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Characterization in phenolic compounds and antioxidant properties of *Glycyrrhiza glabra* L. rhizomes and roots

Running title: Phenolic compounds and antioxidant properties of *Glycyrrhiza glabra*.

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Abstract

The present work aims to characterize and quantify the phenolic composition and to evaluate the antioxidant activity of *Glycyrrhiza glabra* L. (commonly known as licorice) rhizomes and roots. The antioxidant potential of its methanol/water extract could be related with flavones (mainly apigenin derivatives), flavanones (mainly liquirintin derivatives), a methylated isoflavone and a chalcone, identified in the extract. Lipid peroxidation inhibition was the most pronounced antioxidant effect (EC_{50}=0.24±0.01 µg/mL and 22.74±2.42 µg/mL in TBARS and β-carotene/linoleate assays, respectively), followed by free radicals scavenging activity (EC_{50}=111.54±6.04 µg/mL) and, finally, reducing power (EC_{50}=128.63±0.21 µg/mL). In this sense, licorice extract could be used as a source of antioxidants for pharmaceutical, cosmetic and/or food industries.

Keywords: Glycyrrhiza glabra L.; Phenolic compounds; HPLC-DAD-ESI/MS; Antioxidant activity

1. Introduction

Environmental factors, such as pollution, smoking, certain drugs, poor diet, sedentary lifestyle and stress-inducing agents, are considered the main external aggressors for human bodies, increasing cell deterioration and, in long term, contributing to aging and several diseases/disorders. Furthermore, the normal metabolism also produces high quantities of oxidant molecules, through different chemical reactions. Commonly known as free radicals, these substances are highly reactive molecules containing one or more unpaired electrons in atomic or molecular orbitals that can join with cellular components and destroy them [1–3].

Plants are widely used to improve health and even to treat various diseases. Currently, there are several studies evidencing these natural matrices as rich sources of biomolecules, which provide numerous health benefits [4–6]. Antioxidant phytochemicals are a good example of these biomolecules, being considered important contributors to protect cells and DNA, once neutralize reactive molecules and even prevent a cascade of reactions that lead to degenerative processes such as aging, neurodegenerative diseases, cancer, cardiovascular diseases, cataracts, rheumatism, ulcers, or atherosclerosis, among others [1,2,7–11].

Among antioxidants, phenolic compounds have been considered important promoters of health and wellbeing, acting as free radical scavengers, metal chelators, singlet oxygen quenchers, inhibitors of lipid peroxidation as well as modulators of the formation of pro-oxidant and pro-inflammatory molecules (leukotrienes, 5-LOX, cytokines) [12–14].

Glycyrrhiza glabra L. (*Fabaceae*), commonly known as licorice, is widely recommended as emollient, for upper respiratory tract infections and dermal affections, as anti-inflammatory, antiulcer, antibacterial, antifungal, antiviral, anti-allergic, and immunostimulant, among other benefits [15–18]. Its antioxidant properties have also been

reported, either in aqueous [7,19,20], ethanol [20–22], methanol [20,23–25] or methanol/water [26,27] extracts. There are several studies that focused on the phenolic characterization of *Glycyrrhiza* sp., [23,24,26,27]. Nevertheless, information on the quantification of these compounds is scarce.

The aim of this work was to characterize and quantify the phenolic composition and evaluate the antioxidant properties in methanol/water extracts of *Glycyrrhiza glabra* L. (rhizomes and roots).

2. Materials and methods

2.1. Samples

Dried rhizomes and roots of *Glycyrrhiza glabra* L. were supplied by Soria Natural (Garray - Soria, Spain). The samples were obtained in the autumn 2012 and certified as clean products, with monitored parameters for pesticides, herbicides, heavy metals and radioactivity. For each analysis, three different samples were used and the assays were performed in triplicate.

