Antioxidative Activities of Aqueous and Ethanolic Extracts of Licorice Roots

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Abstract: The licorice roots, is known as a healthy nutrient for more than 3000 years. The antioxidant activities of licorice roots, aqueous and ethanolic extracts, have been studied by using two different methods (reducing power and chelating ability). It was found that the total phenolic compounds in aqueous and ethanolic extracts of licorice roots were 4.8 and 9.2 mg/100 mg dry extract, respectively. The flavonoids (which are a part of the phenolic compounds) were found to be 2.3 and 6.8 mg/100 mg dry extract in aqueous and ethanolic extract of licorice roots respectively. The ethanolic extract shows high antioxidant activity as compared with aqueous extract. The aqueous and ethanolic extracts of licorice roots show high reducing power ability comparing with their abilities as chelating agents. Thus, this study suggests that licorice extract can be used as a potential source of natural antioxidants.

Key words: Licorice roots, aqueous and ethanolic, extract, antioxidants

INTRODUCTION
Licorice (Glycyrrhiza glabra L.) belongs to the Family Papilionaceae/Fabaceae. It is a traditional medicinal herb grows in the various parts of the world (Biondi et al., 2005). Phytochemical analysis of Glycyrrhiza glabra root extract showed that it contains saponin triterpenes (glycyrrhizin, glycyrrhetic acid and liquiritic acid) and flavonoids (liquiritin, isoflavonoids and formononetin). (Fukai et al., 1998; Arystanova et al., 2001). In the traditional system of medicine, the roots and rhizomes of G. glabra have been employed clinically for their anti-inflammatory, antiulcer, expectorant, antimicrobial and anxiolytic activities (Asl and Hosseinzadeh, 2008). Licorice has been shown to have great antioxidant, free radical scavenging and anticonvulsant activities (Di Mambro and Fonseca, 2005; Nassiri-Asl et al., 2007). The high levels of free radicals in living systems are able to oxidize biomolecules, leading to tissue damage, cell death or various diseases (Okay et al., 2003; Gulcin et al., 2009). Antioxidant compounds can deactivate and scavenge the free radicals. Antioxidants can inhibit the effect of oxidants by donating hydrogen atom or chelating metals (Sen et al., 2000; Gulcin et al., 2003a; Prakash et al., 2007). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used as additives in foods to prevent oxidation of lipids (Gulcin, 2002a, 2006a). Besides, BHA and BHT are restricted by legislative rules because of doubts over their toxic and carcinogenic effects. Therefore, there is a growing request and interest on natural and safer antioxidants in food applications and a growing trend in consumer preferences for natural antioxidants (Eirinastas et al., 2005, 2006a). Natural antioxidants commonly exist on plants which contain polyphenolic compounds (Gulcin et al., 2002b, 2007; Stolova et al., 2007). The root of Glycyrrhiza species is one of the richest sources of biological active compounds such as phenolic and flavanoid compounds (Roth, 2004). The present study has been used to determine the antioxidant activity of aqueous and ethanolic extracts of Licorice roots.

MATERIALS AND METHODS
The roots of G. glabra, were locally obtained, cleaned and ground. Twenty gram of ground material was extracted by 250 mL distilled water or ethanol 95% at boiling point, under reflux for 1 hr. The extractive was filtered and evaporated at 50°C to the compete dryness.

Phytochemical analysis
Determination of total phenolic content: A Folin-ciocalteu’s colorimetric method was used as described by Ayoola et al. (2008) To 0.5 mL of (1 mg/mL) extract a 2.5 ml of a ten-fold diluted Folin-ciocalteu’s reagent and 2 mL of 7.5% sodium carbonate solution were added before the reaction allowed standing for 30 min at room temperature. The absorbance was recorded at 760 nm by using UV/VIS Spectroskan 80 D spectrophotometer. The total phenolic compounds were determined according to gallic acid standard curve (0.01 to 1 mg/mL) (Fig. 1).

