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Effect of harvest time on antioxidant activity of *Glycyrrhiza glabra* root extract and evaluation of its antibacterial activity

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Abstract

Glycyrrhiza glabra roots harvested at two different times (T_1: 20 October 2010 and T_2: 20 January 2010) were analyzed for their chemical composition, and then antioxidant activity and antibacterial effect of ethanolic root extract against some pathogenic bacteria were evaluated. Higher amounts of moisture were detected in T_1 licorice root, whereas higher levels of sugar, protein, ash and phenolic compounds were determined in T_2 licorice root. The antioxidant activity was assessed by DPPH, reducing power and total antioxidant capacity assay and T_2 licorice root was found to possess higher antioxidant activity. Antimicrobial activity was estimated using a microdilution technique (ELISA) against some human pathogenic bacteria. The following bacteria were tested: *Salmonella entritidis* (gr⁻), *Escherichia coli* (gr⁻), *Bacillus cereus* (gr^+) and *Staphylococcus aureus* (gr^+). It exhibited antimicrobial activity against both tested Gram-positive and Gram-negative bacteria.

Introduction

Plant roots are valuable sources of useful chemicals, such as secondary metabolites and enzymes, which areused by the food and pharmaceutical industries (Shabani *et al*., 2009). *Glycyrrhiza glabra*, belongs to genus *Glycyrrhiza* and is commonly called as licorice which is available in India, Spain, Italy, Turkey, Israel, Syria, Iran, China and Russia (İbanoğlu and İbanoğlu, 2000). Licorice roots are important commercial products that grows in subtropical climates in rich soil to a height of four or five feet (Khanzadi and Simpson, 2010). The plant has dark green leaflets and produce spikes of small pea-like flowers in summer. The plant roots are straight pieces of wrinkled fibrous wood, which grow horizontally underground (Khanzadi and Simpson, 2010). The roots and stolons of licorice contain more than 100 various compounds, some of which accumulated in large amounts, Most important of them are triterpene saponins (including glycyrrhizin) and phenolic compounds (Shibata, 2000; Shabani *et al*., 2008). The yellow color of licorice is due to the flavonoid content of the plant, which includes liquiritin, isoliquiritin (a chalcone), and other compounds. The isoflavones glabridin and hispaglabridins A and B have significant antioxidant activity.

Phenolic phytochemicals are important aromatic secondary metabolites in plants. In addition, many phenolic phytochemicals have antioxidative, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and antiinflammatory activities (Kima *et al*., 2002). The content of phytochemical substances is influenced by numerous factors including harvesting time, genotype, cultivation techniques, climatic conditions that occur during the pre-harvest period and the operations carried out during the post-harvest storage (Gao *et al*., 2011). Imeh and Khokhar (2002) underlined various factors, including agronomic, genomic, pre- and post-harvest conditions and processing, which may affect the chemical composition of plant foods in general, and they may have a significant role in determining the phenolic composition and the bioactivity of these compounds in particular. In order to improve the nutritional value and functionality of plant materials or foods, there is an increasing interest to study the effects of various factors on the phytochemical substances including phenolic compounds, essential oil, ascorbic acid, amino acids and carotenoids (Liu *et al*., 2010; Lombardo *et al*., 2010; Gao *et al*., 2011).

The objective of present study wasto investigate the effect of harvest time on chemical composition, total phenolic content and antioxidant capacity of licorice root. Also, in the current study, the antimicrobial potential of ethanolic extract of *Glycyrrhiza glabra* roots was investigated.

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Materials and Methods

Chemicals

Ethanol, potassium ferricyanide, trichloroacetic acid, $FeCl_3$, sulphuric acid, sodium phosphate and ammonium molybdate, Muller Hinton Broth, Barium chloride, Chloramphenicol and H_2SO_4 were purchased from Merck Company (Germany). Gallic acid, Folin-Ciocalteu, 2,2-diphenyl-1picryl-hydrazyl (DPPH) and standards (glycyrrhizin and BHT) were purchased from sigma chemical co, USA. Sodium carbonate was from Merck. All chemicals used were of analytical grade.

