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Original article

Antioxidant potencies and chemical compositions of essential oils of two endemic species grow in Turkey: *Astragalus oocephalus* subsp. *stachyophorus* and *Astragalus sericans*

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Summary. *Astragalus* species are both economic and medicinal plants that are used in the world for years. We investigated essential oil compositions and antioxidant activities of the essential oils from dried aerial parts of two endemic *Astragalus* taxa, which are *Astragalus sericans* Freyn & Sint. (AS) and *Astragalus oocephalus* Boiss subsp. *stachyophorus* Hub.-Mor. & Chamb. (AOS). The essential oils isolated by Clevenger apparatus and GC-FID and GC-MS analysis resulted in the identification of twenty seven and thirty nine compounds representing 94.79 and 96.76 % of the total oil, respectively. The main compounds were α-Pinene (19.18%), 1,5,5-Trimethyl-6-methylene-cyclohexene (16.34%), and 2,5,5-Trimethyl-1,3,6-heptatriene (9.64%) in *A. sericans* essential oil, whereas Camphor (18.25%), γ-Terpineol (16.74%), 1,8-Cineole (16.35%), and α-Pinene (12.82%) were the major constituents of *A. oocephalus* subsp. *stachyophorus*. It was concluding that the essential oils; α-Pinene chemotype in *A. sericans* and Camphor chemotype in *A. oocephalus* subsp. *stachyophorus* was in plants from eastern Anatolian region of Turkey. Inhibition of lipid peroxidation was 70.36±0.75% for AS and 37.22±0.23% for AOS at concentration of 200 μg/ml. Reducing powers of essential oils and their scavenging effects were effective when compared with Vit. E and BHT. IC_{50} values were 44.43±1.09 μg/ml for AS and 134.22±1.31 μg/ml for AOS. These essential oils can be used in medicinal and pharmaceutical purposes.

Key words: essential oils, GC-MS, antioxidant activity, *Astragalus*

Introduction

Plant essential oils are so valuable for many industrial areas including pharmaceutical, perfume, cosmetic and food, because these essential oils have natural preservative effects and have therapeutic properties such as cytotoxic, antioxidant, antinociceptive, antimicrobial, antiproliferative, antifungal and insecticidal activities (1-3). The 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 (4, 5). There is a rising interest in endemic species because they may be used for the production of raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (6). Some plants synthesize large amounts of vitamin C, vitamin E, and carotenoids, which are wellknown antioxidants. Phenolic compounds are also widely distributed in

plants and have been found to possess antioxidative potential as well (7).

Some of *Astragalus* species are used as a stabilizer, thickener and emulsifier in food, pharmaceutical and cosmetic industries (8). Medicinal and economical importance of various species of the *Astragalus* genus have been made it attractive for chemical constituents analysis in recent years (9-11).

Many researchers have published papers on the essential oil compositions of various *Astragalus* species, but unfortunately there is no information about the essential oil composition and bio-availability of *A. oocephalus* subsp. *stachyophorus* and *A. sericans*. These endemic species can be useful in many industrial field such as perfume, cosmetic, food and agricultural as well as therapeutic applications for medical purpose.

For the reasons mentioned above, the essential oils of these particular species were selected for the phytochemical and biological activity assays. A comparative GC-FID/MS analysis and antioxidant activities were conducted on two endemic *Astragalus* taxa grows in its natural habitat of Turkey's eastern regions for the first time.

Material and Method

Plant material

During the flowering stage the aerial parts of *A. oocephalus* subsp. *stachyophorus* and *A. sericans* species were collected in July 2014, from Şabanlı and Yelesen villages surrounding of Bingöl province in eastern part of Turkey. The plant materials were identified with volume 3 of Flora of Turkey and East Aegean Islands (12), and were deposited in the Herbarium of the Department of Biology, University of Bingol, Turkey (Voucher No: BIN3241 for *A. oocephalus* subsp. *stachyophorus* and BIN3242 for *A. sericans*).

Isolation of essential oil

Essential oils from aerial part of AOS and AS were obtained by hydrodistillation for 4 h, using 100 g of plant material in a Clevenger-type apparatus.

The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, stored in an amber vial at low temperature (4°C) prior to analysis.

