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LETTER FROM THE EDITOR

Dear Subscribers,

It is with great pleasure and satisfaction that I announce the 20th anniversary of the Italian Journal of Food Science. Over the years, the Journal has evolved to attain its current stable and sound position among the world's chief scientific Food Science journals.

The ISI Impact Factors of IJFS have only varied slightly since 1992, the latest being 0.506. This certainly indicates that, thanks to a careful selection of top quality papers, a small journal can play an important role in a specific international editorial field. This could not have been achieved without the hard work of our Staff and, in particular, of our Co-Editor Sister Mary Traynor, whom I would especially like to thank for her unstinting assistance and great expertise.

To mark this special occasion, Chiriotti Editori has enthusiastically adopted my suggestion to put the Journal on the net! Starting with this first issue of 2008, and in addition to the standard printed version, all of our paid subscribers will be receiving a personal username and password to enable them to access the Journal directly from the net. At the same time, for a small fee, we extend this service to all our foreign readers, who can download the pdf version of IJFS.

The success of any scientific Journal is mainly dependent on the scientists who select it for the publication of their work and ideas and on the reviewers who evaluate the papers. We wish to thank the authors who have given us the opportunity to publish their work and to the many reviewers who have contributed their time and effort to improve the papers with their comments and suggestions.

We are extremely grateful to all of you for having chosen our Journal, thus having enabled us to achieve so much and to view the future with great optimism.

Sincerely yours

Paolo Fantozzi
Editor-in-Chief

PAPER

TOTAL PHENOLICS, MINERAL CONTENTS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *GLYCYRRHIZA GLABRA* L. ROOTS GROWN WILD IN TURKEY

POLIFENOLI TOTALI, MINERALI, ANTIOSSIDANTI E LE PROPRIETÀ ANTIBATTERICHE DELLE RADICI DI *GLYCYRRHIZA GLABRA* L. SELVATICA CRESCIUTA IN TURCHIA

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ABSTRACT

The total phenolic content, macro and micro elements (N, P, K, Ca, Mg, Na, Fe, Mn, Zn and Cu), antioxidant and antibacterial activities of roots of wild grown *Glycyrrhiza glabra* are reported. The amount of total phenolics was 12.88 μgGAE/mg DW in *Glycyrrhiza glabra* roots. The average N, P, K, Ca, Mg, Fe, Mn, Zn, Na and Cu composition in licorice roots was 2.80%, 175 mg/100 g,

RIASSUNTO

Sono riportati il contenuto in polifenoli totali, in macro e micro elementi (N, P, K, Ca, Mg, Na, Fe, Mn, Zn e Cu), in antiossidanti e le proprietà antibatteriche delle radici di *Glycyrrhiza glabra* selvatica. Nelle radici di *Glycyrrhiza glabra* la concentrazione di polifenoli totali era 12.88 μgGAE/mg di peso secco. La composizione media in N, P, K, Ca, Mg, Fe, Mn, Zn, Na e Cu nella liquirizia era, rispet-

- Key words: antibacterial activity, antioxidant activity, elements, *Glycyrrhiza*, phenolics -

1400 mg/100 g, 147 mg/100 g, 120 mg/100 g, 20 mg/100 g, 6 mg/100 g, 4.4 mg/100 g, 2.1 mg/100 g and 0.1 mg/100 g, respectively. The highest antioxidant activity of root extract was 88.7% at a concentration of 800 μg/mL. The methanol extracts of the roots showed antibacterial activities against *Agrobacterium tumefaciens, Bacillus cereus, Bacillus subtilus* and *Pseudomonas syringae* pv. *tomato*, but none of the water extracts of *Glycyrrhiza glabra* roots showed any antibacterial activity against the these microorganisms.

tivamente, 2,80%, 175 mg/100 g, 1400 mg/100 g, 147 mg/100 g, 120 mg/100 g, 20 mg/100 g, 6 mg/100 g, 4,4 mg/100 g, 2,1 mg/100 g e 0,1 mg/100 g. L'attività antiossidante massima dell'estratto di radice era l'88,7% ad una concentrazione di 800 μg/mL. Gli estratti metanolici presentavano attività antibatteriche contro *Agrobacterium tumefaciens, Bacillus cereus, Bacillus subtilus* e *Pseudomonas syringae* pv. *tomato*, ma nessuno degli estratti acquosi delle radici di *Glycyrrhiza glabra* presentava attività antibatterica contro questi microorganismi.

