British Journal of Pharmaceutical Research
11(1): 1-11, 2016, Article no.BJPR.24363
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antioxidant Activity and Biochemical Screening of two *Glycyrrhiza* L. Species

Yavuz Selim Cakmak[1,2*], Abdurrahman Aktumsek[3], Ahmet Duran[3] and Ozlem Çetin[4]

[1]Department of Biotechnology and Molecular Biology, Faculty of Science and Arts, Aksaray University, Aksaray, Turkey.
[2]Science and Technology Application and Research Center, Aksaray University, Aksaray, Turkey.
[3]Department of Biology, Faculty of Science, Selcuk University, Konya, Turkey.
[4]Department of Biotechnology, Faculty of Science, Selcuk University, Konya, Turkey.

Authors' contributions

This work was carried out in collaboration between all authors. Authors YSC and AA designed the study, managed the literature searches and wrote the first draft of the manuscript. Authors AD and OC obtained and identified plant materials. Author YSC analyses of the study performed the spectroscopy analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/24363
Editor(s):
(1) Wenbin Zeng, School of Pharmaceutical Sciences, Central South University, Hunan, China.
(2) Ke-He Ruan, Director of the Center for Experimental Therapeutics and Pharmacoinformatics (CETP) Professor of Medicinal Chemistry & Pharmacology, Department of Pharmacological and Pharmaceutical Sciences, University of Houston, USA.
Reviewers:
(1) Anonymous, National Research Institute of Animal Production, Poland.
(2) Emanuel Vamanu, University of Agronomical Sciences and Veterinary Medicine Bucharest, Romania.
(3) Said Fatouh Hamed, National Research Centre, Egypt.
Complete Peer review History: http://sciencedomain.org/review-history/13858

Original Research Article

Received 16th January 2016
Accepted 15th March 2016
Published 25th March 2016

ABSTRACT

Aims: *Glycyrrhiza* L. is one of the most widely used plants in traditional medicine since antiquity. For the determining of the biochemical composition and antioxidant activity of methanolic extracts of two *Glycyrrhiza* species different parts.
Study Design: In the current study, antioxidant activities, essential oils and fatty acid compositions of root and aerial parts of *G. iconica* and *G. flavescens* subsp. *flavescens* were examined. To measure antioxidant activities of methanol extracts, different chemical methods were carried out.
Place and Duration of Study: Department of Biology, Physiology and Biochemistry Research Laboratory (December 2011- November 2012).

**Corresponding author: E-mail: yavuzselimcakmak@gmail.com;*

Methodology: The antioxidant capacity tests were designed to evaluate the antioxidant activities of methanol extracts of *Glycyrrhiza* species tested. The extracts will be executed for their possible antioxidant activities by five different test systems namely total antioxidant capacity (phosphomolibdat assay), β-carotene/linoleic acid test system, free radical scavenging (DPPH), ferric and cupric ion reducing power (CUPRAC). Total phenolic and flavonoid concentrations of each extract were also determined by using both Folin-Ciocalteu reagent and aluminum chloride.
Results: The root of *G. iconica* showed the highest activity in all methods. The major components of essential oils from *Glycyrrhiza* species were 1-pentylcyclobutene, naphtalene, hexahydro farnesyl acetone, phytol, dimethylamine and n-hexadecanoic acid. In fatty acid profiles of these two taxa, the main fatty acids were palmitic and linoleic acids.
Conclusion: Our results showed that studied parts of two *Glycyrrhiza* species can be used as an easily accessible source of natural antioxidants that could be important in pharmaceutical industry.

Keywords: Essential oil; fatty acid; medicinal plant; Glycyrrhiza iconica; Glycyrrhiza flavescens subsp. flavescens.

1. INTRODUCTION

Biochemical compounds commonly found in plants are known to have possible benefits, such as antioxidative, anticarcinogenic and antimutagenic activities. Many active constituents, such as phenols, carotenoids and vitamins, are sources of natural antioxidants in plants [1].

The fatty acid profiles of some plants enhance good health as they reduce the risk of some chronic diseases, such as cancer and coronary heart disease. They have high amounts of mono and poly unsaturated fatty acids [2].

Free radicals may cause damage to biomolecules, including proteins, nucleic acids and membrane lipids [3]. Although singlet oxygen atoms are not strictly free radicals, they are considered to be free radicals due to having the capacity to form free radicals in living organisms [4].

Essential oils have beneficial effects, such as antibacterial, antifungal and insecticidal activities. Thus, they are commonly used in the pharmaceutical, food and cosmetic industries [5].

Fabaceae is the most common family after Asteraceae in Turkey's flora and the most economically important family after Poaceae [6,7]. *Glycyrrhiza* L. (Fabaceae) is represented in Turkey by eight native species, and four of them are endemic [6-8].

The roots of *Glycyrrhiza* species are called licorice, and have a characteristic odor and sweet taste; it has been used in China for a few thousand years [9]. The root has many beneficial effects such as antiulcer [10], antitumor [11], antimutagenic [12], protective against hepatotoxicity [13] and antimicrobial activities [14]. Due to the antibacterial properties of the roots, *Glycyrrhiza* species have also been used to relieve chest pain and to treat ulcers in Turkish folk medicine. Moreover, they are used as a sweetener [8].

The objective of the present study was to determine the chemical composition and antioxidant activities of extracts obtained from the aerial and root parts of two *Glycyrrhiza* species growing in Turkey.