Gas chromatography–mass spectrometry (GC-MS)

The essential oils obtained from the aerial parts of AS and AOS were analyzed using a Perkin-Elmer GC-MS system. Gas chromatograph equipped with an automatic liquid sampler coupled to a mass selective detector in the electron impact mode (Ionization energy: 70 eV). GC analysis was performed on HP-5MS 5% phenylmethylsiloxane capillary column (30 m 0.25 mm, i.d., 0.25 μm film thickness) equipped with a flame ionization detector (FID). The oven temperature was set at 60°C for 2 min initially, followed by an increase of 4°C/min to 240°C in a 59 min total run and a solvent delay of 3 min was selected. Injector and detector temperatures were set at 210°C and 280°C, respectively. The samples were injected using a volume of 1 μl and used operating in the split mode injection (1:100). Helium was used as a carrier gas at a constant pressure mode (flow rate: 1 ml/min). All mass spectra were recorded in the scan mode at 70 eV (40–550 m/z). Repeatability was verified by analyzing the sample three times and retention indices were calculated for all components using a homologous series of n-alkanes injected in conditions equal to the sample one. The fragmentation patterns of mass spectra were also compared with those stored in the spectrometer database, spectrometric electronic libraries (WILEY, NIST).

Antioxidant capacity by the ferric thiocyanate (FTC) method

Inhibition of lipid peroxidation of samples was determined according to the ferric thiocyanate method as reported by Osawa and Namiki (13), with some modifications. Each test tube contained 2 ml of samples at 200 μg/ml concentrations, 2 ml an emulsion of linoleic acid in ethanol (2.51%; v/v), 4 ml of phosphate buffer (0.05M; pH 7.0) and 2 ml of distilled water. The solution (10 ml) was mixed and incubated in an oven at 40°C in dark for 9 days. The same solution, without any additives was taken as control samples. At regular intervals during incubation, 0.1 ml aliquot of the mixture was diluted with 9.7 ml of ethanol (75%) followed by the addition of 0.2 ml of ammonium thiocyanate (30%) and 0.1 ml of ferrous chloride (20 mM) in hydrochloric acid (3.5%; v/v). The peroxide level of each sample was determined by reading absorbance

at 500 nm spectrophotometrically. These steps are repeated every 24 h until the control sample reached its maximum. The low absorbance value indicates the efficiency of the test samples to inhibit lipid oxidation. Percent inhibition of lipid peroxidation is calculated by formula: inhibition of lipid peroxidation (%) = (1-S_{abs}/C_{abs}) x 100 where C_{Abs} is the absorbance of the control and S_{Abs} is the absorbance of the samples.

DPPH free radical scavenging activity

The electron-donation abilities of the compounds were measured from the bleaching of the purple-colored methanol solution of DPPH. The free radical-scavenging activity was evaluated as described by Ardestani and Yazdanparast (14), with a slight modification. Various concentrations (50–200 µg/ml) of the samples were prepared and each sample (1 ml) was mixed with 1 ml of 0.4 mM freshly prepared methanolic solution containing DPPH radicals. The resulting solutions were then left to stand at room temperature for 30 min prior to being spectrophotometrically detected at 517 nm. The solution without DPPH served as blank.

The percent DPPH scavenging activity was calculated from the following equation:

$$\%\ \text{DPPH scavenging activity} = \left(\frac{A_c - A_s}{A_c}\right) x100$$

A_c is the absorbance value without sample and A_s the absorbance in the presence of sample. A_s opposed to increasing concentration of specimens decline of absorbance is an indication that destroyed DPPH radical. Antioxidant activity results are expressed as IC_{50} (µg/ml) that reduces the effective concentration of DPPH radicals by half and was calculated by interpolation from linear regression analysis.

Reducing power assay

The reducing property of the samples was determined by using the potassium ferricyanide-ferric chloride method (15). One ml of each sample at different concentrations (50-600 µg/ml) was mixed with 2.5 ml sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, then 2.5 ml 10% trichloroacetic acid was added to mixture was centrifuged at 3000 rpm for 10 min. and 5 ml of the supernatant was mixed with an equal volume of distilled water followed by the addition of 1 ml, 0.1% ferric chloride. The absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power.

Statistical analysis

All experiments were carried out in triplicate. Data were expressed as means ± SD (standard deviation). A one-way analysis of variance (ANOVA) followed by Duncan's post test was used for comparison between the essential oil samples and standard compounds. A difference was considered statistically significant when $p < 0.05$. All the statistical analyses were accomplished using the computer software SPSS 16 for Windows.