2.2. Standards and reagents

Methanol was of analytical grade purity and supplied by Pronalab (Lisbon, Portugal). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). HPLC-grade acetonitrile was obtained from Merck KgaA (Darmstadt, Germany). Formic and acetic acids were purchased from Prolabo (VWR International, France). The phenolic compound standards were from Extrasynthese (Genay, France). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

89

90 *2.3. Extraction procedure*

91 The extraction was performed by stirring the sample (1 g) with 30 mL of
92 methanol/water (80:20, *v/v*) at 25 ºC and 150 rpm for 1 h, and filtered through Whatman
93 No. 4 paper. The final residue was then extracted with an additional 30 mL portion of
94 the extraction solvents mixture. The combined extracts were evaporated at 35 ºC under
95 reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland) and then
96 lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA). The lyophilized extracts
97 were re-dissolved in methanol/water (80:20, *v/v*), performing a stock solution with a
98 concentration of 20 mg/mL, from which several dilutions were prepared.

99

100 *2.4. Analysis of phenolic compounds*

101 Phenolic compounds were determined by HPLC (Hewlett-Packard 1100, Agilent
102 Technologies, Santa Clara, USA) as previously described by the authors [28]. Double
103 online detection was carried out in the diode array detector (DAD) using 280 nm and
104 370 nm as preferred wavelengths and in a mass spectrometer (MS) connected to the
105 HPLC system via the DAD cell outlet. Peaks were tentatively identified based on their
106 UV-vis and mass spectra and comparison with data reported in the literature.
107 Quantification was performed from the areas of the peaks recorded at 280 and 370 nm
108 using calibration curves (1-100 μg/mL) obtained with phenolic standards of the same
109 group. The results were expressed in mg per g of extract.

110

111 *2.5. Evaluation of antioxidant activity*

112 *2.5.1 DPPH radical-scavenging activity (RSA) assay*

The capacity to scavenge the "stable" free radical DPPH, monitored according to the method of Hatano et al., with some modifications[29], was performed by using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc; Winooski, USA). The reaction mixture in each one of the 96-wells consisted of one of the different concentration solutions (30 μL) and methanolic solution (270 μL) containing DPPH radicals ($6x10^{-5}$ mol/L). The mixture was left to stand for 30 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: RSA (%) = $[(A_{DPPH}-A_S)/A_{DPPH}]$ x 100, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50 % of antioxidant activity (EC_{50}) was calculated from the graph of DPPH scavenging activity against extract concentrations. Trolox was used as positive control.

2.5.2. Reducing power (RP) assay

RP was determined according to the method of Oyaizu, with some modifications [30]. The present methodology is based on the capacity to convert Fe^{3+} into Fe^{2+}, measuring the absorbance at 690 nm, by using the microplate Reader mentioned above. At the different concentration solutions (0.5 mL) were added sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1 % w/v, 0.5 mL). The mixture was incubated at 50 ºC for 20 min, and then, trichloroacetic acid (10 % w/v, 0.5 mL) was added. In a 48-wells, the obtained mixture (0.8 mL), and also deionised water (0.8 mL) and ferric chloride (0.1 % w/v, 0.16 mL) was joined, and the absorbance was measured at 690 nm. The extract concentration providing 0.5 of absorbance (EC_{50}) was

calculated from the graph of absorbance at 690 nm against extract concentrations. Trolox was used as positive control.

2.5.3. β-carotene bleaching inhibition (CBI) assay

CBI was evaluated though the β-carotene/linoleate assay. A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 mL). Two millilitres of this solution were pipetted into a round-bottom flask. After the chloroform was removed at 40 ºC under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of the obtained emulsion were transferred into different test tubes containing different concentrations of the samples (0.2 mL). The tubes were shaken and incubated at 50 ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm in a spectrophotometer (AnalytikJena, Jena, Germany). β-Carotene bleaching inhibition was calculated using the following equation: (Abs after 2h of assay/initial Abs) x 100 [31]. The extract concentration providing 50% of antioxidant activity (EC_{50}) was calculated from the graph of β-carotene bleaching inhibition against extract concentrations. Trolox was used as positive control.