2. MATERIALS AND METHODS

2.1 Plant Materials and Chemicals

The aerial and root parts of *G. iconica* Hub.-Mor. and *G. flavescens* subsp. *flavescens* Boiss. were collected from Konya (Sarayonu) and Antakya, Turkey. The plant species were identified by botanist Professor Dr. A. Duran, and voucher specimens (Ö.Çetin-1026 & A.Duran and Ö.Çetin-1031 & A.Duran) have been deposited at Konya University Education Faculty Herbarium, Konya.

Chemicals were purchased from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany), and all chemicals and solvents were of analytical grade.

2.2 Extraction of Essential Oil

To obtain essential oils, the chopped plant samples were subjected to hydrodistillation using a Clevenger apparatus for 3 h with deionized water. The extracted essential oils were dried

with anhydrous sodium sulfate and stored at +4ºC until analysis.

2.3 Gas Chromatography–mass Spectrometry

The GC-MS analyses were performed on an Agilent 7890 GC-MS system using a HP-INNOWAX column (60 m length, 0.25 mm i.d. and 0.25 µm film thickness). Both injector and detector temperatures were 250ºC. The initial temperature was 60ºC for 10 min. Afterwards, the temperature was increased 4ºC/min to 220ºC and held for 10 min, then increased at 1ºC min-1 to 240ºC. Total run time was 80 min. Helium was used as the carrier gas with a flow rate 1.2 mL/min. The mass spectra were recorded at 70 eV. The relative percentages of the separated compounds were calculated from total ion chromatograms. The identification of the oil components was based on the Wiley and Nist mass spectral library. Retention indices (RI) of the compounds were determined relative to the retention times of a series of n-alkanes.

2.4 Preparation of Methanolic Extract

The plant samples were dried at room temperature and then ground using a laboratory mill. The powdered plants (15 g) were mixed with 250 mL methanol and extracted in a Soxhlet apparatus for 6-8 h. The extracts were filtered and the methanol was evaporated at 40ºC in a rotary evaporator. Then the extracts were stored at +4ºC in the dark until analysis.

2.5 Assays for Total Phenolic and Flavonoid Contents

The phenolic content of the extracts was determined according to Slinkard and Singleton [15]: 0.2 mL of extract solution (2 mg/mL) was mixed with 1 mL Folin-Ciocalteu reagent and 2 mL Na_2CO_3 (7.5%). The final volume was brought up to 7 mL with deionized water. The mixture was allowed to stand for 2 h at room temperature and the absorbance was measured at 765 nm with a spectrophotometer (Shimadzu, UV-1800). Gallic acid was used as a standard for the calibration curve. The total phenolic contents of the extracts were determined as gallic acid equivalents (mg GAE/g extract).

The total flavonoid content of the extracts was spectrophotometrically determined according to Arvouet-Grand et al. [16]. Briefly, 1 mL of 2% aluminum trichloride ($AlCl_3$) methanolic solution was mixed with the same volume of extract solution (at 2 mg/mL concentration). The absorbance values of the reaction mixtures were determined at 415 nm after 10 min against a blank. Rutin was used as a standard and the total flavonoid content of the extracts was expressed as mg rutin equivalents per gram of extract (mg RE/g extract).

2.6 Total Antioxidant Capacity

The total antioxidant capacity of the extracts was evaluated using the phosphomolybdenum method according to Prieto et al. [17]: 0.3 mL of extract solution (1 mg/mL) was mixed with 3 mL of reagent solution (6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95ºC for 90 min. Then, the absorbance of the solution was measured at 695 nm against a blank. The antioxidant capacity of the extracts was evaluated as equivalents of ascorbic acid (mg AAE/g).

2.7 Free Radical Scavenging Activity (DPPH, 2, 2-diphenyl-1-picrylhydrazyl)

The free radical scavenging activity of the plant extracts was determined with slight modifications to the method of Kirby and Schmidt [18]: 0.5 mL of various concentrations of the extracts in methanol were added to 3 mL of 6.10^{-5} M of a methanol solution of DPPH. This solution was incubated for 30 min at room temperature in the dark. After incubation, the mixture's absorbance was measured at 517 nm. Inhibition activity was calculated as follows:

$$I(\%) = (A_0-A_1)/A_0 x100$$

where, A_0 is the absorbance of the control, A_1 is the absorbance of the extract/standard. Free radical inhibition (IC_{50}) of extracts was calculated. Lower IC_{50} values indicate higher antioxidant capacities.

2.8 β-carotene / Linoleic Acid Bleaching Assay

The β-carotene bleaching assay is based on rapid discoloration in the absence of an antioxidant [19]. In this assay, the antioxidant activity of the extracts was determined with slight modifications to the procedure described by Sokmen et al. [20]. A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 mL

chloroform and 25 mL linoleic acid and 200 mg Tween 40 were added.

The chloroform was completely evaporated and 100 mL distilled water saturated with oxygen was added with vigorous shaking. Then, 2.5 mL of this reaction mixture was dispensed into test tubes and 350 mL portions (1 mg/mL concentration) of the extracts were added. The reaction mixture was incubated at 50ºC for 2 h. The same procedure was repeated with BHT and BHA as the positive control and blank, respectively.

After the incubation period, the absorbance of the mixtures was measured at 490 nm and the inhibition ratio was calculated.