Plant material

The roots of *Glycyrrhiza glabra* were collected from research farm of Gorgan agricultural research institute, Iran at two different times (T_1: 20 October 2010 and T2: 20 January 2010). After removing dust, the plant materials were dried at 40°C in an oven and then ground to fine powders and passed through the No. 20 mesh sieve and stored in plastic bags until use.

Preparation of ethanolic extract

Samples were soaked in selected solvent (ethanol 80%) for 90 min as recommended by Pan *et al.* (2003). A domestic microwave oven (CE3260F JUNE-2008) was used in this study. The suspensions were placed in microwave oven and heated according to the method mirzapour *et al.* (2010). The extraction conditions were time 5 min and liquid/solid ratio 15:1. The extract was then filtered through Whatman No. 1 filter paper. The resulting extract was lyophilized and used in experiments.

Proximate composition

The proximate composition of *Glycyrrhiza glabra* was determined using the AOAC standard methods (AOAC) methods for protein, fat, ash, moisture and sugar before and after hydrolysis (AOAC, 1990).

HPLC analysis

HPLC analysis was performed, using a Jasco PU-2089 pump equipped with a Jasco MD-2015 diode array detector (DAD), and chromatographic separations were performed on a LiChrospher RP-18 column (4.0 × 250 mm i.d., 5 lm). The composition of solvents and the isocratic elution conditions were as previously described (Shiong and Mei Len, 2003). The DAD detector was operated in the range of 200–650 nm, and the analysis was performed at 254 nm. Components of glycyrrhizin were identified by comparison of their retention times and UV spectra with those of authentic standards (glycyrrhizin), under identical analysis conditions. Solutions, at different concentrations, of each standard, were injected into the HPLC column to check the linearity between concentration and peak areas, and a response factor was calculated. Quantifications of glycyrrhizin were done using these calibration factors.

Determination of total phenolic content

After isolation of the phenolic compounds by the extraction methods described in previous sections, the concentration of total phenolic content was estimated by the Folin-Ciocalteu assay, according to the method presented by Lin *et al.* (2007). Gallic acid was used as standard and results were expressed as mg of Gallic acid per g of dry sample.

DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the method of Blois (1958). Briefly, 1 mL of a 1 mM methanolic solution of DPPH was mixed with 3 mL of extract solution in methanol (containing 50–500 g of dried extract). The mixture was then vortexed vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm by Spectrophotometer (CE 2502) and activity was expressed as percentage of DPPH scavenging relative to control using the equation (1):

$$\text{DPPH scavenging activity\%} = (\text{Absorbance of control} - \text{Absorbance of sample}/\text{Absorbance of control}) \times 100 \quad (1)$$

Reducing power assay

The ability of extracts to reduce iron (III) was assessed by the method of Yildirim *et al.* (2001). The dried extract (200–2000 µg) in 1 ml of the corresponding solvent was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$, 10 g/l], then the mixture was incubated at 50°C for 30 min. After incubation, 2.5 ml of trichloroacetic acid (100 g/l) were added and the mixture was centrifuged at 1650 g for 10 min. Finally, 2.5 ml of the supernatant solution were mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (1g/l) and the absorbance was measured at 700 nm. High absorbance indicates high reducing power.

Total antioxidant capacity

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Arabshahi and Urooj, 2007). An aliquot of 0.1 ml

of sample solution (containing 200–2000 µg of dried extract in corresponding solvent) was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against a blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples.

Antimicrobial activity

In order to obtain quantitative data for EtOH extract (T_2 licorice root), the modified microdilution technique was used (Kukić *et al*., 2008). The following bacteria were tested: *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 29213), *Salmonela entritidis* (WHO) and *Bacillus cereus* (ATCC 11778). The bacterial suspension was adjusted with mac farland turbidity standard No. 1 to a concentration of approximately 1×10^6 CFU/ML.