INTRODUCTION

The genus *Glycyrrhiza* includes about 20 species native to Europe, Asia, North and South America and Australia. The generic name is derived from The Greek glukos (sweet) and riza (root). The two most commonly grown species are European licorice (*Glycyrrhiza glabra* L.) and the Chinese species (*Glycyrrhiza uralensis* Fisch.); European licorice has a rich historical tradition (BOWN, 1995).

The plant is an upright, hairless perennial. It is 1.2-1.8 m tall with pinnate leaves of 9-17 leaflets and it had pale-blue, pea-like flowers (BOWN, 1995). The medicinal properties of licorice have been known for thousands of years, and the root and root extract were used in ancient China, India, Egypt, Greece and Rome (TSUKIYAMA *et al.*, 2002). The main ingredient of *Glycyrrhiza glabra* is glycyrrhizin, a substance 50-times sweeter than sucrose, with cortisone-like effects. It is a very sweet, moist, soothing herb. It is an anti-inflammatory and expectorant; it controls coughing, and has some hormonal effects. It also detoxifies and protects the liver (BAYTOP, 1996; TSUKIYAMA *et al.*, 2002).

The main *Glycyrrhiza glabra* producing countries are Turkey (the largest exporter), followed by Afghanistan, Azerbaijan, Iran, Pakistan, Russia and Syria (LANGE, 1998). The roots are boiled to extract the familiar black substance that is dried and then sold as the licorice confectionery. Licorice is also the basis for most proprietary laxatives; The extracts are also used to flavor tobacco, beer, soft drinks and pharmaceutical products. It is used as a foaming agent in beer and fire extinguishers (ATES and ERDOGRUL, 2003; BOWN, 1995).

In terms of diversity the Eastern Anatolia region is one of the richest licorice centers in Turkey. For centuries, the people in this area have been using herbal medicine, including licorice, to treat some common diseases (OZTURK *et al.*, 2000).

Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases (HALLIWELL, 1997; SIES, 1993). The search for natural antioxidants is therefore very important. While earlier research on antioxidants was mostly focused on isolated pure compounds, recent studies have been directed more toward natural formulations (HAGERMAN *et al.*, 1998; HARAMAKI and PACKER, 1995; NAIK *et al.*, 2003). Plant products are also known to possess potential for food preservation and the antiox-

idant capacity of plant extracts is closely related to their phenolic compound contents (LIM and QUAH, 2007).

Recently, the growing acceptance of traditional medicine as an alternative form for health care and the development of microbial resistance to the currently available antibiotics have led researchers to investigate the antibacterial activity of some medicinal plants (KUMARASAMY *et al.*, 2002; SCRINIVASAN *et al.*, 2001). Some plants are known to produce certain bioactive molecules which react with other organisms in the environment to inhibit bacterial or fungal growth (antibiotic activity). Traditionally used medicinal plants produce a variety of compounds that are known to have therapeutic properties (BRUNETTON, 1995; CHOPRA *et al.*, 1992; COWAN, 1999).

Information is lacking on the nutritional value of *Glycyrrhiza glabra* accessions from different regions of the world. To date there have been few studies on the bioactive compounds of *Glycyrrhiza glabra*. The bioactive compounds, particularly those with antibacterial properties of wild-growing *Glycyrrhiza glabra* have not been reported in detail. The present work reports the results of an investigation on the bioactive contents, total phenolics, mineral contents and antioxidant and antibacterial activities of *Glycyrrhiza glabra* in relation its potential as a food source and natural preservative.