2.9 Ferric Ion Reducing Power

The ferric reducing power method was applied with slight modifications to the method of Oyaizu [21]. Different concentrations of extracts were mixed with 2.5 mL of 0.2 M phosphate buffer and potassium ferricyanide, and then 2.5 mL of the 1% mixture was incubated at 50ºC for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid was added. Then, 2.5 mL of the reaction mixture was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The solution's absorbance was measured at 700 nm. The reducing power of the samples increased with the absorbance value. The same procedure was applied with BHA and BHT. The EC_{50} value (the effective concentration at which the absorbance was 0.5) was calculated for the extract, BHA and BHT.

2.10 CUPRAC Assay

The cupric ion reducing capacity of the extracts of *G. iconica* and *G. flavescens* subsp. *flavescens* was determined according to the method of Apak et al. [22]: 1 mL each of 10 mM $CuCl_2$, 7.5 mM neocuproine and NH_4Ac buffer (1 M, pH 7.0) solutions were added into a test tube. Then, 0.5 mL of different concentrations of the extracts were mixed and the total volume was brought up to 4.1 mL with deionized water. The mixture's absorbance was recorded against a blank at 450 nm after 30 min incubation at room temperature.

2.11 Extraction of Oils

The oil extraction from the dried and powdered aerial and root parts of the plants (10 g) was carried with diethyl ether at boiling point (34ºC) for 6 h with a Soxhlet extractor. Diethyl ether was also used as the solvent. The solvent was evaporated with a rotary evaporator.

2.12 Fatty Acid Methyl Esters (FAMEs) Preparation

The fatty acids in the oil were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14% BF_3 (v/v) in methanol [23].

2.13 Gas Chromatographic Analysis

Fatty acid composition analyses were performed with an Agilent 6890 N model gas chromatograph (GC) using a flame ionization detector (FID) that was fitted to an HP-88 capillary column (100 m length, 0.25 mm i.d. and 0.2 µm film thickness). The injector and detector temperatures were set at 240 and 250ºC, respectively. The oven was held at 160ºC for 2 min. Thereafter, the temperature was increased up to 185ºC at a rate of 4ºC/min and then increased up to 200ºC at a rate of 1ºC/min. It was held at 200ºC for 46.75 min. The total run time was 70 min. Helium was used as the carrier gas (1 mL/min).

Identification of fatty acids was determined by comparing sample FAME peak relative retention times with those obtained from Alltech and Accu standards. Results were expressed as FID response areas at relative percentages. Each reported result was obtained from the average value of three GC analyses, and is given as mean±S.D. The atherogenic index (AI) and thrombogenicity index (TI) were calculated according to Ulbricht and Southgate [24]. AI = [12:0 + (4 x 14:0) + 16:0]/[(ω6 + ω3) PUFA + 18:1 + other MUFA] and TI = [14:0 + 16:0 + 18:0]/[0.5 x 18:1 + 0.5 x other MUFA + 0.5 x ω6 PUFA + 3 x ω3 PUFA + (ω3 PUFA/ ω6 PUFA)].

2.14 Statistical Information

Three replicates were prepared for all of the antioxidant activity tests and fatty acid composition analyses. The results of these analyses were given as means and their standard deviations (means±SD).

3. RESULTS AND DISCUSSION

3.1 Composition of Essential Oil

This work identified 50 known compounds in four hydrodistilled essential oils from different parts of

the plants as shown in Table 1. The aerial and root parts of *G. flavescens* subsp. *flavescens* were representing 98.857%, 96.580% total mass, respectively, whereas accounted for 97.443%, 95.919% of total mass in the aerial and root parts of *G. iconica*, respectively.

The major components of the volatile oil obtained from the aerial parts of *G. flavescens* subsp. *flavescens* were hexahydro farnesyl acetone (29.502%), phytol (10.357%) and n-hexadecanoic acid (7.288%). However, in the roots, 1-phenylcyclobutene (30.450%), dimethylamine (18.170%) and 4-pyridinecarbonitrile (13.772) were the major constituents. Similarly, the essential oil composition of the aerial parts of *G. iconica* included n-hexadecanoic acid (30.204%), phytol (17.650%) and hexahydro farnesyl acetone (9.554%). The composition of the root oil of *G. iconica* was characterized by the abundance of naphthalene (63.838%) together with n-hexadecanoic acid (6.932%) and furan, 2 pentyl (3.290%). Miyazawa and Kameoka [25] reported that octanoic acid (11.4%), paeonol (8.9%), octadecane (8.6%), benzaldehyde (7.5%), α-terpineol (7.5%) and 4-terpineol (7.2%) were the main constituents of G. glabra L. var. glandulifera from China. In the study of Fu et al. [26], *G. uralensis* was found to be mainly composed of β-cadinene (12.28%), β-caryophyllene (10.04%), γ-cadinene (9.49%), α-cadinene (4.43%), caryophyllene oxide (3.65%) and α-gurjunene (3.48%).

According to Zhang et al. [27], over the 50% of the total essential oil constituents of *G. pallidiflora* consisted of 5-(2-propenyl)-1,3-benzodioxole, 3-7dimethyl-1,6-octadien-3-ol, [1R-(1R*.4Z.9S*)]-4.11.11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene and 2.3.6-trimethyl-1.6-heptadiene.