Minimum inhibitory concentrations (MICs) determination was performed by a serial dilution technique, using 96-well microtiter plates. Extract were dissolved in distilled water (0.1-4 mg/ml). Extracts were sterilized over a membrane filter unit of 0.2 µm of pore size. The bacterial suspension applied in a final volume of 100 µl/well. The extracts (0.1–4.0 mg/ml) were added to broth medium with bacterial suspension. The microplates were incubated for 24 h at 37°C. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations which completely inhibited bacterial growth (MICs).The minimum bactericidal concentrations (MBCs) were determined by serial sub-cultivation of 2 µl in microtiter plates containing 100 µl of broth per well and further incubation for 24 h at 37°C. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. Suspension and Muller Hinton Broth (MHB) was used as a negative control, while chloramphenicol (100 ppm) and suspension was used as a positive control.

Results and Discussion

Chemical composition at different harvest times

Chemical compositions of licorice root at different harvest time are shown in Table 1. The content of protein increased from 5.30% ($p < 0.05$) in T_1 licorice root to 6.65% in T_2 licorice root. There were no significant differences between fat of licorice root harvested at T_1 and T_2 Times. The results of Table 1 show that the effect of harvest season on the sugar before and after hydrolysis is significant. As can be seen from the Table 1, the T_2 licorice root possessed higher sugar before and after hydrolyze compared to T_1 licorice root. Sugar compounds which are produced in different periods of plant growth are consumed by the plant metabolism and the excess is stored in the root. In the early growing season when plants have a high metabolism, sugar stored in plant roots is reduced and when plant metabolism is reduced, then sugar level will rise (Omidbaigi, 2004). Also, according to results, the ash content of T_2 licorice root was higher while the moisture content was lower compared to T_1 licorice root.

Table 1. Proximate composition and phyto-consttuents of *Glycyrrhiza glabra* root extract

Composition (%)	Harvest time	
	T_1	T_2
Fat	1.95±0.212[a]	2.45±0.07[a]
Moisture	10.00±1.414[a]	3.40±0.424[b]
Ash	4.58±0.0212[b]	7.40±0.141[a]
Protein	5.30±0.141[b]	6.65±0.212[a]
Sugar before hydrolyze	8.10±0.141[b]	9.30±0.141[a]
Sugar after hydrolyze	11.40±0.141[b]	12.40±0.141[a]
Total phenolic (mg GA eq/g dry sample)	45.853±0.48[b]	54.266±0.64[a]
Glycyrrhizin (%)	0.8867±0.02[b]	1.112±0.07[a]

Values in the same column followed by different letters are significantly different ($p < 0.05$).

Table 2. Minimum inhibitory and bactericidal concentrations (MICs and MBCs) of licorice root extract (mg/ml)

Bacteria	MICs	MBCs
Bacillus cereus	1.0	3.0
Staphylococcus aureus	0.8	0.9
Salmonella entritidis	0.8	0.9
Escherichia coli	0.8	0.9

Phenolic content at different harvest times

Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, phenolic contents of plants may contribute directly to their antioxidant action. In the present study, phenolic content of licorice root was determined by Folin-Ciocalteau method. The phenolic content was in coordinately influenced by harvest time (Table 1). The contents of phenolics increased by 18.3% in T_2 licorice root compared to T_1 licorice root. It is known that the content of phenolic compounds in plant materials varies greatly with, for example processing, genotype, harvest time, growing environment and conditions. Variation of phenolic concentration in licorice root affirms the influence of both harvest time and climate factors on production and release of these metabolites. The variation in content of phenolic

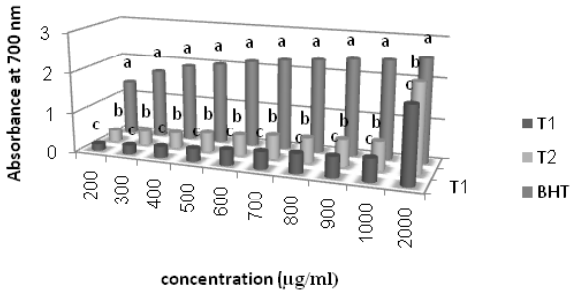

Figure 1. Reducing powers of ethanolic extracts of T_1 and T_2 licorice root and BHT. Values with different letters are significantly different ($p < 0.05$) from each other.