2.5.4. Lipid peroxidation inhibition (LPI) through thiobarbituric acid reactive substances (TBARS) assay

LPI was evaluated by the decreasing in thiobarbituric acid reactive substances (TBARS). Porcine brains were obtained from official slaughtering animals, dissected, and homogenized with a Polytron in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate, which was centrifuged at 3000*g* for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with the different solution

concentrations (0.2 mL) in the presence of $FeSO_4$ (10 μM; 0.1 mL) and ascorbic acid (0.1 mM; 0.1 mL) at 37 ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28 % w/v, 0.5 mL), followed by thiobarbituric acid (TBA, 2 %, w/v, 0.38 mL), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A – B)/A] × 100 %, where A and B were the absorbance of the control and the compound solution, respectively [32]. The extract concentration providing 50% of antioxidant activity (EC_{50}) was calculated from the graph of TBARS formation inhibition against extract concentrations. Trolox was used as positive control.

3. Results and discussion

3.1. Characterization of the phenolic compounds

The phenolic profile of *Glycyrrhiza glabra*, obtained after methanol/water extraction, and recorded at 280 and 370 nm is shown in **Figure 1**; compound characteristics and tentative identities are presented in **Table 1**. Eleven compounds were detected corresponding to the groups of flavones, flavanones and chalcones, as well as a possible isoflavone.

Compound 1 presented a pseudomolecular ion $[M\text{-}H]^-$ at *m/z* 593, releasing MS^2 fragment ions at *m/z* 443 (loss of 120 u), 383 (apigenin + 113 u) and 353 (apigenin + 83 u), whereas no relevant fragments derived from the loss of complete hexosyl (-162 u) or pentosyl residues (-132 u) were detected. This fragmentation behaviour is characteristic of di-*C*-glycosylated flavones [33]. The compound was tentatively identified as apigenin-

6,8-di-*C*-glucoside (vicenin-2) owing to its previous description in Traditional Chinese Medicine Formulae containing *Glycyrrhiza* roots and rhizomes [34,35].

Compound 2 presented a pseudomolecular ion $[M-H]^-$ at *m/z* 563. A compound with the same mass was reported in licorice (dried roots and rhizomes of *Glycyrrhiza* species) by Xu et al. (2013) and identified as the di-*C*-glycosylflavone isoschaftoside (i.e., 6-*C*-arabinopyranosyl-8-*C*-glucopyranosylapigenin). However, the MS^2 fragmentation pattern of the compound observed in our samples would not match such a structure, but it points to the pentosyl residue is *O*-attached to a *C*-glycosylating hexose. This assumption is supported by the characteristic fragment detected at *m/z* 413 ($[M-150]^-$), which according to [33] would be typical from that type of substitution. Further, the fragment ion at *m/z* 443 ($[M-120]^-$) supported the presence of a *C*-attached hexose, whilst the absence of an ion $[(M-H)-90]^-$ suggested a 6-*C* attachment [33]. The pentose should not be attached on positions 6'', 4'' or 3'' of the hexose, otherwise the fragment $[(M-H)-120]^-$ would not be produced. As for the rest of fragment ions, the one at *m/z* 323 $[(M-H)-150-90]^-$ would result from the partial loss of the *C*-attached hexose from the ion at *m/z* 413, whereas those at *m/z* 311 $[aglycone+41]^-$ and 293 $[aglycone+41-18]^-$ are associated to mono-*C*-glycosyl derivatives *O*-glycosylated on 2'' [36]. All in all, peak 2 was tentatively assigned as apigenin 2''-*O*-pentosyl-6-*C*-hexoside.

Compound 4 showed a pseudomolecular ion ($[M-H]^-$ at *m/z* 577) and a UV spectrum coherent the *C*-glycosylflavones commonly reported in *Glycyrrhiza* species isoviolanthin (apigenin-6-*C*-rhamnoside-8-*C*-glucoside) [34,35,37–39] or violanthin (apigenin-6-*C*-glucoside-8-*C*-rhamnoside) [34,35]. The data obtained in this study do not allow to conclude about the precise pattern of sugar substitution, so that the compound was just identified as (iso)violanthin. Compound 5 also corresponded to another flavone

that was tentatively assigned as a methylapigenin *O*-hexoside based on its UV and mass spectral data.