Table 1. Essential oil composition of the different parts of *Glycyrrhiza* species

Compounds			*G. flavescens* subsp. *flavescens*		*G. iconica*	
	RT[a]	RI[b]	Aerial	Root	Aerial	Root
Hexanal	14.324	1089	-	1.125	1.061	2.262
1-Cyclopropyl-2-propanone	18.840	1188	-	0.681	0.125	0.229
(E)-2-hexanal	20.574	1227	-	-	1.424	0.324
Furan, 2-pentyl	21.036	1237	-	0.823	0.333	3.290
Methylheptenone	25.500	1345	-	0.197	0.234	0.449
1-Hexanol	25.809	1354	-	-	-	0.537
Hexadecane	27.551	1399	-	0.086	1.044	0.906
(E)-2-octenal	29.026	1441	-	-	-	0.475
Butanal	29.451	1453	0.766	-	0.305	-
1-Hexanol, 3-methyl	30.818	1492	0.620	0.204	-	0.489
3,5-Octadien-2-one	32.160	1533	0.479	0.222	0.802	0.542
Benzaldehyde	32.581	1546	1.535	9.427	1.069	0.320
(E)-2-Nonenal	32.680	1549	2.757	0.173	-	0.882
Homoserine	33.046	1560	0.616	0.173	0.180	0.321
Hexane 3,3-dimethyl	34.293	1600	0.608	-	0.650	0.486
4-terpineol	34.805	1617	2.990	0.274	0.261	0.230
Carane, 4,5-epoxy-,trans	35.605	1643	1.641	-	0.753	-
1-Phenylcyclobutene	35.749	1648	0.692	30.450	-	-
1-Nonanol	36.209	1663	0.438	0.271	0.218	0.555
Safranal	36.385	1670	1.297	-	0.547	-
Acetophenone	36.597	1677	0.926	6.075	-	-
Isononane	37.211	1697	1.089	0.104	0.207	0.249
β-Terpinene	37.591	1711	1.830	-	0.295	0.716
2,3-Dihydroanisole	37.950	1723	0.870	0.583	-	0.382
Isooctanol	39.171	1767	-	-	-	0.376
Naphthalene	39.407	1775	0.924	0.265	9.380	63.838
2-Benzothiophene	41.241	1842	0.762	-	4.112	0.695
2,4,6-Trihydroxytoluene	41.405	1849	2.128	1.615	-	1.458
Geraniol	41.551	1854	0.963	-	-	-

Compounds			*G. flavescens* subsp. *flavescens*		*G. iconica*	
	RT[a]	RI[b]	Aerial	Root	Aerial	Root
Geranyl acetone	41.963	1869	1.405	0.196	0.624	0.400
β-Methylnaphthalene	42.474	1889	-	0.683	-	1.388
β-Ionone	44.419	1964	5.693	0.124	2.870	1.323
1-Tridecene	44.631	1973	0.375	0.176	-	0.383
1-Phenyl-1H-pyrazol-3-amine	45.539	2009	3.682	-	0.252	-
m-Cresol	45.840	2021	1.201	0.206	1.245	0.220
Benzene, 1-ethyl-2,3-dimethyl	46.222	2037	1.212	-	0.157	-
Hexahydro farnesyl acetone	48.616	2137	29.502	0.275	9.554	0.980
Phthalimidine	49.068	2156	0.905	0.371	2.390	-
Acetaldehyde	49.803	2188	1.164	0.974	1.221	0.508
2-methoxy-4-vinyl phenol	50.694	2226	0.719	0.079	1.494	0.754
Propane	52.270	2294	0.528	-	2.246	-
1-Tetracosanol	54.860	2385	-	2.055	-	-
2-(4-Methoxyphenyl)-5-methyl-[1.3.4]oxadiazol	56.730	2443	-	2.273	-	-
Pthalic acid, isobutyl nonyl ester	61.230	2564	4.846	0.462	3.877	3.020
Phytol	63.582	2618	10.357	0.443	17.650	-
Benzyl Benzoat	65.686	2663	-	1.567	-	-
2-Propenamide	67.285	2696	1.231	-	-	-
4-Pyridinecarbonitrile	69.625	2745	-	13.772	-	-
Dimethylamine	77.946	2903	4.240	18.170	-	-
n-Hexadecanoic acid	78.767	2918	7.288	0.644	30.204	6.932
Total identified			98.279	95.218	96.784	95.919

[a]*RT: Retention time*
[b]*RI: Retention indices*

3.2 Antioxidant Activity

3.2.1 Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The TPC and TFC extraction yields of the different parts obtained from the two *Glycyrrhiza* species are given in Table 2. The total phenolic contents of the samples ranged from 143.12 to 309.18 mg GAE/g. The highest phenolic content was identified in the root of *G. iconica*, while the lowest was in the aerial parts. The phenolic content of both ethanol and aqueous extracts of different parts of *G. glabra* were reported by Tohma and Gulçin [28] to range from 75.7 to 185.7 µg GAE. Methanol has been considered as the best extraction solvent for phenolics [29], therefore, the results in this study were greater than those of Tohma and Gulçin [28].

