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Original Research Article

Phenolic content and biological activities of *Lycium barbarum* L (Solanaceae) fruits (Goji berries) cultivated in Konya, Turkey

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Abstract

Purpose: *To evaluate the phenolic content and biological activities of Lycium barbarum fruits cultivated in Turkey.*
Methods: *Phenolic compounds in the water and methanol extracts of the fruits were determined by liquid chromatography-mass spectrometry (LC-MS/MS). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and superoxide radical scavenging activities and ferric-reducing antioxidant power (FRAP) assays were used to evaluate the antioxidant potential of the fruits. The acetylcholinesterase (AChE) inhibitory activity of the fruits was evaluated by Ellman assay.*
Results: *LC-MS/MS results showed that all the extracts contained phenolic compounds including flavonoids, phenolic acids, anthocyanins and polyphenols. Some anthocyanins, namely, cyanidin-3-O-glucoside, cyanidin chloride, pelargonin chloride, pelargonidin chloride, and pelargonidin-3-0-glucoside were identified in the fruits for the first time. Pelargonidin-3-O-glucoside and cyanidin-3-O-glucoside were the main anthocyanins in the water extract with levels of 119.60 ± 12.04 and 1112.25 ± 125.40 mg/kg, respectively. The results indicated that the extracts possessed good radical scavenging and ferric-reducing activities.*
Conclusion: *The results show that Lycium barbarum cultivated in Konya is a good source of the phenolic compounds, and thus may be exploited for commercial production of the antioxidants.*

Keywords: *Lycium barbarum, Goji berries, Antioxidant activity, Acetylcholinesterase, Flavonoids*

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INTRODUCTION

The fruits of *Lycium barbarum* L. (Solanaceae) gained popularity in Western countries at the beginning of the 21st century, but have long been used in traditional Chinese medicine since the time of the legendary emperor Shen Nung. It has been used as dietary supplement for longevity, in addition to it has analgesic, antitussive, antipyretic, hypnotic, hepatoprotective and diuretic effects [1-3].

L. barbarum fruits (Goji berries) have come to be considered a popular functional food with antioxidant and nutritive properties [4,5], being used in traditional Chinese medicine for the

treatment of dry cough, fatigue, headaches, abdominal pain, infertility, blurred vision and diminished visual acuity [6,7]. Goji berries started to gain popularity at the beginning of the last century and became commercially available in Europe and North America, and *L. barbarum* is today cultivated extensively in different parts of the world [2,3].

Recent studies have shown that the fruits contains polysaccharide, carotenoid, flavonoid, vitamin and essential oil, and has hepatoprotective, hypoglysemic, hypolipidemic, anticancer, immunostimulant, antiphatic and neuroprotective properties [2]. The profile of the plant has been raised as a valuable asset to our country based on the strong neuroprotective properties of the compound based on its polysaccharide content, and as a result of findings indicating that the water extract prevents neurodegeneration and can be a source of protection for people at risk of developing Alzheimer's disease, giving the increasing incidence of Alzheimer's in the country [8, 9]. In Turkey, eight species of *Lycium*, known colloquially as wolfberry, grow naturally, aside from the one endemic species. Studies of the chemical contents and biological activity potential of the fruits of *L. barbarum* and *L. ruthenicum* have shown that these two species are also rich in phenols, and have strong antioxidant effects [10,11].

In the present study, making use of LC/MSMS, we identified the phenolic contents of the water and methanol extracts of *L. barbarum* fruits of Chinese origin that were cultivated in Konya, determining their total phenol and flavonoid content, ferric ion-reducing potential, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide-radical scavenging effects, and acetylcholinesterase (AChE) inhibitory effect. There have to date been no studies of *L. barbarum* cultivated in Turkey.

EXPERIMENTAL

Chemical agents

All compounds used as standards in LC–MS/MS analysis were obtained from Sigma-Aldrich, Germany. All solutions were HPLC grade. The compounds used for biological activity were analytical grade.

Plant material and preparation of the extracts

L. barbarum plants were cultivated in Temmuz Organic Certified Product Production Farm in Konya, central Anatolia of Turkey, by Muammer Sen (Pharmacist). Fruits of *L. barbarum* were collected from the farm in July 2014 and air-dried at room temperature under shade.