MATERIALS AND METHODS

Mature licorice (*Glycyrrhiza glabra* L.) plants were harvested manually from ten sites near the city of Erzurum in the Eastern Anatolia region of Turkey; the soil types at the sites were similar. At least 30 individual plants were pooled to form a single plant sample. They were put in a portable refrigerator for transportation to the laboratory (2-3 h). The samples from different locations were combined prior to analysis. Samples were dried at 50°C in an oven and the roots were then ground to a fine powder with a mortar and pestle and kept at room temperature prior to extraction for studies on the antioxidant activity and total phenolics.

The concentration of total phenolics in the ethanol extract of *Glycyrrhiza glabra* plants was determined by the Folin-Ciocalteau colorimetric method (GULCIN *et al.*, 2002). One mL of the extract solution (containing 1 mg) in ethanol was pipetted into a flask. Then, 46 mL of distilled water and 1 mL of Folin-Ciocalteau reagent were added and mixed thoroughly. The mixture was left to stand for 3 min and then 3.0 mL of 2% sodium carbonate were added. After 120 min of incubation at ambient temperature with shaking, the absorbance was measured at 760 nm. Measurements were carried out in duplicate and the calibration curve was made using gallic acid; the results are expressed as μgGAE/mg.

The antioxidant activity of *Glycyrrhiza glabra* ethanol extracts was determined according to the β-carotene bleaching method described by KAUR and KAPOOR (2002) with some modifications. Ten mg of ground sample were mixed with 10 mL of ethanol and stirred for 30 min on a magnetic stirrer. The suspension was filtered through Whatman No. 1 filter paper. Final solutions were used as stock solution for the antioxidant activity. Four mL of β-carotene solution (0.1 mg in 1 mL chloroform), 40 mg of linoleic acid and 400 mg of Tween 40 were transferred to a round-bottom flask. The mixture was then evaporated at 50 °C using a rotary evaporator to remove chloroform. One-hundred mL of oxygenated distilled water were added slowly to the residue and vigorously agitated to give a stable emulsion. Twenty-five, 50, 75, 100, 200, 400 and 800 μL of extracts were then added to 3 mL aliquots of β-carotene/linoleic acid emulsion. As the emulsion was

added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer (Thermo, Nicolet 100 UV, London, UK). The mixtures were incubated at 50°C for 90 min. The measurements were taken at 15 min intervals for 90 min. Ethanol was used as the control. A blank, devoid of β-carotene, was prepared for background subtraction. BHA was used as a standard. All samples were assayed in triplicate. The degradation rate (DR) was calculated according to first order kinetics, using the following equation based on: ln (a/b) x $1/t = DR_{sample}$ or $Dr_{standard}$ where ln is natural log, a is the initial absorbance (470 nm) at time 0, b is the absorbance (470 nm) at 100 min and t is time.

Antioxidant activity (AA) was expressed as the percentage of inhibition relative to the control, using the formula: AA = ($DR_{control}$-DR_{sample} or $_{standard}$/$DR_{control}$) x 100

Total N was determined by the micro-Kjeldahl method. In order to determine the mineral composition, samples were burned with a nitric acid and perchloric acid solution, on a hot plate, at 200°C. The absorbance of the extract was then measured using an Atomic Absorbance Spectrophotometer (Perkin Elmer, Waltham/MA, USA). The quantities of minerals were calculated with a standard curve for each element. The phosphorus content of the extract was analyzed by determining the absorbance of the yellow color, obtained from the Barton reaction, using a spectrophotometer (Thermo, Nicolet 100 UV, London, UK) at a 680 nm wavelength; the results were compared to the standard curve (JAMES, 1995).