The flavonoid content of the studied species ranged from 143.85 to 285.73 mg RE/g (Table 2). Tohma and Gulçin [28] showed that the total flavonoid contents were 4.4 (aerial parts) and 4.2 (root) µg quercetin equivalents in ethanolic extracts from *G. glabra*; and, 2.6 (aerial parts) to 5.1 (root) µg quercetin equivalents in aqueous extracts from *G. glabra*. Visavadiya et al. [30] found that in a *G. glabra* ethanol extract the levels of total phenolic and flavonoid contents were 36.50 (mg GAE/g) and 12.75 (mg quercetin equivalents/g), respectively. Additionally, the total polyphenol and flavonoid contents of aqueous extracts obtained from G. glabra were determined to be 47.41 (mg GAE/g) and 17.47 (mg quercetin equivalents/g), respectively.

3.2.2 Total antioxidant capacity

The highest activity was observed in the root of *G. iconica* (Table 2). The roots of *G. flavescens* subsp. *flavescens* and *G. iconica* (258.44 and 325.55 mg AAE/g, respectively) showed higher activity than the aerial parts (239.84 and 161.92

mg AAE/g, respectively). In our study showed similarly high levels both of the phenolic content and the antioxidant capacity. Kumaran and Karunakaran [31] have shown that a high total phenol content increased the antioxidant activity. The total antioxidant capacity of plant extracts may be attributed to their chemical composition and phenolic content [32].

3.2.3 Free radical scavenging activity (DPPH, 2, 2-diphenyl-1-picrylhydrazyl)

Using the DPPH method, the highest free radical scavenging activity was observed in the roots of *G. iconica* (IC_{50}= 90.34 µg/mL), followed by the roots of *G. flavescens* subsp. *flavescens* (IC_{50}= 127.15 µg/mL), the aerial parts of *G. flavescens* subsp. *flavescens* (IC_{50}= 146.03 µg/mL) and the aerial parts of *G. iconica* (IC_{50}= 288.37 µg/mL) (Table 3), in that order. The scavenging ability of the positive control (BHT, IC_{50}= 34.06 µg/mL) was higher than all the plant samples. In another our study, *D. pontica* leaves water extracts showed highest scavenging activity with 0.08 mg/ml IC_{50} value [33]. Ozsoy et al. [34] worked *C. antiochia* var. *praealta* methanol and chloroform extracts. The authors indicated that it has 5.10 mg/ml scavenging activity of DPPH radical. Our samples have higher activity than their studied plant.

3.2.4 β-carotene/linoleic acid bleaching assay

β-carotene/linoleic acid bleaching assay measured the ability of an plant extract for the inhibition of lipid peroxidation [35]. In the β-carotene bleaching assay, inhibition values of extracts were between 85.58% and 96.16% in the different parts of *G. flavescens* subsp. *flavescens* and *G. iconica* (Table 3). When compared to the inhibition capacity of BHA (92.82%) and BHT (95.76%), the inhibitory activities of the extracts and synthetic antioxidants were similar. Sanda et al. [33] reported that the metanolic extract were higher than water extract. Especially methanol extract of leaves have strongest inhibition ratio with 89.96%. The results of current study have more effective inhibiton activity than *G. echinata* [36].

3.2.5 Ferric ion reducing power

The root of *G. iconica* showed the highest ferric ion reducing power activity, with an EC_{50} value of 156.647 µg/mL (Table 3). This value was followed by the aerial extract of *G. flavescens* subsp. *flavescens*, the root extract of *G. flavescens* subsp. *flavescens* and the aerial extract of *G. iconica* with EC_{50} values of 162.764, 170.439 and 361.364 µg/mL, respectively. The results of our study which worked on *G. echinata* have lower ferric ion reducing power activity than current study [36]. Karakoca et al. [37] determined high reducing activity in methanol extract of *Onobrychis armena*.

3.2.6 CUPRAC assay

The roots of *G. iconica* and *G. flavescens* subsp. *flavescens* had the highest cupric ion reducing power activity, which was followed by the aerial parts of *G. flavescens* subsp. *flavescens* and *G. iconica* (Fig. 1). Similarly, the reducing power of the *G. glabra* root was determined to be higher than the aerial parts in the study of Tohma and Gulçin [28].

3.3 Fatty Acid Composition

In the current study, 22 fatty acids were identified in the extracted oils obtained from *Glycyrrhiza* species (Table 4). The total oil contents of the different parts of studied plants varied between 1.593 and 7.621%. In general, the major fatty acids in the composition of *Glycyrrhiza* species were palmitic acid (16.20-23.30%) and linoleic acid (10.95-30.78%). Similarly, linoleic acid has been reported to be a major fatty acid in *G. uralensis* [38] and *G. glabra* [39]. Linoleic acid is found in the seeds of most plants. Moreover, it is the precursor of arachidonic acid and eicosanoids derived from arachidonic acid [40]. A higher intake of linoleic acid reduces the risk of coronary artery disease [41]. Saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA) were between 26.57-44.58, 7.44-23.00 and 39.77-57.66%, respectively. The amounts of unsaturated fatty acids (UFA) were higher than the saturated fatty acids in all samples, and ranged from 55.42 to 73.44%. Consuming dietary plant oils is not associated with an increased risk of disease because the saturated fatty acids are lower than the unsaturated fatty acids [42]. The dietetic quality of lipids is indicated by the atherogenic index (AI) and thrombogenic index (TI) [24]. The root oil of *G. flavescens* subsp. *flavescens* showed the lowest AI (0.42) and TI (0.27) values.