Compounds 3, 6, 7 and 10 presented the same pseudomolecular ion $[M-H]^-$ at *m/z* 549, all of them releasing a main MS^2 fragment at *m/z* 255, from the loss of 132+162 u (pentosyl+hexosyl residues), pointing to the correspond different apiosyl-glucosides of (iso)liquiritigenin, consistently reported to occur as major flavonoids in licorice [34,35,37–43]. The fragmentation patterns do not allow to distinguish between liquiritigenin (a flavanone) and isoliquiritigenin (a chalcone), so that they were assigned as derived from one or another based on their UV spectra, showing maxima at 272-276 nm plus a shoulder around 316-318 nm (peaks 3, 6 and 7) or 362 nm (peak 10), respectively. Liquiritin apioside (i.e., liquiritigenin 4'-*O*-apiosyl-glucoside) has been widely reported to occur in *Glycyrrhiza* species [34,35,37–45], although other isomers have also been described, such as liquiritigenin 7-*O*-apiosyl-glucoside [40,42,43] and liquiritigenin-7-*O*-apiosyl-4'-*O*-glucoside [34]. The results obtained herein do not allow concluding about the precise location of the sugar moieties, so that they were just identified as liquiritigenin apiosyl-glucoside isomers. Furthermore, as the carbon at position 2 is asymmetric the possibility of different stereoisomers may be also envisaged.

As previously indicated, compound 10 should correspond to a derivative of the chalcone isoliquiritigenin bearing pentosyl+hexosyl residues. Two main isomers possessing that structure have been widely reported in *Glycyrrhiza* species: licuroside (also designed as licuraside; isoliquiritigenin-4'-*O*-apiosyl-glucoside) and neolicuroside (isoliquiritigenin-4-*O*-apiosyl-glucoside) [34,35,37,39,41–43,45]. As for the liquiritigenin derivatives, it was not possible to conclude about the precise location of the glycosyl groups, so that compound 10 was assigned as (neo)licuroside.

Compound 8 presented a pseudomolecular ion $[M-H]^-$ at *m/z* 565 releasing a fragment ion at *m/z* 271 (-294 u, loss of a pentosyl and hexosyl moieties), and a UV spectrum coherent with a flavanone. These characteristics match the structure of naringenin-7-*O*-apiosyl-glucoside reported in *Radix Glycyrrhizae* by Wang et al. (2014), so that this identity was tentatively assumed for the compound.

Compound 9 ($[M-H]^-$ at *m/z* 561) was tentatively identified as glycyroside (i.e., formononetin-7-*O*-apiosylglucoside) owing to the previous identification of that isoflavone in Radix *Glycyrrhizae* by Wang et al. (2014). The presence of formononetin derivatives in *Glycyrrhiza* species has also been reported by various authors [35,41,42,45,46].

Finally, it was not possible to identify compound 11 with a pseudomolecular ion $[M-H]^-$ at *m/z* 591 that released two fragments at *m/z* 297 (-294, loss of a pentosyl and hexosyl moieties) and 282 (further loss of -15 u of a methyl residue), although its UV spectra with a maximum at 372 nm pointed to a chalcone aglycone.

Among the eleven phenolic compounds detected, liquiritin apioside isomers were the most abundant. Many papers have been published profiling phenolic compounds in *G. glabra* samples from different origins and using different extraction methodologies, some of them cited in the previous discussion [34,35,37–46]. However, from all of them, only Montoro et al. (2011) presented quantitative results, although they cannot be compared with ours results since they are expressed differently (mg/g of dry plant), thus these authors revealed liquiritin apioside as the main flavonoid present in their sample, which is in agreement with the sample studied herein. In our case, the results were expressed in mg/g of extract in order to relate the amounts of phenolic compounds found in the extract to the antioxidant activity. Therefore this study will add new data related to the quantification of these compounds, which are scarce in literature.

3.2. Evaluation of antioxidant activity

Figure 1 shows the results of the antioxidant potential of the licorice extract using different assays: DPPH radical scavenging activity (RSA), reducing power (RP), β-carotene bleaching inhibition (CBI) and lipid peroxidation inhibition (LPI) in brain cell homogenates. The most pronounced effect was observed for LPI assay (EC_{50}=0.24±0.01 μg/mL), followed by CBI (EC_{50}=22.74±2.42 μg/mL). RSA and RP presented higher EC_{50} values (meaning lower antioxidant activity), respectively, 111.54±6.04 μg/mL and 128.63±0.21 μg/mL.