Results and Discussion

Chemical compositions of essential oils

The essential oils with yellowish color and the relaxing scent produced from the dried aerial parts of AS and AOS. The mean values of yields (w/w %) of AS and AOS oils were 0.21% and 0.17% respectively. The chemical compositions of AS and AOS essential oils were analyzed by GC and GC–MS and resulted in identification and quantification of twenty seven and thirty nine compounds representing 94.79 and 96.76 % of the total oil, respectively, as listed in Table 1 according to their retention indices.

The main components of the AS oils were α-Pinene (19.18%), 1,5,5-Trimethyl-6-methylene-cyclohexene (16.34%), and 2,5,5-Trimethyl-1,3,6-heptatriene (9.64%). The presence of 1,8-Cineole (8.72%), Camphor (8.02%), 1,6-dimethylhepta-1,3,5-triene (6.25%) and *p*-Cymene (4.08%) was also important for the AS oil profile. The chemical composition of AS oils, when compared to other *Astragalus* species, shows many differences with *A. sahendi* from Iran (16), *A. microcephalus* grow in Karadj (17), *A. hamzaoglui* a Turkey endemic taxon (18), but also similar components such as α-Pinene, Camphene and δ-Cadinene have been detected in *A. schahrudensis* oils (19). These differences might have been derived from many different reasons

Table 1. Essential oil composition of *A. sericans* and *A. oocephalus* subsp. *stachyophorus* (%)

Compound[a]	R.I.[b]	AS (%)[c]	AOS (%)[c]
Butanoic acid	833	3.46	-
Pentanoic acid	855	3.06	-
Cyclohexanol	877	-	0.10
2,5,5-Trimethyl-1,3,6-heptatriene	914	**9.64**	-
Tricyclene	922	-	0.50
α-Pinene	943	**19.18**	**12.82**
Camphene	952	3.42	3.94
β-Pinene	979	0.55	0.40
α-Terpinene	1016	-	0.04
iso-Sylvestrene	1019	-	4.20
o-Cymene	1021	-	1.21
p-Cymene	1025	4.08	-
β-Phellandrene	1026	1.29	2.15
Limonene	1030	-	0.25
1,8-Cineole	1031	8.72	**16.35**
1,4-Cyclohexadiene,	1045	-	0.60
γ-Terpinene	1060	0.30	0.33
Camphenilone	1084	-	0.29
cis-Thujone	1109	-	0.14
trans-Thujone	1122	-	0.31
trans-Sabinol	1140	-	2.55
Benzenemethanol	1142	1.26	-
cis-Sabinol	1143	-	3.36
Camphor	1146	8.02	**18.25**
α-Ocimene	1050	-	0.02
1,6-dimethylhepta-1,3,5-triene	1170	6.25	-
α-Thujenal	1182	-	0.39
Azulene	1192	-	0.04
γ-Terpineol	1199	-	**16.74**
Verbenone	1205	-	0.03
Piperitone	1253	-	0.11
Isobornyl acetate	1286	0.46	-
Borneol	1287	-	5.28
L-Bornyl acetate	1289	-	5.84
3,5-Heptadienal,2-ethylidene-6-methyl	1292	0.58	-

Table 1 (continued). Essential oil composition of *A. sericans* and *A. oocephalus* subsp. *stachyophorus* (%)

Compound[a]	R.I.[b]	AS (%)[c]	AOS (%)[c]
p-cymen-7-ol	1300	-0.14	
α-Cubebene	1351	0.60	-
α-Copaene	1360	2.82	0.02
β-Maaliene	1378	-0.03	
cis-α-Bergamotene	1415	-0.03	
E-Caryophyllene	1419	-0.08	
Isobornyl n-butanoate	1434	-0.02	
Aromadendrene	1435	-0.01	
trans-Muurola-3,5-diene	1450	0.26	-
1,5,5-Trimethyl-6-methylene-cyclohexene	1465	**16.34**	-
Germacrene D	1480	0.36	0.03
4-Methyl-2-(3-methyl-2-butenyl)-furan	1481	0.38	-
trans-Cadina-1,4-diene	1496	0.31	-
α-Muurolene	1499	0.24	-
β-Bisabolene	1506	-0.10	
γ-Cadinene	1515	1.16	-
δ-Cadinene	1524	0.85	-
β-sesquiphellandrene	1526	-0.02	
trans-Calamenene	1539	1.01	-
Z-Nerolidol acetate	1678	-0.02	
Caryophylene oxide	1972	0.19	0.02
Total		**94.79**	**96.76**

-: not detected; [a]Identification of compounds was made by the calculation of their R.I and by GC–MS analysis; [b]R.I.: retention indices (HP-5 column); [c]%: percentage calculated by GC-FID on non-polar capillary column HP-5.

include climatological factors, nutritional status, genetic and especially the locality and growth stage of the plants (20, 21).