Table 2. Extraction yield, total phenolics, flavonoid contents and antioxidant capacities of methanolic extracts obtained from *Glycyrrhiza* species

Sample	Part	Yield (%)	TPC[a] (mg GAE/g)	TFC[b] (mg RE/g)	TAC[c] (mg AAE/g)
G. flavescens subsp. *flavescens*	Aerial	15.40	259.94±4.58[d]	285.73±2.16	239.84±6.98
	Root	26.98	286.45±10.05	175.12±8.77	258.44±0.84
G. iconica	Aerial	29.62	143.12±4.55	238.43±3.48	161.92±3.68
	Root	28.18	309.18±1.36	143.85±7.36	325.55±8.67

[a]*TPC: Total phenolic content (mg GAE g^{-1} extract).*
[b]*TFC: Total flavonoid content (mg RE g^{-1} extract).*
[c]*TAC: Total antioxidant capacity (mg AE $^{-1}$ exract).*
[d] *Values are reported as means±S.D. of three parallel measurements*

Table 3. Free radical scavenging, linoleic acid inhibition, ferric reducing power activity of *Glycyrrhiza* species

	Parts	IC_{50}[a] (µg ml^{-1})	Inhibition[b] (%)	EC_{50}[c] (µg ml^{-1})
G. flavescens subsp. *flavescens*	Aerial	146.03±6.87[d]	91.95±0.15	162.764±8.29
	Root	127.15±5.3	95.40±0.63	170.439±8.99
G. iconica	Aerial	288.37±1.37	85.58±0.21	361.364±5.14
	Root	90.34±1.64	96.16±0.07	156.647±5.04
BHA		-	92.82±0.15	-
BHT		34.06±0.38	95.76±0.26	24.348±7.852

[a] *Results of free radical scavenging activity (DPPH assay).*
[b] *Results of β-carotene/linoleic acid test system.*
[c] *Results of ferric ion reducing power.*
[d] *Values are reported as means±S.D. of three parallel measurements*

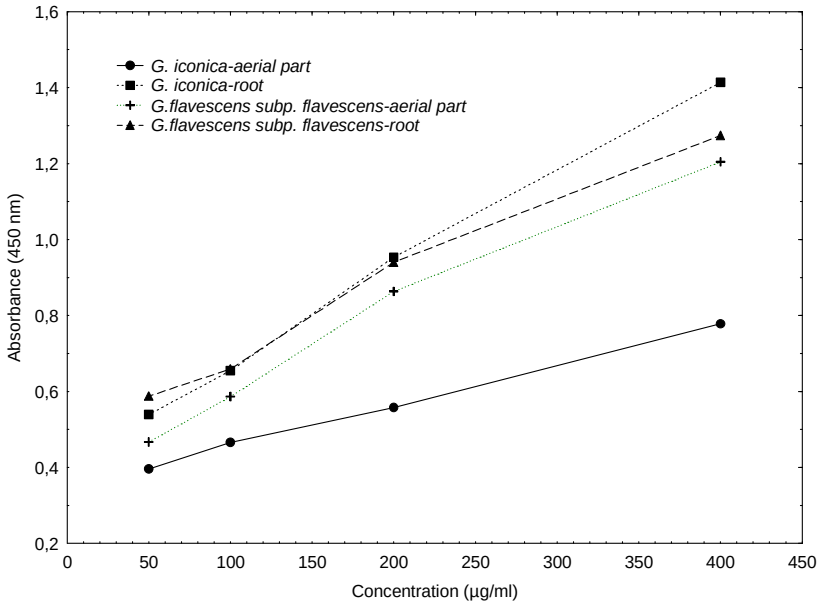

Fig. 1. Cupric reducing antioxidant capacity (CUPRAC) of *Glycyrrhiza* species

Table 4. Fatty acid composition of the different parts of *Glycyrrhiza* species (%)

Fatty acids	*G. flavescens* subsp. *flavescens*		*G. iconica*	
	Aerial	**Root**	**Aerial**	**Root**
C 12:0	1.06±0.01[a]	0.21±0.04	1.07±0.03	0.32±0.01
C 13:0	0.21±0.01	0.33±0.01	0.07±0.01	0.09±0.03
C 14:0	3.55±0.01	1.37±0.01	1.74±0.01	1.18±0.02
C 15:0	1.93±0.02	1.30±0.01	0.25±0.01	0.87±0.01
C 16:0	23.20±0.33	16.20±0.08	23.30±0.01	19.07±0.14
C 17:0	1.65±0.04	1.49±0.01	1.78±0.03	1.10±0.01
C 18:0	8.20±0.17	4.70±0.01	7.20±0.01	4.39±0.01
C 20:0	4.76±0.08	0.96±0.03	7.17±0.04	3.11±0.01
C 21:0	0.03±0.01	0.03±0.01	0.06±0.04	1.88±0.01
Σ SFA[b]	44.58±0.66	26.57±0.08	42.62±0.01	32.01±0.15
C 14:1ω5	2.11±0.04	0.52±0.01	0.41±0.05	0.53±0.01
C 15:1ω5	0.07±0.01	0.10±0.01	0.04±0.01	0.05±0.01
C 16:1ω7	0.86±0.07	0.63±0.01	1.12±0.01	1.45±0.01
C 17:1ω8	0.18±0.12	0.33±0.02	0.07±0.01	0.09±0.01
C 18:1ω9	9.12±0.18	19.15±0.12	4.62±0.02	5.24±0.01
C 18:1ω7	0.96±0.02	1.27±0.01	0.31±0.02	1.32±0.01
C 20:1ω9	2.16±0.01	0.83±0.06	0.83±0.04	1.58±0.07
C 22:1ω9	0.20±0.01	0.19±0.02	0.07±0.01	0.09±0.02
Σ MUFA[b]	15.65±0.06	23.00±0.04	7.44±0.06	10.34±0.08
C 18:2ω6	10.95±0.21	21.54±0.11	14.43±0.17	30.78±0.05
C 18:3ω6	17.51±0.37	3.90±0.01	25.80±0.21	6.94±0.01
C 18:3ω3	0.62±0.02	0.27±0.04	0.48±0.01	0.59±0.01
C 20:4ω6	4.96±1.49	3.28±0.01	4.37±0.05	17.50±0.08
C 22:6ω3	5.75±0.17	21.46±0.21	4.87±0.03	1.86±0.03
Σ PUFA[b]	39.77±0.72	50.44±0.13	49.94±0.06	57.66±0.07
Σ UFA[b]	55.42±0.66	73.44±0.09	57.38±0.01	68.00±0.15
Σ EFA[b]	28.45±0.58	25.44±0.12	40.23±0.04	37.71±0.04
AI[c]	0.86±0.03	0.42±0.01	0.73±0.01	0.65±0.01
TI[c]	0.91±0.01	0.27±0.01	0.92±0.01	0.99±0.01
Oil content	1.593	7.621	3.349	5.642