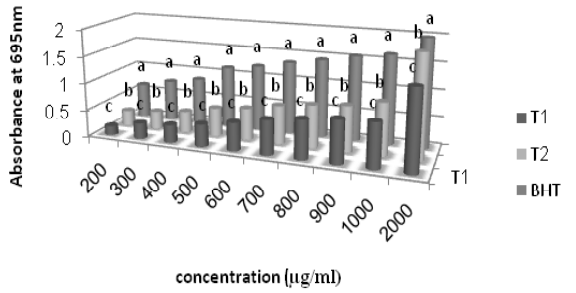

Figure 2. Total antioxidant activities of ethanolic extracts of T_1 and T_2 licorice root. BHT was used as positive control. Values with different letters are significantly different ($p < 0.05$) from each other.

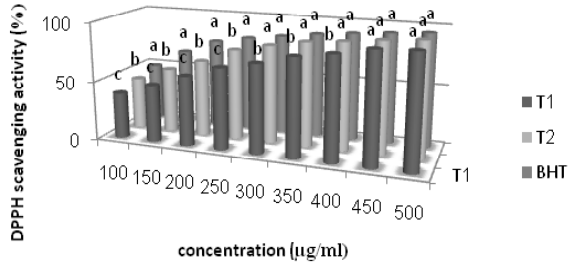

Figure 3. DPPH radical scavenging activities of ethanolic extracts of T_1 and T_2 licorice root. BHT was used as positive controls. Values with different letters are significantly different ($p < 0.05$) from each other.

compounds correlates with the metabolic balance between biosynthesis and further catabolism of plants and external environmental disturbances (Liu *et al.*, 2010; Gao *et al*., 2011). Previous studies have concluded that low temperature stress resulted in an accelerated build up of phenolic compounds in peach, tomato, sweet potato and Hayward kiwifruit (Toor and Savage, 2006; Padda and Picha, 2008; Tavarini *et al*., 2008; Meng *et al.*, 2009). The vacuoles in plant cells form the main compartment in which phenolic compounds accumulate (Toor and Savage, 2006). At chilling temperatures, the permeability of the cell membranes and the activity of membrane bound enzymes are changed, which causes an accumulation of toxic intermediates (Toor and Savage, 2006) in the cells. This creates physiological stress in plant cells and, therefore, the level of phenylalanine ammonia lyase (PAL), an important enzyme involved in phenolic biosynthesis (Morello *et al.*, 2005) increases considerably, thus accumulates more phenolic compounds. Various stresses such as irradiation, wounding, nutrient deficiencies, herbicide treatment, lower temperature exposure and viral, fungal and insect attacks can increase PAL synthesis or PAL activity in various plants (Morello *et al.*, 2005).

Antioxidant activity

The antioxidant activity is influenced by many factors, which cannot be fully described with one single reaction system. Thus, utilizing multiple assays to evaluate antioxidant activity tend to be necessary and may provide exclusive information on their multiple abilities to scavenge different radicals. In the present study, DPPH radicals scavenging capacity, reducing power and total antioxidant capacity assays were employed to test the antioxidant activity of phenolics extracted from licorice root and the results are shown in Figures 1, 2 and 3.

Reducing powerassay

In the present study, the reducing power was assessed based on measurement of Fe^{+3} to Fe^{+2} transformations and compared to that of BHT, which is known to be a strong reducing agent. Fig. 1 shows the reducing power of the T_1 and T_2 licorice root extract as a function of their concentration. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The reducing power of all these test compounds increased in a concentration-dependent manner. T_2 Licorice root extract which contained higher amount of total phenolics, showed higher reducing power, compared to T_1 licorice root extract with lower amount of total phenolics. Similar relations between iron (III) reducing activity and total phenol content have been reported in the literature. However the correlation may not be always linear (Yildirim *et al*., 2000; Arabshahi and Urooj, 2007). The reducing power of both licorice extract was markedly lower than that of BHT. At the high concentration (2 mg/ml), licorice root extract showed better reducing capacity (Figure 1).