The LPI capacity, accessed by using the TBARS assay, measures the malondialdehyde (MDA) formed as the split product of an endoperoxide of unsaturated fatty acids resulting from oxidation of a lipid substrate. The MDA is reacted with thiobarbituric acid (TBA) to form a pink pigment (TBARS) that is measured spectrophotometrically at 532 nm [32].

$MDA + TBA \rightarrow MDA\text{-}TBA_2$

$MDA + TBA + A \rightarrow MDA + TBA_2$

This procedure involves two distinct steps: the substrate is oxidized with the addition of a transition metal ion such as copper or iron or a free radical source such as 2,2'-azobis (2-amidinopropane) dihydrochloride, and then the extent of oxidation is determined by addition of TBA and spectrophotometric measurement of the product ($MDA\text{-}TBA_2$). Oxidation is inhibited by the addition of an antioxidant and, therefore, a reduction in the absorbance is observed. In the present experiment, the studied methanol/water extract exerted strong inhibitory effects of lipid oxidation (*e.g.*, exponential inhibition of TBARS formation, being these effects achieved at extremely low concentrations), which is in agreement with the results obtained by Jiang et al.[47] that reported the

efficacy of licorice ethanolic extract to prevent lipid oxidation and protect sensory attributes of ground pork.

Concerning to the CBI assay, and taking into account the basis of the method, β-carotene undergoes a rapid discoloration in the absence of an antioxidant since the free linoleic acid radical attacks the β-carotene molecule, which loses the double bonds and, consequently, loses its characteristic orange colour. Antioxidants can donate hydrogen atoms to quench radicals and prevent decolourization of carotenoids [48], through the following reactions:

β-carotene–H (orange) + ROO• → β-carotene• (bleached) + ROOH

β-carotene–H (orange) + ROO• + AH → β-carotene–H (orange) + ROOH + A•

The decolourization of β-carotene can be monitored by spectrophotometry at 470 nm [49]. Regarding the obtained results for the CBI activity of the studied methanol/water extract, a more pronounced effect (EC_{50}=23 μg/mL) was observed than the one reported by Ercisli et al. (2008) for ethanolic extracts of licorice roots collected in Turkey (EC_{58}=75 μg/mL). The results reported by these authors ranged between 28.3% (25 μg/mL) and 88.7% (800 μg/mL).

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), a stable organic nitrogen radical which presents a deep purple colour, allows the determination and quantification of the reducing capacity of antioxidants toward DPPH. Representing the DPPH radical by X• and the donor molecule by AH (being mainly phenolic compounds, they are proton donators), the primary reaction is:

X• + AH → XH + A•

In the present reaction, XH is the reduced form and A• is the free radical produced in this first step. This latter radical will then undergo further reactions, which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorized)

by one molecule of the reductant [50]. When a solution of DPPH• is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of colour. Upon reduction, the colour of DPPH• solution fades and this colour change is conveniently monitored measuring the absorbance decrease at 515-528 nm [51]. Thus, by using the present assay, the free radicals scavenger effect of licorice was accessed. The RSA obtained for the studied methanol/water extract (EC_{50}=112 µg/mL) was similar to some of the values reported by Cheel et al. (2012) for similar extracts prepared from samples harvested at different times (February- EC_{70}=100 µg/mL, May- EC_{60}=30 µg/mL, August- EC_{50}=50 µg/mL, November- EC_{50}=30 µg/mL), and by Cheel et al. (2010) for extracts obtained by infusion (EC_{49}=100 µg/mL). However, it was lower than the RSA described by Tohma & Gulçin (2010) for aqueous (EC_{52}=62 µg/mL) and ethanol (EC_{54}=50 µg/mL) extracts obtained from roots of Turkish licorice samples.