[a] *Values are reported as means±S.D. of three parallel measurements.*
[b] *SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, UFA: Unsaturated fatty acids, EFA: Essential fatty acids*
[c] *AI: Atherogenic index, TI: Thrombogenicity index*

4. CONCLUSION

According to the results obtained in the current study, the methanolic extracts of two *Glycyrrhiza* species could be potential sources of natural antioxidants and a possible food supplement as they possessed strong antioxidant properties. The *Glycyrrhiza* species generally have high antioxidant activities. The root extract of *G. iconica* showed the highest activity in all the antioxidant capacity tests. Additionally, the phenolic content of this sample was higher than in the others. Moreover, the *Glycyrrhiza* species had high UFA and EFA contents. Hence, this species may be useful in the pharmaceutical and food industries.

FUNDING SOURCE

This research was supported financially as a project (project no: 111t614). We thank to the scientific and technological research council of turkey (tubitak) for financial support.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Larson RA. The antioxidants of higher plants. Phytochemistry. 1988;4:969-78.
2. Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence, Nutr. Cancer. 1992;18:1-29.
3. Halliwell, B. Antioxidants in human health and disease. Ann. Rev. Nutr. 1996;16: 33-49.
4. Aruoma OI. Free radicals, oxidative stress and antioxidants in human health and disease. J. Am. Oil Chem. Soc. 1998;75: 199-12.
5. Bakkali F, Averbeck S, Averbeck D, Waomar M. Biological effects of essential oils-A review. Food Chem. Toxicol. 2008; 46:446-75.
6. Chamberlain DF. *Glycyrrhiza*. In flora of Turkey and the East Aegean Islands; Davis PH, Ed.; Edinburgh University Press: Edinburgh. 1970;260-63.
7. Davis PH, Mill RR, Tan K. Flora of Turkey and the East Aegean Islands; Davis PH, Mill RR, Tan K, Eds.; Edinburgh: Edinburgh University Press. 1988;10:1.
8. Sümbül H, Tufan Ö, Düşen O, Göktürk RS, A new taxon *Glycyrrhiza* L. (Fabaceae) from southwest Anatolia. Isr. J. Plant Sci. 2003;51:71-74.
9. Vaya J, Tamir S, Somjen D. Estrogen-like activity of licorice root extract and its constituents. Oxid. Stress Dis. 2004;14: 615-34.
10. Van Marle J, Aarsen PN, Lind A, van Weeren-Kramer J. Deglycyrrhizinized licorice (DGL) and the renewal of rat stomach epithelium. Eur. J. Pharm. 1981; 72:219-25.
11. Nishino H, Yoshioka K, Iwashima A, Takizawa H, Konishi S, Okamoto H, et al., Glycyrrhetic-Acid inhibits tumor-promoting activity of teleocidin and 12-O-tetradecanoylphorbol-13-acetate in two-stage mouse skin carcinogensis. Jpn. J. Cancer Res. 1986;77:33-38.
12. Zani F, Cuzzoni MT, Daglia M, Benvenuti S, Vampa G, Mazza P, Inhibition of mutagenicity in *Salmonella typhimurium* by *Glycyrrhiza glabra* extract, glycyrrhizinic acid, 18α- and 18β-glycyrrhetinic acids. Planta Medica. 1993;59:502-07.
13. Nose M, Ito M, Kamimura K, Shimizu M, Ogihara Y. A comparison of the antihepatotoxic activity between glycyrrhizin and glycyrrhetinic acid. Planta Medica. 1994;60:136-139.
14. Haraguchi H, Tanimoto K, Tamura Y, Mizutani K, Kinoshita T. Mode of antibacterial action of retrochalcones from *Glycyrrhiza* inflate. Phytochemistry. 1998, 48:125-129.
15. Slinkard K, Singleton VL. Total phenol analysis: Automation and comparison with manual methods. Am. J. Enol. Viticult. 1977;28:49-55.
16. Arvouet-Grand A, Vennat B, Pourrat A, Legret P, Standardisation d'un extrait de propolis et identification des principaux constituants. Journal de Pharmacie de Belgique. 1994;49:462-68.
17. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphor molybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem. 1999;269:337-41.
18. Kirby AJ, Schmidt RJ. The antioxidant activity of Chinese herbs for eczema and of placebo herbs. J. Ethnopharm. 1997; 56:103-08.
19. Kulisic T, Radonic A, Katalinic V, Milos M, Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem. 2004;85:633-40.
20. Sokmen A, Gulluce M, Akpulat HA, Daferera D, Tepe B, Polissiou M, Sokmen M, Sahin F. The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food Control. 2004;15:627-34.
21. Oyaizu M. Studies on products of browning reactions: Antioxidative activities of browning reaction prepared from glucosamine. Jpn. J. Nutr. 1986;44:307-15.
22. Apak R, Guclu K, Ozyurek M, Karademir SE, Ercag E. The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. Int. J. Food Sci. Nutr. 2006;57:292-304.
23. IUPAC. Standards methods for analysis of oils, fats and derivatives. In C. Paquot Ed.; Oxford Pergamon Press: Great Britain. 1979;59-66.
24. Ulbricht TLV, Southgate DAT. Coronary heart disease seven dietary factors. Lancet. 1991;338:985-92.
25. Miyazawa M, Kameoka H, Volatile flavour components of *Glycyrrhiza* Radix (*Glycyrrhiza glabra* L. var. *glandulifera*