Determination of total flavonoid content: The total flavonoids in aqueous and ethanolic extracts were determined according to (Zhisben et al., 1999). One milliliter extract solution (1 mg/mL) was placed in 10 mL volumetric flask. Five milliliter of distilled water and 0.3 mL of 5% NaNO2 solution were added. After 5 min 0.6 mL of 10% AlCl3 was added. Two milliliter of 1M NaOH solution was added after another 5 min and the volume was made up to 10 mL with distilled water. The mixture was mixed thoroughly and the absorbance was
Antioxidant activity

Reducing power assay: The reducing power was estimated as described by (Chou et al., 2009). One milliliter of extract (0.5-10 mg/mL) was mixed with 2.5 mL of 1% potassium ferric cyanide and 2.5 mL of 0.2 M (pH 6.6) of sodium phosphate buffer and incubated at 50°C for 20 min. To stop the reaction, 2.5 mL of 1% trichloroacetic acid (TCA) was added to the mixture and centrifuge for 10 min at 3000 rpm. 0.5 mL of the supernatant was mixed with 1 mL of 1% ferric chloride and stand for 10 min. The absorbance was measured at 700 nm. EHT used as standard.

Chelating ability assay: Chelating ability was determined according to (Su et al., 2008) with some modification. One milliliter of extract (0.5-10 mg/mL) was mixed with 0.2 mL of ferric chloride of 2 mM and 0.2 mL of 8-Hydroxyquinoline (5 mM). After 10 min at room temperature, the absorbance was determined at 562 nm. The EDTA-Na, was used as reference.

RESULTS AND DISCUSSION

The root of Glycyrrhiza species is one of the richest sources of biological active compounds such as phenolic and flavonoid compounds (Roth, 2004). Phenolic compounds are very essential for plants due to their quenching ability because of the presence of hydroxyl groups (Elmastas et al., 2006). They belong to a class of antioxidant compounds which act as free radicals inhibitors (Ebrahimzadeh et al., 2010). Table 1, shows the percentages of total phenolic compounds and flavonoids which are represent the main antioxidant compounds in aqueous and ethanolic extracts of licorice roots. The total phenolic compounds which expressed as gallic acid and flavonoids as catechins were determined according to standard curves, phenols were determined by Folin-Ciocalteu’s colorimetric method and flavonoids by aluminum chloride colorimetric method. As shown in Fig. 3, the high percentages of the total phenolic and flavonoids in alcoholic extract mean that, the ethanol as extracting solvent (according to the chemical composition of phenolic compounds) is more effective than water (Syeda et al., 2008).

Several analytical methods have been developed to determine the antioxidant capacity of natural substances in vitro. However, the antioxidant activity of plant extracts cannot be evaluated using only one method, due to the complex composition of the phytochemical and oxidative processes (Inchuen et al., 2010). In this study, reducing power and chelating ability methods were used to evaluate the antioxidant activity, the results were summarized in Fig. 4 and 5.

In reducing power assay, Fe (II) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action (Nabavi et al., 2009) as the presence of antioxidants in...
Fig. 4: Reducing power of aqueous and ethanolic extracts of licorice roots as compared with BHT at the same concentration

Fig. 5: Chelating ability of aqueous and ethanolic extracts of licorice roots as compared with EDTA at the same concentration

the sample would result in the reducing Fe$^{2+}$ to Fe$^{3+}$ by donating an electron. Amount of Fe$^{2+}$ complex can then be monitored by measuring the formation of Perl’s Prussian blue at 700 nm. Higher absorbance at 700 nm indicates greater reductive ability (Chung et al., 2002). Figure 4 shows the reducing power of aqueous and ethanolic extracts of licorice roots (as compared with BHT). From these results we can find a proportional relationship between the reducing power and extract concentrations. Here, the ethanolic extract was shown more reducing power than aqueous extract. Higher reducing power might be attributed to higher amounts of total phenolic and flavonoid compounds. These results were associated with previous researches which also stated that the reducing power was increased as the total phenolics increased (Siddhuraju and Becker, 2003; Sultana et al., 2007).

Metal chelating activity is significant as it reduces the concentration of the catalyzing transition metal in lipid peroxidation through the Fenton reaction (Hseu et al., 2008). As shown in Fig. 5, the ferrous ion chelating activity increased with the increasing concentration. The strongest iron chelating activity was noticed at a concentration of 10 mg/mL, also the abilities of aqueous and ethanolic extracts of licorice roots, as chelating agents (comparing with EDTA as a reference) are less than their abilities as reducing power. Many papers were reported that the metal chelating potency plays a minor role in the overall antioxidant activities of some polyphenols (Rice-Evans et al., 1996).

Conclusion: Conclusively, polyphenols and flavonoids in licorice extracts are powerful antioxidant. The ethanol extract was more effective than the water extract as natural antioxidant and its efficiency increased by increasing its concentration in all method used. As a result, we are fully recommended the extract of licorice roots as a natural preservative in the food systems.

REFERENCES