Water extraction

The powdered sample (5 g) was extracted with 100 mL ultra-filtered water at 80 °C for 20 min in a water bath shaker. After cooling the extract was centrifuged at 5,000 rpm for 10 min and filtered by a Millipore filter with a 0.45 µM nylon membrane under vacuum at 23 °C. The water extract was lyophilized and stored at -20 °C [12].

Methanol extraction

The sample (5 g) was extracted with 100 mL 80 % methanol at 35 °C for 24 h in a water bath shaker. Other procedures were the same as in the water extraction method. The solvent was evaporated under vacuum at 40 – 45 °C. The crude methanol extract was lyophilized and stored at -20 °C [12].

LC-MS/MS measurement

Preparation of test solution and LC–MS/MS conditions

A hundred mg of each extract was dissolved in 5 mL of methanol–water (50:50 v/v) in a volumetric flask, from which 1 mL was transferred into another 5 mL of volumetric flask. The detailed description of method was given in literature [13]. Experiments were performed by a Zivak® HPLC and Zivak® Tandem Gold Triple quadrupole (Istanbul, Turkey) mass spectrometer equipped with a Macherey–Nagel Nucleoder C18 Gravity column (125 x 2 mm i.d., 5 µm particle size). The mobile phase was composed of methanol (A, 0.1 % formic acid) and water (B, 0.1 % formic acid), the gradient programme of which was 0–3.00 min 100 % B, 3.01– 13.00 min 30 % A - 70 % B and finally 13.01–20.00 min 100 % B. The flow rate of the mobile phase was 0.3 mL/min, and the column temperature was set to 25 °C. The injection volume was 10 µL.

Total phenolic and flavonoid content

Folin- Ciocalteu assay was used to determinate the phenolic content of the extracts. [14]. Gallic acid used as a standard and total content of phenolic compounds in the extracts was expressed as mg gallic acid equivalent (GAE)/g extract.

Colorimetric determination of total flavonoid content was based on the procedure of Sakanaka *et al* [15]. A standard curve of

catechin was used and the results were expressed as mg of catechin equivalents (CE) per g of the extract.

Antioxidant activity

DPPH radical scavenging activity

The DPPH radical scavenging activities of the extracts were assayed as described by Brand-Williams *et al* [16]. Alpha-tocopherol and quercetin were used as standards and methanol was used as a control. The absorbance of the sample (A) and the control (A_0) were measured at 517 nm. The ability to scavenge DPPH radical (I) was calculated using Eq 1.

I (%) = {(1-A)/A_0}100 …….. (1)

Superoxide radical scavenging activity

The effects of the extracts on generation of superoxide radicals were determined by the NBT reduction method [17]. Quercetin was used as a standard. The absorbance of the sample (A) and the control (A_0) were measured at 560 nm. The abilities of the extracts to scavenge the superoxide radical (I) were calculated by comparing the results of the sample with those of controls not treated with the extract using Eq 2.

I (%) = {(1-A)/A_0}100 …….. (2)

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the procedure of Benzie and Strain [18]. Alpha-tocopherol and quercetin were used as standards. Reducing abilities of the extracts were presented as mM Fe2+ equivalents using the standard curve was constructed with FeSO4.7H2O solution (0.125 - 2 mM).

AChE inhibitory activity

The extracts were screened for their AChE inhibitory activities through the method of Ellman *et al* [19] with a slight modification. Galantamine was used as a standard and a control sample where no inhibitor was used. The reaction rates of the control (A_0) and sample (A) were measured at 412 nm. The percent inhibition of the enzyme activity (I) was calculated using Eq 3.

I (%) = {(1-A)/A_0}100 …….. (3)

Statistical analysis

All determinations were done in triplicate. The results were evaluated using unpaired *t* test with NCSS statistical analyses software (version 10.0) and expressed as mean ± standard deviation (SD). Differences at $p < 0.05$ were considered to be significant.

RESULTS

LC-MS/MS

The LC-MS/MS results are presented in Table 1, while the validation and uncertainty parameters for seconder metabolites are shown in Table 2. The water extract was enriched with fumaric acid, p-coumaric acid, gallic acid, chlorogenic acid and ascorbic acid, and also contains epicatechin, epigallocatechin and epigallocatechin gallate. The methanol extract is *rich in flavonoids* (quercetin, isoquercetin, luteolin-7-O-glucoside, kaempferol-3-O-rutino-side) and anthocyanins (pelargonin, pelargonidin, pelargonidin 3-O-glucoside, cyanidin, cyanidin-3-O-glucoside), and fumaric acid, pyrogallol, chlorogenic acid, epicatechin and epigallocatechin are all present.