RP assay, widely used due to its specificity to access the electron-donating potential of antioxidants, and consequent reduction of yellow ferric form to blue ferrous form [52,53]. Antioxidant species Fe (III) or $Fe(CN)_6^{3-}$, when in the present of composite ferricyanide reagent, favors its reduction, and either Fe(II) or $Fe(CN)_6^{4-}$ is formed, and combining with a reagent component - Prussian blue, $KFe[Fe(CN)_6]$, a coloured product is produced. In this sense, by using Fe^{3+} in conjunction with $Fe(CN)_6^{3-}$, while oxidizing agent, any of the follow two reaction pair could occurs, despite the ending coloured product to be the same [54]:

Fe^{3+} + antioxidant ∅ Fe^{2+} + oxidized antioxidant,

$Fe^{2+} + Fe(CN)_6^{3-}$ ∅ $Fe[Fe(CN)_6]^-$

The resultant blue colour is linearly correlated with the total reducing potential of electron-donating antioxidants, being measured spectrophotometrically at 700 nm [55]. The RP value obtained in the present study (EC_{50}=129 µg/mL) was similar to the one described by Tohma & Gulçin (2010) for aqueous (EC_{45}=62 mg/mL) and ethanolic (EC_{76}=50 mg/mL) extracts.

Numerous reports have confirmed the association between phenolic compounds and bioactive properties. Regarding *G. glabra*, flavonoids saponins, coumarins, and stilbenoids have been related with its bioactive properties. Until now, licochalcone A, B, C, D and echinatin, some isoflavones and derivatives, such as glabridin, an isoflavan, hispaglabridin A, hispaglabridin B and 4′-*O*-methylglabridin, but also some chalcones, namely isoprenylchalcone derivative and isoliquiritigenin, were described as possessing potent antioxidant effects, not only inhibiting lipid peroxidation but also acting as radical scavengers and oxidative process preventers [18,27,56,57]. Regarding our study, it is feasible to attribute the antioxidant potential observed for the tested extract to the most abundant phenolic compounds identified, namely liquiritigenin apiosyl-glucosides. Nevertheless, it is important to highlight that plant extracts are usually much more effective than isolated compounds, as it was proved by Cheel et al. (2010) for the case of licorice aqueous extract. The authors verified that, despite in some assays licorice extract evidenced a weak antioxidant activity, the major components identified (liquiritin and glycyrrhizin) presented negligible or even no effects.

Overall, licorice extract could be used as a source of antioxidants for pharmaceutical, cosmetic and/or food industries. Regarding its antioxidants contribution in daily diet, further studies are necessary in order to elucidate the mechanisms of *in vivo* antioxidant action, bioavailability and involved metabolic pathways.

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466 **Figure Legends**

467 **Figure 1.** Phenolic profile of *Glycyrrhiza glabra* L. methanol/water extract at 280 nm

468 (A) and 370 nm (B).

469 **Figure 2.** Antioxidant activity of the *G. glabra* methanol/water extract evaluated by A)

470 DPPH scavenging activity (EC_{50}=111.54±6.04 µg/mL), B) reducing power

471 (EC_{50}=128.63±0.21 µg/mL), C) β-carotene bleaching activity (22.74±2.42 µg/mL) and

472 D) TBARS inhibition (0.24±0.01 µg/mL).

Table 1. Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification and quantification of phenolic compounds in *Glycyrrhiza glabra* methanol/water extract.

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Identification	Quantification (mg/g)
1	9.5	336	593	473(25),383(12),353(23)	Apigenin-6,8-di-*C*-glycoside	0.61±0.04
2	13.9	336	563	443(13),413(4),323(4),311(3),293(3)	Apigenin 2''-*O*-pentosyl-6-*C*-hexoside	0.99±0.04
3	16.2	272,sh316	549	429(23),417(15),255(29)	Liquiritigenin apiosyl-glucoside isomer	4.41±0.10
4	16.3	272/320	577	559(5),503(12),415(5)	(Iso)violanthin	0.48±0.01
5	16.6	334	445	283(100),268(10)	Methyl apigenin-*O*-hexoside	0.84±0.02
6	17.1	276,sh316	549	429(3),417(15),255(29)	Liquiritigenin apiosyl-glucoside isomer	4.02±0.04
7	17.3	276,sh318	549	429(5),417(11),255(55)	Liquiritigenin apiosyl-glucoside isomer	3.85±0.02
8	20.7	284,sh336	565	271(100)	Naringenin-7-*O*-apiosylglucoside	0.43±0.02
9	26.0	252,sh300	561	267(100),252(10)	Formononetin-7-*O*-apiosylglucoside	1.23±0.02
10	26.7	362	549	417(5),255(59)	(Neo)licuroside	0.14±0.01
11	27.8	250,sh292,372	591	297(100),282(46)	Unknown (chalcone derivative)	nq
					Total phenolic compounds	17.00±0.09

n.q. not quantified.