To test the antibacterial activity, *Pseudomonas syringae* pv. *syringae, Pseudomonas syringae* pv. *tomato, Bacillus subtilis, Bacillus cereus* GC subgroup A, *Yersinia enterocolita, Vibrio cholerae* non 01, *Coryneum diphteriae, Yersinia frederiksenii, Yersinia pseudotuberculosis, Salmonella typhimurium* GC subgroup A, *Serratia liquefaciens, Pseudomonas corrugate, Xanthomonas campestris campestris, Agrobacterium tumefaciens* and *Pseudomonas aeruginosa* were used. The bacteria, maintained on Nutrient Agar (Merck, Darmstadt, Germany) were supplied by The Microbiology Laboratory of The Agricultural Faculty of Ataturk University, Erzurum, Turkey. The identity of the bacteria used in this study was confirmed by The Microbial Identification System in the Biotechnology Application and Research Center at Ataturk University. The dried and powdered licorice root materials (400 g) were extracted successively in a Soxhlet extractor with methanol (MeOH) for 72 h at a temperature not exceeding the boiling point of the solvent (OZTURK and ERCISLI, 2007). The extracts were filtered using Whatman filter paper (No. 1) and then concentrated in vacuo at 40°C using a rotary evaporator. The residues obtained were used for the antibacterial tests. Antibacterial activities were determined using the disk diffusion method in which 100 μL of suspension containing 10^8 CFU/mL of bacteria were spread on nutrient agar (NA) medium. Sterile, 6 mm diameter filter paper discs were impregnated with 10 μg of sterile test material and placed on nutrient agar. Negative controls were prepared using the same solvents as those used to dissolve the plant extracts. Ofloxacin (5 μg/disc), sulbactam (30 μg) + cefoperazona (75 μg) (105 μg/disc) and/or netilmicin (30 μg/disc) were used as positive standards. The bacteria-inoculated plates were incubated at 27°C for 24 h. The diameter of the clear zone around the disc was measured and the antibacterial activity is expressed in mm. Five discs per plate and three plates were used, and each test was run in triplicate (DJIPA *et al.*, 2000).

The minimum inhibition concentration (MIC) values were also determined. The inocula of bacteria were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. *Glycyrrhiza glabra*

root extracts dissolved in 0.5% dimethylsulfoxide (DMSO) were first diluted at the highest concentration (500 μg/mL) to be tested, and then serial two-fold dilutions were made in a concentration range from 3.90 to 500 μg/mL in 10 mL sterile test tubes containing nutrient broth. The MIC values of extracts against bacterial strains were determined based on a micro-well dilution method (OZTURK and ERCISLI, 2007). The 96-well plates were prepared by dispensing 95 μL of nutrient broth and 5 μL of the inoculum into each well. One-hundred μL of the extracts initially prepared at the 500 μg /mL concentration were added to the first wells. One-hundred μL of the serial dilutions were then transferred into six consecutive wells. The last well containing 195 μL of nutrient broth without any compounds and 5 μL of the inoculum on each strip was used as the negative control. The final volume in each well was 200 μL. Maxipime (Bristol-Myers Squibb, New York, USA) at the concentration range of 500-3.9 μg /mL was prepared in nutrient broth and used as the standard drug for positive control. The contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated at the appropriate temperatures for 24 h. Microbial growth was determined by absorbance at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc., Highland Park, VT, USA) and confirmed by plating 5 μL samples from clear wells onto nutrient agar medium. The extract tested in this study was screened twice against each organism. The MIC of each extract was taken as the lowest concentration that showed no growth (OZTURK and ERCISLI, 2007).

All reagents were of analytical grade and obtained from Sigma Chemical Co. (St Louis, MO, USA).

The experiment was a completely randomized design with four replications. Data were subjected to analysis of variance (ANOVA) and the means were separated by a Duncan multiple range test at the $p<0.05$ significant level.

RESULTS AND DISCUSSION

The antioxidant activity of *Glycyrrhiza glabra* roots is reported in Table 1. The differences among *Glycyrrhiza glabra* root extract concentrations and BHA concentrations were statistically significant ($p<0.05$). Antioxidant activity was found to be 28.3% at 25 μg/mL extract concentrations and increased to 66.4%

Table 1 - Antioxidant activity in *Glycyrrhiza glabra* root extract and BHA.