Total phenolic and flavonoid compounds

The results of the present study indicate that the methanol extract has higher phenol and flavonoid content than the water extract (Table 3).

Antioxidant activity

In the present study, the antioxidant activity of *L. barbarum* extracts were determined through the use of free radical (DPPH and superoxide) scavenging and FRAP assays. The results are presented in Table 4 and Figure 1, 2.

AChE inhibitory activity

L. barbarum fruits have for many years been used to prevent Alzheimer's disease. It can be understood from the result of the present study that the extracts do not inhibit the AChE enzyme.

DISCUSSION

Furthermore, pelargonidin chloride, pelargonin chloride, pelargonidin-3-0-glucoside, cyanidin chloride and cyanidin-3-O-glucoside were identified for the first time in cultivated *L. barbarum* fruits, and cyanidin-3-O-glucoside and pelargonidin-3-O-glucoside were detected as major anthocyanins in the water extract.

Caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, hyperoside, gallic acid, catechin and

Table 1: Content of secondary metabolites (mg/kg dry extract weight) of the extracts

Compound	Methanol extract (mg/kg)	Water extract (mg/kg)
Fumaric acid	338.00±31.33	126.72±11.75
Pyrogallol	Nd	8.97±1.10
p-Coumaric acid	77.81±8.71	Nd
Gallic acid	2.95±0.47	Nd
1.3-Cynarin	Nd	Nd
Ascorbic acid	37.92±4.36	Nd
Chlorogenic acid	60.72±8.57	55.56±7.84
Quercetin	Nd	2.12±0.36
Isoquercetin	Nd	2.90±0.65
Epicatechin	8.63±0.68	18.28±1.45
Epigallocatechin	5.37±0.61	0.66±0.07
Epigallocatechin Gallate	5.23±0.47	Nd
Luteolin-7-O-Glucoside	Nd	8.89±0.99
Kaempferol-3-O-Rutinoside	13.37±1.29	16.62±1.60
Pelargonin Chloride	Nd	3.35±0.38
Pelargonidin Chloride	Nd	19.52±1.91
Cyanidin Chloride	1.53±0.18	11.40±1.33
Cyanidin-3-O-Glucoside	Nd	1112.25±125.40
Pelargonidin-3-O-Glucoside	Nd	119.60±12.04
Procyanidin B2	Nd	Nd
Punicalagin	Nd	Nd
Delphinidin-3-O-Glucoside	Nd	Nd

Nd = not determined

Table 2: Validation and uncertainty parameters for secondary metobolites

Compound	Linear range	R^2	LOD/LOQ (ppb)	RSD (%)
Fumaric Acid	y=0.056x+0.0176	0.9912	0.07/.023	5.44
Pyrogallol	y=0.039x+0.014	0.9876	0.04/0.15	5.47
p-Coumaric Acid	y=0.289x+0.153	0.9873	0.36/1.21	6.39
Gallic Acid	y=0.401x-0.02	0.9986	0.55/1.82	7.23
1.3-Cynarin	y=0.465x+0.091	0.9909	0.40/1.33	4.22
Ascorbic Acid	y=0.011x+0.0099	0.9914	0.02/0.07	8.72
Chlorogenic Acid	y=0.262x-0.0004	0.9981	0.32/1.06	5.45
Quercetin	y=0.1108x+0.069	0.9810	0.30/1.01	11.4
Isoquercetin	y=0.325x+0.060	0.9960	0.29/0.96	5.07
Epicatechin	y=0.0294x-0.0001	0.9963	0.02/0.07	3.62
Epigallocatechin	y=0.035x+0.0218	0.9875	0.04/0.13	5.53
Epigallocatechin Gallate	y=0.117x-0.006	0.9957	0.09/0.32	4.79
Luteolin-7-O-Glucoside	y=0.135x+0.024	0.9957	0.20/0.68	8.56
Kaempferol-3-O-Rutinoside	y=0.108x+0.013	0.9978	0.16/0.55	8.15
Pelorgonin Chloride	y=0.189x+0.04	0.9927	0.17/0.56	4.45
Pelargonidin Chloride	y=0.036x+0.0047	0.9940	0.01/0.05	2.21
Cyanidin Chloride	y=0.0081x+0.0026	0.9881	0.02/0.06	11.6
Cyanidin-3-O-Glucoside	y=0.353x+0.1004	0.9912	0.09/0.29	1.37
Pelargonidin-3-O-Glucoside	y=0.282x+0.04	0.9932	0.12/0.40	2.49
Procyanidine B2	y=0.0493x+0.0067	0.9902	0.09/0.31	10.6
Punicalagine	y=0.0028x-0.0016	0.9816	0.01/0.03	7.31
Delphinidine-3-O-Glucoside	y=0.0145x-0.0008	0.9943	0.004/0.01	1.43

