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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 antioxidative 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, glycyrrhetinic acid and liquirtic acid) and flavonoids (liquirtin, 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 (Oktay *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 (Elmastas *et al*., 2005, 2006a). Natural antioxidants commonly exist on plants which contain polyphenolic compounds (Gulcin *et al*., 2002b, 2007; Stoilova *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 Spectroscan 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% $NaNo_2$ solution were added. After 5 min 0.6 mL of 10% $AlCl_3$ 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

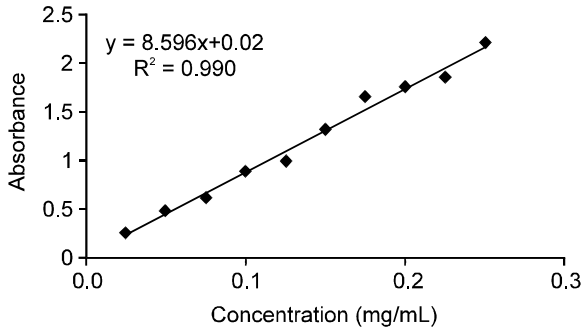

Fig. 1: Concentration-response curve for gallic acid at 760 nm

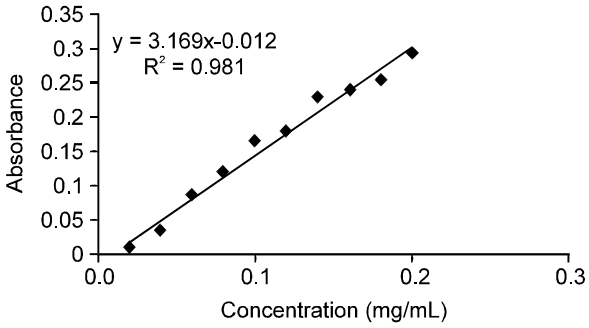

Fig. 2: Concentration-response curve for catechin at 510 nm

measured at 510 nm. The total flavonoids were expressed as µg catechin equivalents per gram dry matter according to catechin standard curve (Fig. 2).

Antioxidant activity

Reducing power assay: The reducing power was estimated as described by (Chou *et al*., 2009). One milliliter extract of (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. BHT used as standard.

Chelating ability assay: Chelating ability was determined according to (Su *et al*., 2008) with some modification. One milliliter of (0.5-10 mg/mL) extract was mixed with 0.2 mL ferric chloride of 2 mM and 0.2 mL 8-Hydroxyquinoline (5 mM). After 10 min at room temperature, the absorbance was determined at 562 nm. The EDTA-Na_2 was used as reference.

RESULTS AND DISCUSSION

The root of Glycyrrhiza species is one of the richest sources of biological active compounds such as

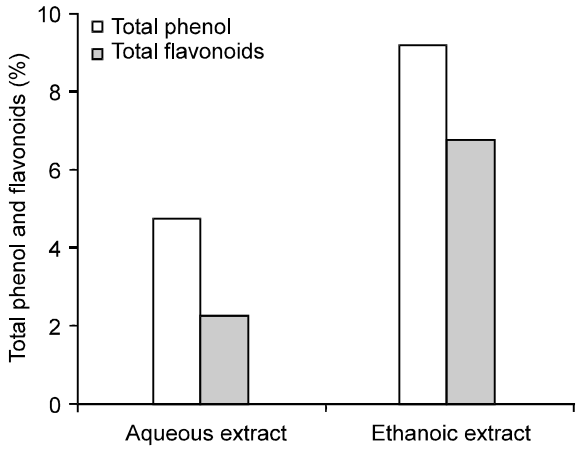

Fig. 3: Comparison of total phenol and flavonoid contents of licorice roots extracts

Table 1: Phenolic and Flavonoids content in licorice roots extracts

Extraction	Type of plant	Phenolic content (%)	Flavonoids content (%)
Aqueous	Licorice roots	4.8	2.3
Methanolic		9.2	6.8

phenolic and flavanoid 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 (III) 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

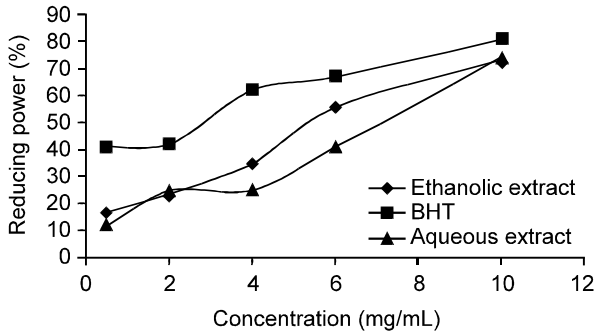

Fig. 4: Reducing power of aqueous and ethanolic extracts of licorice roots as compared with BHT at the same concentration