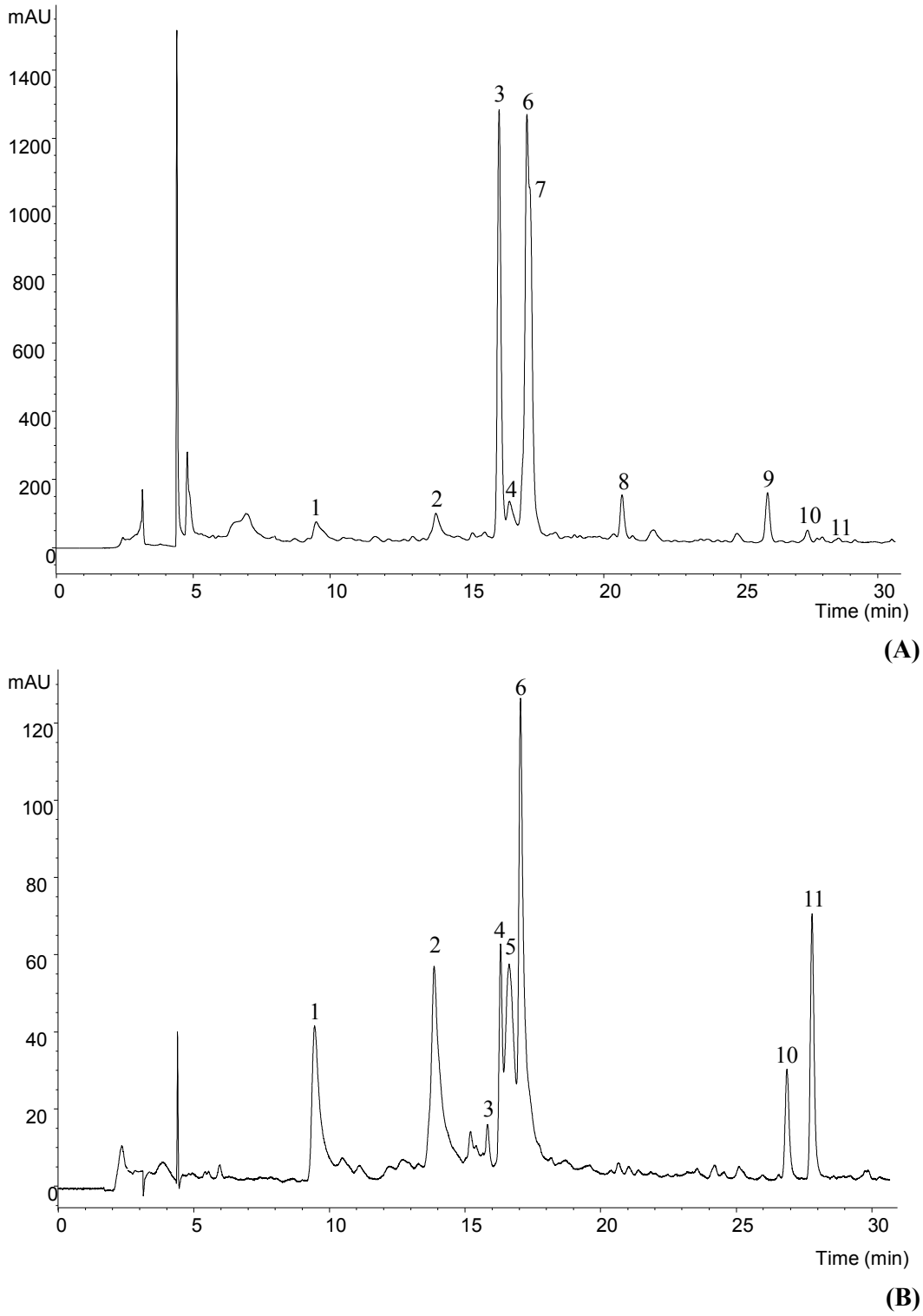

Figure 1. Phenolic profile of *Glycyrrhiza glabra* L. methanol/water extract at 280 nm (A) and 370 nm (B).