Table 3: Total phenolic contents (PC) and total flavonoid contents (FC) of *L. barbarum* extracts

Extract	PC (mg GAE/g extract)	FC (mg CE/g extract)
Water extract	8.16 ± 0.46^{a}	1.78 ± 0.06^{a}
Methanol extract	9.04 ± 0.67^{a}	2.63 ± 0.11^{b}

Values are mean ± standard deviation; Values with different letters in the same column are significantly ($p < 0.05$) different

Table 4: Antioxidant activities of *L. barbarum* extracts and standards

Extract	DPPH EC_{50} (mg/mL) [A]	Superoxide (% inhibition) [B]	FRAP value (mM Fe^{2+}) [C]
Water extract	22.64 ± 1.44[a]	47.24 ± 0.76	2.93 ± 0.25[a,b]
Methanol extract	18.19 ± 0.22[b]	Nd	2.62 ± 0.12[a]
Quercetin	0.06 ± 0.002[c]	89.65 ± 0.18	2.79 ± 0.03[a]
α- Tocopherol	0.25 ± 0.003[d]	-	3.31 ± 0.06[b]

Values are mean ± standard deviation. Different letters in the same column indicate a significant difference ($p < 0.05$). Nd = Not determined. [A] The EC_{50} value (mg/mL) is the effective concentration at which the DPPH radicals are scavenged by 50%. [B] % inhibition values at 40 mg/mL concentration for the water extract and at 1.25 mg/mL concentration for quercetin. [C] FRAP values for the extracts at 40 mg/mL, for quercetin at 0.125 mg/mL and for α-tocopherol at 1 mg/mL concentration

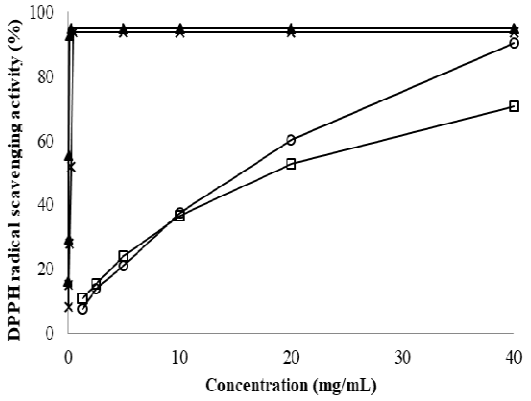

Figure 1: DPPH radical scavenging activities of *L. barbarum* extracts and standards (▲, quercetin; x, α-tocopherol; □, water extract; ○, methanol extract)

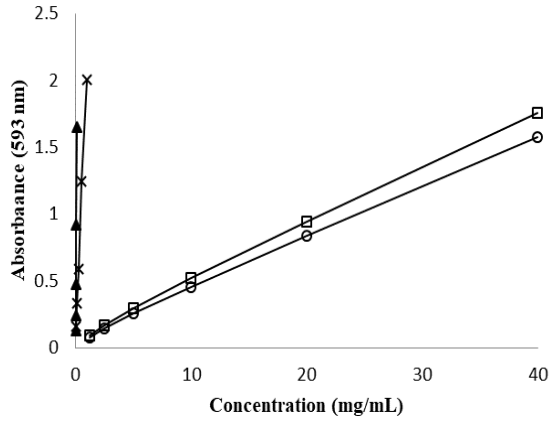

Figure 2: Reducing powers of *L. barbarum* extracts and standards (▲, quercetin; x, α-tocopherol; □, water extract; ○, methanol extract)