Total antioxidant capacity assay

The assay is based on the reduction of MO (VI) to MO (V) by the extract and subsequent formation of a green phosphate/MO (V) complex at acidic pH. The total antioxidant activity was measured and compared with that of BHT. The high absorbance

values indicated that the sample possessed significant antioxidant activity. According to the result (Figure 2), there was significant difference between total antioxidant capacity of T_1 and T_2 licorice root extract ($p < 0.05$). The total antioxidant capacity of licorice root extracts increased with increasing the concentration of samples. Total antioxidant capacity of T_1 and T_2 licorice root extract and standard at each concentration exhibited the following order: BHT>T_2>T_1. It has been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic compound (Ferreira *et al*., 2007).

Free radical scavenging activity

The DPPH assay, which measures the ability of compounds to transfer labile H-atoms to radicals, is the commonest method of antioxidant activity evaluation. The abstraction of hydrogen by this stable free radical is known to lead to bleaching with a maximum absorption band around 515-528 nm and can be easily measured spectrophotometrically. Figure 3, illustrates a significant ($p < 0.05$) decrease in the concentration of DPPH radical due to the scavenging ability of the extracts of licorice root and standard. BHT was used as standard. The scavenging effect of licorice root extracts and standards on the DPPH radical decreased in this order: BHT>T_2>T_1. These results indicate that both licorice extracts had notable effect on scavenging free radical. Free radical scavenging activity also increased with increasing concentration. Gao *et al.* (2011) compared two sample of Sphallerocarpus gracilis root harvested at two different times (T_1:12-10-2009 and T_2:15-04-2010). The results showed a significant increase ($p < 0.05$) in the DPPH radicals scavenging activity of free and conjugated phenolics along with a decrease ($p < 0.05$) of insoluble cell-wall-bound phenolics in T_2 samples.

HPLC analysis of phenolic extracts at different harvest times

In licorice root extract, glycyrrhizin is the main phenolic compound, which contains five hydroxyl groups. The difference between the amounts of glycyrrhizin in licorice root harvested at different time is shown in Table 1. Analysis of variance (Table 1) showed that the harvest season had significant effect on the amount of glycyrrhizin ($p < 0.05$). So, the higher and lower, amount were detected at January (1.112% of dry matter) and October (0.8867% of dry matter), respectively. This result is supported by previous work (Gao *et al*., 2011), that reported high variability in the content of the investigated phenolic compounds with the harvest time. Also, Gao *et al.* (2011) investigated the effect of harvest time on phenolic compound in *S. gracilis* root collected at T_1 (12-10-2009) and T_2 (15-04-2010) times. They reported an increase in the levels of individual phenolic acids in *S. gracilis* root collected at T_2 time. The trend of increase in most of the individual phenolic acids was parallel with the changes in phenolic content. It was concluded that it can be due to the phenolic metabolism resulting from low temperature exposure. A similar result also was found with Globe artichoke and higher phenolic acid content was detected in samples harvested in spring (Lombardo *et al*., 2010). The sweet potato root tissue exposed to low temperature storage showed an increase in individual phenolic acids (Padda and Picha, 2008).