The chemical class distribution of AOS essential oils showed significant differences in qualitative and quantitative from the AS oil composition. 1,8-Cineole (16.35%), α-Pinene (12.82%), Camphor (18.25%) and γ-Terpineol (16.74%) were the most abundant in AOS

essential oil. In addition, L-Bornyl acetate (5.84%) and Borneol (5.28%) have been found in significant amounts in AOS oil and both compounds were reported previously as a member of the essential oil in *A. schahrudensis* (19, 22). It is possible to conclude from the results of present study that, α-Pinene/1,5,5-Trimethyl-6-methylene-cyclohexene and Camphor/γ-Terpineol are the most probable types of the essential oil in AS and AOS, respectively. Some of the *Astragalus* species showed different types of essential oil including, 6,10,14-trimethylpentadecan-2-one/hexadecanoic acid in *A. gombiformis* Pomel (23) and α-Terpineol/2-Pentadecanone, 6,10,14-trimethyl in *A. sahendi* (16). Because of these particularities, it is important to enlarge the sampling number and locations of these plant taxa to conclude the chemotype patterns of this genus.

Antioxidant potencies

Medicinal and beneficial properties of plants intimately related to their natural antioxidant contents. Therefore, antioxidant capacity of medicinal plants is widely used as a scale for identifying the bioavailability of herbs (24). To assess the antioxidant potencies of AS and AOS essential oils were proved on ferric thiocyanate method, reducing power and DPPH assays and their antioxidant activities were compared with those of selected reference antioxidants (BHT and Vit. E) for the three tests are presented in Table 2.

To determine the inhibition of lipid peroxidation, the FTC method is used extensively. Peroxides are formed during the linoleic acid oxidation, which react with Fe^{2+} to form Fe^{3+}. The latter ions form a complex with SCN^- and this complex has a maximum absorbance at 500 nm (25). The effects of AS, AOS, Vit. E and BHT on inhibition of lipid peroxidation are shown in Fig 1. The inhibition of lipid peroxidation of 70.36±0.75% and 37.22±0.23 % for AS and AOS, and 94.21±0.41% and 50.15±0.53% for BHT and Vit. E, respectively (Table 2). The essential oil of AS (70.36±0.75%) showed a higher inhibition of lipid peroxidation than Vit. E. At a concentration of 200 μg/ml, the inhibition of lipid peroxidation by AS was 70.36±0.75% at 192 h, while the same concentration and time period the inhibition of lipid peroxidation by Vit. E was 50.15±0.53%.

In biological damages, free radicals play significant roles, and DPPH is extensively used to determine the free radical scavenging activity of natural antioxidants (26). DPPH, which is a radical itself with a purple color, changes into a stable compound with a yellow color by reacting with an antioxidant, and the extent of the reaction depends on the hydrogen donating ability of the antioxidant (9). A dose-dependent assay was performed and both samples exhibited dose-dependent increase in activity. When compared with standards, at a concentration of 200 μg/ml, the scavenging ability of essential oils from AS and AOS on DPPH radical decreased in following order: 98.27% (BHT) > 89.53% (AS) > 89.23% (Vit. E) > 60.69% (AOS). It is clear from Fig. 2 that AS showed strong DPPH radical scavenging ability compared with standards Vit. E and BHT. There were significant differences ($p < 0.05$) between the scavenging ability of AS and Vit. E at the same concentration. The AOS had the moderate scavenging ability ($p < 0.05$).

IC_{50} value defined as the amount of sample which is necessary to scavenge 50% of DPPH present in the test solution. The lower IC_{50} value means higher DPPH radical scavenging activity (24). IC_{50} values for AS and AOS were 44.43±1.09 and 134.22±1.31 μg/ml, respectively, while BHT and Vit. E had an IC_{50} of 1.48±0.04 and 24.57±0.90 μg/ml, respectively ($p < 0.05$) (Table 2).

Previous studies have been reported that the reducing power of a compound may associated with its potential antioxidant activity (27, 28). In the presence of AS and AOS, the Fe^{3+}-Fe^{2+} transformation was examined for evaluation of the reducing power. High absorbance indicates high reducing power. The reducing power activity of AS and AOS was showed in Table 2 and compared with that of BHT and Vit. E. AS and AOS were capable of reducing power in a concentration dependent manner. The absorbance of Fe^{3+}-Fe^{2+} transformation of 600 μg/ml concentration of AS, AOS, BHT and Vit. E were found as 0.394±0.03, 0.464±0.04, 0.767±0.03 and 0.550±0.07, respectively. The sequence for reducing power was BHT > Vit. E > AOS > AS ($p < 0.05$).