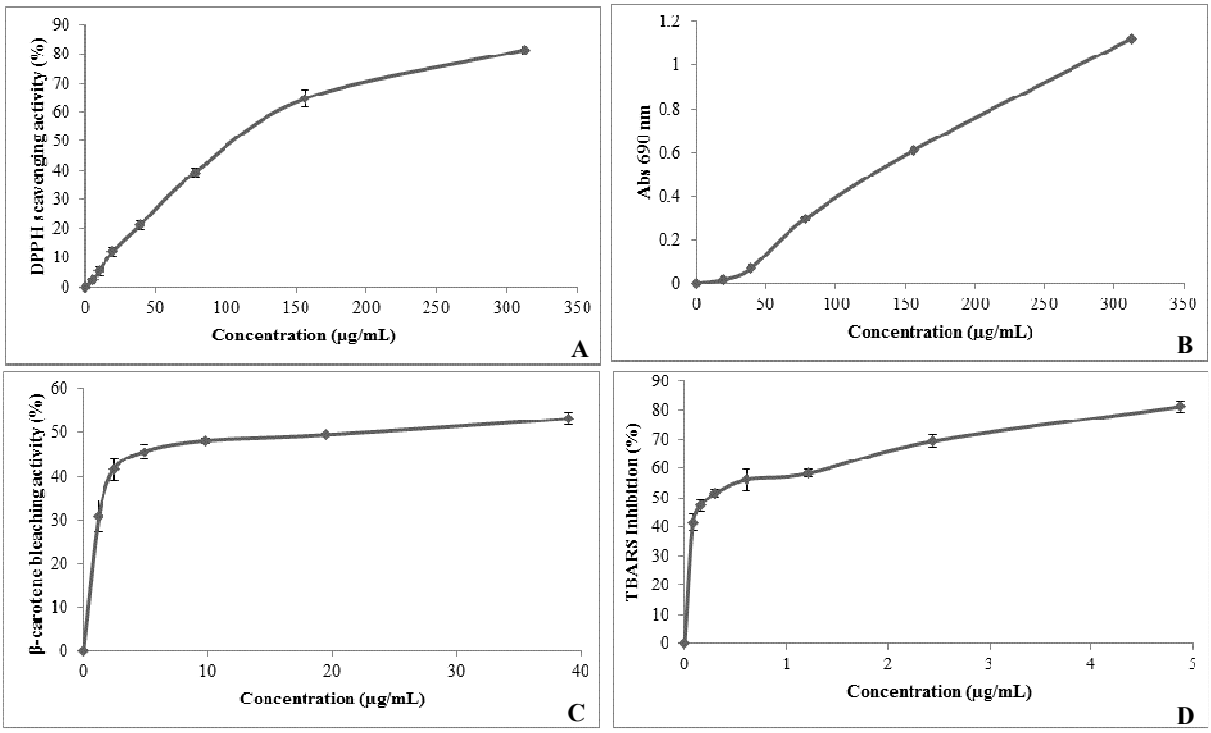

Figure 2. Antioxidant activity of the *G. glabra* methanol/water extract evaluated by A) DPPH scavenging activity (EC_{50}=111.54±6.04 µg/mL), B) reducing power (EC_{50}=128.63±0.21 µg/mL), C) β-carotene bleaching activity (22.74±2.42 µg/mL) and D) TBARS inhibition (0.24±0.01 µg/mL). The EC_{50} values obtained for trolox were: DPPH scavenging activity (EC_{50}=41.43±1.27 µg/mL), B) reducing power (EC_{50}=41.68±0.28 µg/mL), C) β-carotene bleaching activity (18.21±1.12 µg/mL) and D) TBARS inhibition (22.84±0.74 µg/mL).