Antibacterial activity

The antimicrobial activity of *Glycyrrhiza glabra* is well known (Gupta *et al.*, 2006). The results of antibacterial activity (Table 2) showed that *B. cereus* (with MICs 1.0 ± 0.0 mg/ml and MBCs of 3.0 ± 0.0 mg/ml) was the most resistant species. Also, *E. coli*, *S. entridis* and *S. aureus* showed higher sensitivity with the MICs of 0.8 ± 0.0 mg/ml and MBCs of 0.9 ± 0.0 mg/ml. The root extract exhibited antimicrobial activity against both Gram-positiveand Gram-negative bacteria.This antimicrobial activity can be due to alkaloid, saponins, flavonoids, tannin, glycosides and phenols found in ethanolic extract. These phytochemical groups are known to posses' antimicrobial compounds (Meghashri and Shubha, 2009). Mitscher *et al.* (1980) studied the antibacterial activity of the *G. glabra* extract and reported that it had high activity against *Staphylococcus aureus* ATCC13709 and *Candida albicans* ATCC 10231 strains. Demizu *et al.* (1988) examined a flavonoid in licorice-root for antibacterial activity and found that its antibacterial activity was region-specific. Its high activity against gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) was also reported.

Conclusion

The environmental condition influences the growth of herbs and therefore the content of their active ingredients. So, the picking of plant is cost-effective when the concentration of active ingredients reaches to optimal level. The results of present work demonstrated that the chemical composition, phenolics content and antioxidant activity of licorice root vary with the harvest time. Data of the present study indicated higher amounts of sugar, protein

and ash, as well as phenolics in T_2 licorice root compared to T_1 licorice root, while higher levels of moisture were found in the later one. In general, the antioxidant activity of the phenolic extracts from licorice root collected at T_2 time was higher than those from licorice root collected at T_1 time. There were significant differences between DPPH radicals scavenging capacity and reducing power and total antioxidant activity of two licorice root extract with higher value in T_2 one. Also, according to the results, the extract of licorice root revealed considerable antimicrobial activity against some pathogenic bacteria.

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References

Arabshahi-Delouee, S. and Urooj, A. 2007. Antioxidant properties of various solvent extracts of mulberry (*Morusindica* L.) leaves. Food Chemistry 102: 1233–1240.

Blois, M. S. 1958. Antioxidant determinations by the use of a stable free radical. Nature 26: 1199–1200.

Demizu, S., Kajiyama, K., Takahashi, K., Hiraga, Y., Yamamoto, S., Tamura, Y. and Okada, K. 1988. Antioxidant and antimicrobial constituents of licorice: isolation and structure elucidation of a new benzofuran derivative. Chemical and Pharmaceutical Bulletin 36: 3474-3479.

Gao, C. Y., Lu, Y.H., Tian, C. R., Xu, J. G., Guo, X.P., Zhou, R. and Guo Hao. 2011.Main nutrients, phenolics, antioxidant activity, DNA damage protective effect and microstructure of *Sphallerocarpus gracilis* root at different harvest time. Food Chemistry 127:615-622.

Gupta, V. K., Fatima, A., Faridi, U., Negi, A. S., Shanker, K., Kumar, J. K., Rahuja, N., Luqmana, S., Sisodia, B.S., Saikia, D., Darokar, M.P. and Khanuja, S. P. S. 2006. Antimicrobial potential of *Glycyrrhiza glabra* roots. CIMAP Communication, No, 39J.

Helrich, K. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC), 15th Edit.

İbanoğlu, E. and İbanoğlu, S. 2000. Foaming behaviour of liquorice (*Glycyrrhiza glabra*) extract. Food Chemistry 70: 333-336.

Imeh, U. and Khokhar, S. 2002. Distribution of conjugated and free phenols in fruits: Antioxidant activity and cultivar variations. Journal of Agricultural and Food Chemistry 50: 6301–6306.

Ferreira, I. C. F. R., Baptista, P., Vilas-Boas, M. and Barros L. 2007. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. Food Chemistry 100: 1511–1516.

Khanzadi, F. K. and Simpson, T.J. 2010. Effect of gamma irradiation on the antimicrobial and free radical scavenging activities of *Glycyrrhiza glabra* root. Radiation Physics and Chemistry 79: 507–512.

Kima, D O., Jeongb, S W. and Leea, C. Y.2002. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry 81: 321–326.

Kukić, J., Popović, V., Petrović, S., Mucaji, P., Ćirić, A., Stojković, D. and Soković, M. 2008. Antioxidant and antimicrobial activity of *Cynara cardunculus* extracts. Food Chemistry 107: 861–868.