epicatechin were reported in the fruits of *L. barbarum* cultivated in Italy [20]. Vulić *et al* detected compounds of gallic acid, protocatechuic acid, catechin, vanillic acid, chlorogenic acid, coumaric acid, caffeic acid, ferulic acid and rutin in the fruits of *L. barbarum* cultivated in Serbia [21]. Benchennouf *et al.* identified a number of bioactive compounds, namely esters of hydrocinnamic and dihydroxybenzoic acids with quinic acids, quercetin 3-O-hexosecoumaric ester and quercetin 3-O-hexose-O-hexose-O-rhamnose, coumaric, isoferulic and caffeic acids and their derivatives in the fruits of *L. barbarum* cultivated in Greece [22]. When the findings of the present study were compared with these studies, it was revealed that the chemical composition of *L. barbarum* differs according to soil content, climatic conditions and cultivation zone.

The phenolic content values obtained in this study were lower than in the studies reported by Benchennouf *et al* and Vulić *et al* [21,22]. These inconsistencies may stem from differences in extraction procedure, although in general, polyphenol extraction from plant material is influenced mainly by solvent polarity [23]. It has been reported that ethyl acetate is the best solvent for the extraction of phenolics from *L. barbarum* fruits [22].

An evaluation of the DPPH radical scavenging effects of the *L. barbarum* extracts revealed the dose-dependent scavenging activities of the extracts, with the methanol extract showing the highest activity at concentrations of 40 mg/mL (90.36 ± 0.39 %), and the water extract showing the lowest activity (70.83 ± 2.79 %) at the same concentration (Figure 1). The DPPH radical scavenging activities of both extracts were found to be lower than the standard. The half maximal effective concentration (EC_{50}) value of the DPPH radical scavenging activities of the extracts are presented in Table 4. The results of the present study show that the methanol extract has a higher phenol and flavonoid content than the water extract, and so has a higher radical scavenging activity. These results concur with the previous observation identifying the strong free radical scavenging activity of *L. barbarum* extracts [21,22]. However, due to differences in the experimental procedures between the studies in the use of a DPPH assay, inconsistent results were observed.

The water extract also demonstrated low superoxide radical scavenging activity (47.24 ± 0.76 %) at a concentration of 40 mg/mL (Table

4), while the superoxide radical scavenging activity of the methanol extract could not be determined.

The ferric ion-reducing potential of the *L. barbarum* extracts increased depending on their concentration (Figure 2), with the concentration at which extracts showed the highest reduction potential being 40 mg/mL. Reducing power of the extracts was expressed as FRAP value (mM Fe^{2+} equivalents), and these values decreased in the order of water extract ≥ methanol extract (Table 4). A high FRAP value indicates high redox potential, and compounds with a high redox potential can donate electrons to free radicals and convert them into a harmless state, ending their radical reactions. Reducing properties of *L. barbarum* cultivated in Greece have been demonstrated by Benchennouf *et al* [22], who reported that reducing ability of the fruit may be related to the phenolic compounds in the extracts.

The results of studies performed to date have shown that *L. barbarum* fruits have a mild antioxidant effect. The inconsistencies between the results of the present study with those in literature may be due to the fact that the fruits used in the present study were from young plants that were yet to complete their adaptation to the culture environment.

Previous literatures about the AChE inhibitory activity have put forward a number of hypotheses related to the neuroprotective effects of *L. barbarum* fruits against neurodegenerative diseases as a result of their detoxification effects, and these suggest that the mechanism of action of the fruits follows a different pathway.

CONCLUSION

The findings of this study indicate that the fruits of *L. barbarum* cultivated in Konya are rich in phenolics and flavonoids compounds with mild antioxidant activities. Therefore, the climate of the central Anatolia is suitable for the cultivation of the Goji berries used for health benefits.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. The study was designed by Esra Eroglu Ozkan and Afife Mat. Esra Eroglu Ozkan, Tugba Yilmaz-Ozden and Gizem Gulsoy Toplan collected and analysed the data. The manuscript was written by Esra Eroglu Ozkan and Tugba Yilmaz-Ozden. All authors have read and approved the manuscript for publication.

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