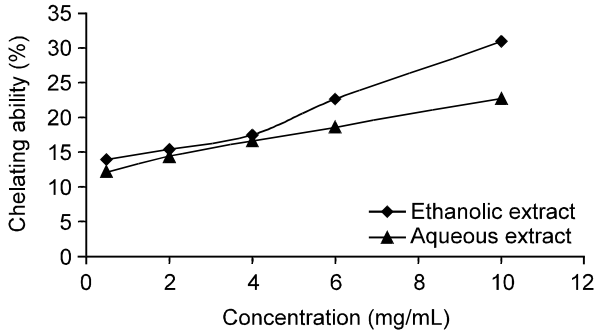

Fig. 5: Chelating ability of aqueous and ethanolic extracts of licorice roots as compared with EDTA at the same concentration

the sample would result in the reducing Fe^{3+} to Fe^{2+} 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

Arystanova, T., M. Irismetov and A. Sophekova, 2001. Chromatographic determination of glycyrrhizinic acid in Glycyrrhiza glabra preparation. Chem. Nat. Com., 37: 89-91.

Asl, M.N. and H. Hosseinzadeh, 2008. Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. Phytotherapy Res., 22: 709-724.

Ayoola, G.A., S.S. Ipav, M.O. Sofidiya, A.A. Adepoju-Bello, H.A. Coker and T.O. Odugbemi, 2008. Phytochemical screening and free radical scavenging activities of the fruits and leaves of Alanblack floribunda oliv (Guttiferae). Int. J. Health Res., 1: 87-93.

Biondi, D.M., C. Rocco and G. Ruberto, 2005. Dihydrostilbene derivatives from Glycyrrhiza glabra leaves. J. Nat. Prod., 68: 1099-1102.

Chou, H.J., J.T. Kuo and E.S. Lin, 2009. Comparative antioxidant properties of water extracts from different parts of beefsteak plant (perilla frutescens). J. Food Drug Anal., 17: 489-496.

Chung, Y.C., C.T. Chang, W.W. Chao, C.F. Lin and S.T. Chou, 2002. Antioxidative activity and safety of the 50 ethanolic extract from red bean fermented by Bacillus subtilis IMR-NK1. J. Agric. Food Chem., 50: 2454-2458.

Di Mambro, V.M. and M.J. Fonseca, 2005. Assays of physical stability and antioxidant activity of a topical formulation added with different plant extract. J. Pharmaceutical and Biomed. Analysis, 37: 287-295.

Ebrahimzadeh, M.A., S.F. Nabavi, S.M. Nabavi and F. Pourmorad, 2010. Nitric oxide radical scavenging potential of some Elburz medicinal plants. Afr. J. Biotechnol., 9: 5212-5217.

Elmastas, M., I. Gulcin, O. Isildak, O.I. Kufrevioglu, K. Ibaogluand and H.Y.A. Enein, 2006. Radical scavenging activity and antioxidant capacity of bay leaf extracts. J. Iran. Chem. Soc., 3: 258-266.

Elmastas, M., I. Gulcin, L. Ozturk and I. Gokce, 2005. Investigation of antioxidant properties of spearmint (*Mentha spicata* L.). Asian J. Chem., 17: 137-148.

Elmastas, M., I. Turkekul, L. Ozturk, I. Gulcin, O. Isildak and H.Y. Aboul-Enein, 2006a. The antioxidant activity of two wild edible mushrooms(Morchella vulgaris and Morchella esculanta). Comb. Chem. High T. Scr., 9: 443-448.

Fukai, T., C. Baosheng, K. Maruno, Y. Migakawa, M. Konoshi, T. Nomura and B. Cai, 1998. An isopernylated flavonone from Glycyrrhiza glabra and re-assay of liquorice phenols. Phytochem., 49: 2005-2013.

Gulcin, I., M.E. Buyukokuro-lu, M. Oktay and O.I. Kufrevio-lu, 2002b. On the *in vitro* antioxidant properties of melatonin. J. Pineal. Res., 33: 167-171.