The *Astragalus* taxa has a variety of pharmacologically active compounds and the most interesting pharmacological properties are hepatoprotective, im-

Table 2. Antioxidant activity of samples in the performed assays

	FRSA[A]	LPI[B]	RPA[C] concentration (µg/ml)					
			50	100	200	300	500	600
AS	44.43±1.09[c]	70.36±0.75[c]	0.102±0.01[a]	0.151±0.02[d]	0.221±0.03[c]	0.257±0.02[a]	0.352±0.02[a]	0.394±0.03[a]
AOS	134.22±1.31[d]	37.22±0.23[a]	0.140±0.01[b]	0.222±0.01[c]	0.268±0.02[d]	0.345±0.05[b]	0.443±0.03[b]	0.464±0.04[b]
BHT	1.48±0.04[a]	94.21±0.41[d]	0.356±0.09[d]	0.451±0.03[b]	0.531±0.03[b]	0.620±0.04[d]	0.736±0.06[d]	0.767±0.03[d]
Vit. E	24.57±0.90[b]	50.15±0.53[b]	0.263±0.07[c]	0.327±0.01[a]	0.433±0.01[a]	0.473±0.01[c]	0.541±0.02[c]	0.550±0.07[c a]

[A]FRSA; DPPH free radical scavenging activity: concentration in microgram per milliliter required to inhibit radical formation by 50%. Results are expressed as means ± standard deviations of three replications determinations; [B]LPI; Percentage of lipid peroxidation inhibition in concentration of 200 µg/ml; [C]RPA; reducing power activity was expressed as absorbance values dependent on concentration. High absorbance at 700 nm indicates high reducing power. Results are expressed as means ± standard deviations of three replications determination. a-d: Differences within columns (samples not connected by the same letter are statistically different at p < 0.05).

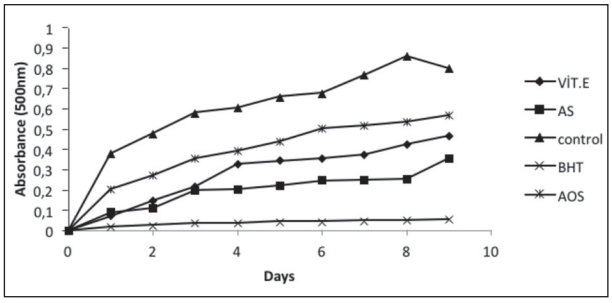

Figure 1. Comparative total antioxidant activities of all samples at concentrations of 200 µg/ml. A low absorbance value represents a high level of antioxidant activity.

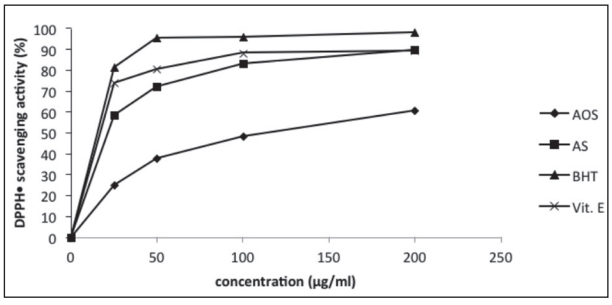

Figure 2. Free radical-scavenging activities of samples measured using the DPPH radical.

munostimulant, antiviral and antioxidative (29). Acetone extract of *Astragalus sinicus* L. seed (30), aqueous extract of *Astragalus mongholicus* (31) showed quite effective antioxidative capacity in various antioxidant assays. There is no work about the chemical compositions and antioxidative effects of *Astragalus sericans* and *Astragalus oocephalus* subsp. *stachyophorus* in literature. Therefore importance of current study becomes more obvious.

Conclusions

Antioxidant activities of essential oils is one of the most examined features, important for both food preservation and control of human diseases. According to literature data, this is the first study on the chemical composition and antioxidant properties of the essential oils of *Astragalus sericans* and *Astragalus oocephalus* subsp. *stachyophorus*. These results suggested that the essential oils of this *Astragalus* species could be a new potential source as inhibitory substances against some skin pathogens and food-borne spoilage, and be a candidate to be used in the pharmaceutical and food industries.

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