BHA Concentration (μg/mL)	Antioxidant activity (%)	Root extracts Concentration (μg/mL)	Antioxidant activity (%)
25	31.7±3.7^{d}	25	28.3±5.5^{c}
50	63.3±5.7^{c}	50	39.6±6.6^{c}
75	80.4±6.9^{b}	75	58.5±7.3^{b}
100	98.4±8.7^{a}	100	66.4±7.7^{b}
		200	83.1±8.3^{a}
		400	87.4±8.7^{a}
		800	88.7±8.4^{a}

Values (on dry basis) represent, the means of a triplicate determination (n=3) ±SD. Values in a column with different superscripts are significantly different at $p<0.05$.

at 100 μg/mL and finally reached 88.7% at 800 μg/mL in *Glycyrrhiza glabra* root extract. The antioxidant activity of BHA was 31.7% at 25 μg/mL and reached nearly 100% (98.4%) at the 100 μg/mL concentration. This suggests that *Glycyrrhiza glabra* root extract may contain high concentrations of active compounds. OKADA *et al.* (1989) reported that *Glycyrrhiza glabra* roots from Russia and China had antioxidant constituents. The present results show that *Glycyrrhiza glabra* roots have a strong antioxidant capacity and may be important for both the human diet and food safety when mixed with the other foods. The antioxidant activity of *Glycyrrhiza glabra* roots was previously reported in Ayurvedic medicine in India (NAIK *et al.*, 2003). HARAGUCHI *et al.*, (1998a) reported that the other *Glycyrrhiza* species, *Glycyrrhiza inflata* also had antioxidative and superoxide scavenging activities. Studies on the antioxidant content of *Glycyrrhiza* plants are being carried out in various parts of the world. As expected, there is an enormous variation in the antioxidant content due to difference in climatic conditions and the plants used. From these results, it can be suggested that the consumption of *Glycyrrhiza glabra* roots may offer some dietary benefits since they contain antioxidant constituents, which can protect against lipid peroxidation and scavenge free radicals. This report further suggests that *Glycyrrhiza glabra* may have beneficial chemopreventive effects in addition to being a potential new source of natural antioxidants.

The total phenolic compounds were determined by the Folin-Ciocalteau method. This method provides a simple, inexpensive, quick and useful estimate of total phenolics. Phytochemicals, especially phenolics, are thought to be most important bioactive compounds for health benefits. Plant extracts, which contain different classes of polyphenols are very attractive not only in modern phytotherapy but also in the food industry. Therefore, the total phenolic content of *Glycyrrhiza glabra* root extracts was investigated.

Total phenolics in *Glycyrrhiza glabra* roots were found to be 12.88 μgGAE/mg DW. The results for total phenolics of *Glycyrrhiza glabra* root extracts clearly show that it is one of the richest phenolic sources. HATANO *et al.* (2000) previously reported that *Glycyrrhiza glabra* roots are rich in phenolic constituents and the variability in phenolic content in different plants could be due to the plants used, environmental factors and the collection period. Since phenolics are one of the most important groups of compounds acting as primary antioxidants, it was reasonable to determine the total phenolic content in *Glycyrrhiza glabra*. Phenolics have also played an important role against chronic diseases (NIJVELDT *et al.*, 2001).

The mineral content of *Glycyrrhiza glabra* root is made up of the elements N, P, K, Ca, Mg, Fe, Mn, Zn, Na and Cu in varying concentrations which are reported in Table 2.

To date there have been no reports in the literature related to the mineral composition of *Glycyrrhiza glabra* root; this study is the first in this area. The mineral composition of the plants depends not only on the species or variety,

Table 2 - Mineral contents (mg/100g) in *Glycyrrhiza glabra* roots.

N (%)	P	K	Ca	Mg	Fe	Mn	Zn	Na	Cu
2.80±0.4	175±23	1400±169	147±16	120±14	20±3	6±1	4.4±1	2.1±0.3	0.1±0.0
Values (on dry basis) represent the means of a triplicate determination (n=3) ±SD.									

Table 3 - Antibacterial activity of *Glycyrrhiza glabra* extracts against some bacteria.