Regel et Herder) from China. Frag. J. 1990;5(3):157-60.
26. Fu YJ, Wang W, Zu YG, Reichling J, Suschke U, Composition and antimicrobial activity study on essential oil from the aerial parts of Chinese licorice (*Glycyrrhiza uralensis* Fisch.). J. Indian Chem. Soc. 2009;86:1218-23.
27. Zhang J, Ma J, Wang Y, Yao J, Yang Y. Analysis of leaf volatile chemical components of *Glycyrrhiza pallidiflora*. Acta Pratacult Sin. 2004;13(3):103-05.
28. Tohma HŞ, Gulçin İ, Antioxidant and radical scavenging activity of aerial parts and roots of Turkish liquorice (*Glycyrrhiza glabra* L.). Int J Food Prop. 2010;13: 657-71.
29. Kallithraka S, Garciaviguera C, Bridle P, Bakker JI. Survey of solvents for the extraction of grape seed phenolics. Phytochem Analysis. 1995;6:265-67.
30. Visavadiya NP, Soni B, Dalwadi N, Evaluation of antioxidant and anti-atherogenic properties of *Glycyrrhiza glabra* root using in vitro models. Int J Food Sci Nutr. 2009;60(2):135-49.
31. Kumaran A, Karunakaran RJ, *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT-Food Sci Technol. 2007;40:344-52.
32. Prasad KN, Yang B, Dong X, Jiang G, Zhang H, Xie H, Jiang Y. Flavonoid contents and antioxidant activities from *Cinnamomum* species. Innovative Food Science and Emerging Technologies. 2009;10:627-32.
33. Sanda MA, Zengin G, Aktumsek A, Cakmak YS. Evaluation of antioxidant potential of two *Daphne* species (*D. gnidioides* and *D. pontica*) from Turkey. Emir J Food Agric. 2015;27(6):488-494.
34. Ozsoy N, Kultur S, Yilmaz-Ozden T, Celik BO, Can A, Melikoglu G. Antioxidant, anti-inflammatory, acetylcholinesterase inhibitory and antimicrobial activities of Turkish endemic *Centaurea antiochia* var. *Praealta*. J Food Biochem. 2015;39:771-776.
35. Burda S, Oleszek W. Antioxidant and antiradical activities of flavonoids. J Agric Food Chem. 2001;49: 2774-2779.
36. Cakmak YS, Aktumsek A, Duran A. Studies on antioxidant activity, volatile compound and fatty acid composition of different parts of *Glycyrrhiza echinata* L. EXCLI J. 2012;11:178-187.
37. Karakoca K, Asan-Ozusaglam M, Cakmak YS, Teksen M. Phenolic compounds, biological and antioxidant activities of *Onobrychis armena* Boiss. & Huet flower and root extracts. Chiang Mai J Sci. 2015;42(2):376-392.
38. Fu YJ, Wang W, Zu YG, Suschke U, Reichling J, Schwarz G. Supercritical fluid extraction of seed oil from Chinese Licorice (*Glycyrrhiza uralensis* Fisch.): Chemical composition and antibacterial activity. South Afr J Chem. 2007;60:67-70.
39. Yunusova SG, Danilov VT, Yunusov MS, Murinov YI, Tsyrlina EM, Straek R. Chemistry of natural compounds and bioorganic chemistry-Lipids of *Glycyrrhiza-glabra* root. Russ. Chem. Bull. 1995; 44(2):359-62.
40. Whelan J, The health implications of changing linoleic acid intakes. Prostagland Leukotr Essent Fatty Acids. 2008;79(3-5): 165-67.
41. Djoussé L, Pankow JS, Eckfeldt HJ, Folsom AR, Hopkins PN, Province MA, Hong Y, Ellison RC, Relation between dietary linolenic acid and coronary artery disease in the National Heart, Lung, and Blood Institute Family Heart Study. Am J Clin Nutr. 2001;74:612-19.
42. Coulston AM. The role of dietary fats in plant-based diets. Am J Clin Nutr. 1999; 70(3):512-15.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13858