Liu, W., Zu, Y. G., Fu, Y. J., Kong, Y., Ma, W. and Yang, M. 2010.Variation in contents of phenolic compounds during growth and post-harvest storage of pigeon pea seedlings. Food Chemistry 121: 732–739.

Lin, J.Y. and Tang, C. Y. 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chemistry 101:140-147.

Lombardo, S., Pandino, G., Mauromicale, G., Knodler, M., Carle, R. and Schieber, A. 2010. Influence of genotype, harvest time and plant part on polyphenoliccomposition of globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori]. Food Chemistry 119: 1175–1181.

Meghashri, H. and Shubha, G. 2009. *In Vitro* antifungal and antibacterial activities of root extract of *Glycyrrhiza Glabra*. Journal of Applied Sciences Research 5: 1436-1439.

Meng, X., Han, J., Wang, Q. and Tian, S. 2009. Changes in physiology and quality of peach fruit treated by methyl jasmonate under low temperature stress. Food Chemistry 114: 1028–1035.

Mitscher, L., Park, Y. H. and Clark, D.1980. Antimicrobial agents from higher plants. Antimicrobial isoflavanoids and related substances from *Glycyrrhiza glabr*a L. var. *fypica.* Journal of Natural Products 43: 259-269.

Mirzapour, M., Hamedi, M. and Rahimipanah, M. 2010. Sunflower oil stabilization by Persian walnut leaves extract during oven storage test. Food Science and Technology Research 16:443-446.

Morello, J. R., Romero, M. P., Ramo, T. and Motilva, M. J. 2005. Evaluation of L-phenylalanine ammonia-lyase activity and phenolic profile in olive drupe (*Olea europaea* L.) from fruit setting period to harvesting time. Plant Science 168: 65–72.

Omidbaigi, R., 2004. Production and Processing of Medicinal Plants. Behnashr Press 3: 379p.

Padda, M. S. and Picha, D. H. 2008. Effect of low temperature storage on phenolic composition and antioxidant activity of sweet potatoes. Postharvest Biology and Technology 47: 176–180.

Pan, X., Niu, G. and Liu, H. 2003. Microwave-assisted extraction of tea polyphenols, tea caffeine from green tea leaves. Chemical Engineering and Processing 42: 129-133.

Shabani, L., Ehsanpour A. A., Asghari, G. and Emami, J. 2009. Glycyrrhizin production by *in vitro* cultured

Glycyrrhiza glabra elicited by Methyl Jasmonate and salicylic acid. Russian Journal of Plant Physiology.56: 621–626.

ShiOng, E. and Mei Len, S. 2003. Pressurized hot water extraction of berberin, baicalein and glycyrrhizin in medicinal plants. Analytical Chemical Acta 482:81-89.

Shibata, S. 2000. A Drug over the Millennia: Pharmacognosy, Chemistry, and Pharmacology of Licorice, Yakugaku zasshi-J. Pharmaceutical Society of Japan. 120: 849–862.

Tavarini, S., Degl'Innocenti, E., Remorini, D., Massai, R. and Guidi, L. 2008. Antioxidant capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after storage of Hayward kiwifruit. Food Chemistry 107:282–288.

Toor, R. K. and Savage, G. P. 2006. Changes in major antioxidant components of tomatoes during post-harvest storage. Food Chemistry 99: 724–727.

Yildirim, A., Mavi, A. and Kara, A. A. 2001. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. Journal of Agricultural and Food Chemistry 49: 4083–4089.

Yildirim, A., Mavi, A., Oktay, A. A., Algur, . F. and Bilaloglu, V. 2000. Comparison of antioxidant and antimicrobial activity of tilia (*Tilia argenta* Desf. Ex. D.C.), sage (*Salvia triloba* L.) and black tea (*Camellia sinensis* L.) extracts. Journal of Agricultural and Food Chemistry 48: 5030–5034.