and *S. kronenburgii* are effective in inhibiting reactive oxygen species and xanthine oxidase. It was seen that the *Artemisia* essential oil is more effective than *Salvia*'s in inhibiting the superoxide radical and xanthine oxidase; on the other hand, the Salvia essential oil is effective in scavenging the hydroxyl radical and DPPH. In accordance with all these results, it is observed that these plants are antioxidant and can be used in treating numerous diseases caused by reactive oxygen species. It may even be used in treating such diseases as gout just as allopurinol that is an xanthine oxidase inhibitor. However, these prospective cogitations need to be sup-

ported by *in vivo* studies.

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