Gulcin, I., M.E. Buyukokuroglu, M. Oktay and O.I. Kufrevioglu, 2003a. Antioxidant and analgesic activities of turpentine of Pinus nigra Arn. Subsp. pallsiana (Lamb.) Holmboe. J. Ethnopharmacol., 86: 51-58.

Gulcin, I., R. Elias, A. Gepdiremen, K. Taoubi and E. Koksal, 2009. Antioxidant secoiridoids from fringe tree (*Chionanthus virginicus* L.). Wood Sci. Technol., 43: 195-212.

Gulcin, I., M. Elmastas and H.Y. Aboul-Enein, 2007. Determination of antioxidant and radical scavenging activity of basil (Ocimum basilicum) assayed by different methodologies. Phytother. Res., 21: 354-361.

Gulcin, I., V. Mshvildadze, A. Gepdiremen and R. Elias, 2006a. Antioxidant activity of a triterpenoid glycoside isolated from the berries of Hedera colchica: 3-O-(β-D-glucopyranosyl)-hederagenin. Phytother. Res., 20: 130-134.

Gulcin, I., M. Oktay, O.I. Küfrevioglu and A. Aslan, 2002a. Determinations of antioxidant activity of lichen *Cetraria islandica* (L) Ach. J. Ethnopharmacol., 79: 325-329.

Hseu, Y., W. Chang, C. Chen, L. Liao, C. Huang, F. Lu, Y. Chia, H. Hsu, J. Wu and H. Yang, 2008. Antioxidant activities of Toona Sinensis leaves extracts using different antioxidant models. Food and Chem. Toxicol., 46: 105-114.

Inchuen, S., W. Narkrugsa and P. Pornchaloempong, 2010. Effect of drying methods on chemical composition, color and antioxidant properties of Thai red curry powder. Kasetsart J. Natural Sci., 44: 142-151.

Nabavi, S.M., M.A. Ebrahimzadeh, S.F. Nabavi, M. Fazelian and B. Eslami, 2009. *In vitro* antioxidant and free radical scavenging activity of Diospyros lotus and Pyrus boissieriana growing in Iran. Pharmacognocy Magazine, 4: 123-127.

Nassiri-Asl, M., S. Saroukhani and F. Zamansoltani, 2007. Anticonvulsant effects of aqueous extract of Glycyrrhiza glabra root in PTZ-induced seizure in mice. Int. J. Pharmacol., 3: 432-434.

Oktay, M., I. Gulcin and O.I. Kufrevioglu, 2003. Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. Lebensm. Wissen. Technol., 36: 263-271.

Prakash, D., G. Upadhyay, B.N. Singh and H.B. Singh, 2007. Antioxidant and free radical-scavenging activities of seeds and agri-wastes of some varieties of soybean (Glycine max). Food Chem., 104: 783-790.

Rice-Evans, C.A., N.J. Miller and G. Paganga, 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biol. and Med., 20: 933-956.

Roth, K., 2004. Licorice spirals. Chemie in unserer Zeit, 38: 202-207.

Sen, C.K., L. Packer and O. Hanninen, 2000. Handbook of oxidants and antioxidants in exercise (1st edn). Elsevier Science B.V. Amsterdam. Sci., 44: 142-151.

Siddhuraju, P. and K. Becker, 2003. Antioxidant properties of various extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera lam.) leaves. J. Agric. Food Chem., 51: 2144-2155.

Stoilova, I., A. Krastanov, A. Stoyanova, P. Denev and S. Gargova, 2007. Antioxidant activity of a ginger extract (Zingiber officinale). Food Chem., 102: 764-770.

Su, M.S., Y.T. Shyu and P.J. Chein, 2008. Antioxidant activities of citrus herbal product extract, Food Cem., 111: 892-896.

Sultana, B., F. Anwar and R. Przybylski, 2007. Antioxidant activity of phenolic components present in barks of Azadirachta indica, Terminalia arjuna, Acacia nilotica and Eugenia jambolana Lam. Trees. Food Chem., 104: 1106-1114.

Syeda, B.B., I.B. Muhammad and M. Shahabuddin, 2008. Antioxidative activity of extracts from Fenugreek seeds (Trigonella foenumgraecum). Pak. J. Anal. Environ. Chem., 9: 78-83.

Zhisben, J., T. Mengcheng and W. Jianming , 1999. The determination of flavonoid contents In mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555-559.