Bacterial species	Inhibition zone in diameter (mm) of *Glycyrrhiza glabra* extracts (10 µg/disc)	Water extract	Negative control MEOH	Positive control (mm)			MIC (µg/mL)	
				Net	Sfc	Ofx	extract	Standard drug Maxipime
Pseudomonas syringae pv. *syringae*	-	-	-	16	-	16	3.90	250
Pseudomonas syringae pv. *tomato*	8	-	-	20	-	-	125	250
Bacillus subtilis	7	-	-	30	40	26	3.90	7.81
Bacillus cereus	8	-	-	19	19	21	62.50	31.25
Yersinia enterocolita	-	-	-	-	19	-	15.62	31.25
Vibrio cholerae	-	-	-	16	39	21	125	62.50
Coryneum diphteriae	-	-	-	-	-	-	3.90	-
Yersinia frederiksenii	-	-	-	12	19	18	7.81	31.25
Yersinia pseudotuberculosis	-	-	-	17	12	16	125	31.25
Salmonella typhimurium	-	-	-	-	9	-	31.25	-
Serratia liquefaciens	-	-	-	14	-	10	15.62	62.50
Pseudomonas corrugata	-	-	-	13	10	23	3.90	-
Xanthomonas compestris compestris	-	-	-	9	15	15	125	500
Agrobaceterium tumefaciens	7	-	-	16	25	23	3.90	-
Pseudomonas aeruginosa	-	-	-	14	12	17	125	250

but also on the growing conditions such as soil type and geographical location.

The antibacterial activity of *Glycyrrhiza glabra* root against several bacteria is shown in Table 3. The *Glycyrrhiza glabra* root extract showed antibacterial activity against *Pseudomonas syringae* pv. *tomato*, *Bacillus subtilus*, *Bacillus cereus* and *Agrobaceterium tumefaciens* (7-11 mm inhibition zone). However, none of the aqueous extracts showed any antibacterial activity against the microorganisms studied. The extracts did not show any antibacterial activity against *Coryneum diphteriae*, *Pseudomonas aeruginosa*, *Pseudomonas corrugate*, *Pseudomonas syringae pv. syringae*, *Salmonella typhimurium*, *Serratia liquefaciens*, *Vibrio chlororae*, *Yersinia enterocolita*, *Yersinia frederiksenii*, *Yersinia pseudotuberculosis* or *Xanthomonas compestris compestris*.

MIC values for the methanol extract of *Glycyrrhiza glabra* and standard drug (Maxipime) were in the range of 3.90-125 µg/mL and 7.81-500 µg/mL, respectively (Table 3). *Pseudomonas syringae* pv. *syringae*, *Bacillus subtilis*, *Coryneum diphteriae*, *Pseudomonas corrugate* and *Agrobaceterium tumefaciens* were the most sensitive to the plant extract with MIC values lower (3.90 µg/mL) than the standard drug. *Pseudomonas syringae* pv. *tomato*, *Yersinia enterocolita*, *Yersinia frederiksenii*, *Serratia liquefaciens*, *Pseudomonas aeruginos-*

sa and *Xanthomonas compestris compestris* were also sensitive with MIC values that were lower than the standard antibiotic (Maxipime).

For centuries, the root and rhizome of *Glycyrrhiza spp.* have been used as a medicine because of its wide-ranging therapeutic properties. TSUKIYAMI *et al.* (2002) reported that licochalcone obtained from *Glycyrrhiza spp.* roots possessed antibacterial activity particularly against all gram-positive bacteria, including *Bacillus* spp. It was previously reported that *Glycyrrhiza glabra* root extracts showed various antibacterial activities against certain microorganisms tested (ATES and OZDOGRUL, 2003; HARAGUCHI *et al.*, 1998b; OKADA *et al.*, 1989). The genera *Bacillus* are a well known spore-forming bacteria and *Bacillus cereus* is a toxin-producing bacteria. Therefore the root of *Glycyrrhiza glabra* could be used to prevent food contamination since the recent trend in food processing is to avoid the use of chemical preservatives. Now, natural antimicrobial alternatives are needed.

In conclusion, wild *Glycyrrhiza glabra* plants have a high total phenolic content and show high antioxidant and antibacterial activities. Wild *Glycyrrhiza glabra* plants could provide a new source of antioxidant and antibacterial agents and can be used for food and medicinal purposes. Planting date and variety factors must also be studied in an attempt to improve and domesticate *Glycyrrhiza glabra* as a major agricultural crop.

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