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Standardization of Infusion of *Lycium barbarum* Grown by Organic Farming Methods and Enzyme Inhibitory and Antioxidant Activities

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ABSTRACT
The cultivation of medicinal and aromatic plants with conventional and organic farming techniques in order to protect biodiversity due to depleted natural resources is becoming increasingly common. The expense of organic farming techniques necessitates more careful selection of the plants to be grown. Evaluation of the bioactivity and phytochemical contents of these plants is important for the pharmaceutical and food industry. In this study, the antioxidant, antidiabetic, anticholesterolemic and antiobesity activities of the extracts obtained from the fruit, root and leaves of *Lycium barbarum* grown with organic farming techniques using infusion and decoction techniques were evaluated *in vitro*. The phytochemical contents of the extracts were investigated by spectroscopic and chromatographic techniques. In the study in which five different antioxidant activity methods were used, *L. barbarum* root decoction showed a strong antioxidant effect in almost all methods. While none of the extracts exerted an inhibitory effect on the α-glucosidase enzyme, the leaf infusion of the plant at 2 mg mL^{-1} concentration caused strong inhibitions especially on pancreatic lipase (62.16±3.33%) and pancreatic cholesterol esterase (93.98±0.54%) enzymes compared to the reference compounds. *L. barbarum* leaf infusion was standardized by RP-HPLC technique on the basis of chlorogenic acid (1.339±0.056 g $100g^{-1}$ dry extract) and quercetin-3-*O*-glucoside (1.801±0.042 g $100g^{-1}$ dry extract) as markers. The findings displayed that leaf infusions of *L. barbarum* grown with organic farming techniques could be the source of natural product development studies for hypercholesterolemia and obesity control, and the extract could be standardized using chlorogenic acid and quercetin-3-*O*-glucoside as markers.

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Organik Tarım Yöntemleri ile Yetiştirilmiş *Lycium barbarum* İnfüzyonunun Standardizasyonu ve Antioksidan ve Enzim İnhibitör Etkileri

ÖZET
Tükenen doğal kaynaklar sebebiyle biyoçeşitliliği korumak maksatlı tıbbi ve aromatik bitkilerin konvansiyonel ve organik tarım teknikleri ile yetiştirilmesi giderek yaygınlaşmaktadır. Organik tarım tekniklerinin pahalı olması yetiştirilmesi düşünülen bitkilerin daha dikkatli seçilmesini gerektir. Bu bitkilerin biyoaktivitelerinin ve fitokimyasal içeriklerinin de değerlendirilmesi ilaç ve gıda sanayi için önem arz etmektedir. Bu çalışmada, organik tarım teknikleri ile yetiştirilen *Lycium barbarum*'un meyve, kök ve yapraklarından infüzyon ve dekoksiyon teknikleri kullanılarak elde edilen ekstrelerin antioksidan, antidiyabetik, antikolesterolemik ve antiobezite aktiviteleri *in vitro* olarak değerlendirilmiştir. Ekstrelerin fitokimyasal içerikleri spektroskopik ve kromatografik tekniklerle incelenmiştir. Yaprak infüzyonunun en yüksek toplam fenol (72.53±9.13 mg GAE g^{-1} ekstre) ve toplam flavonoid (14.92±0.53 mg QE g^{-1} ekstre) içeriğine sahip olduğu bulunmuştur. Beş farklı

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antioksidan aktivite yönteminin (ABTS ve DPPH radikal süpürücü aktivite, metal bağlama kapasitesi, toplam antioksidan kapasite ve demir indirgeme gücü) kullanıldığı çalışmada, tüm bulgular değerlendirildiğinde *L. barbarum* kök dekoksiyonu, toplam antioksidan kapasite yöntemi dışındaki tüm yöntemlerde güçlü bir antioksidan etki göstermiştir. Ekstrelerin hiçbiri *α*-glukozidaz enzimi üzerinde inhibitör etki göstermezken, 2 mg mL^{-1} konsantrasyonda bitkinin yaprak infüzyonu referans bileşiklerle (orlistat ve simvastatin) karşılaştırıldığında özellikle pankreatik lipazı ve pankreatik kolesterol esteraz enzimleri üzerinde güçlü inhibisyonlara neden olmuştur. Bu sonuçlar ışığında *L. barbarum* yaprağı infüzyonu, markör olarak klorojenik asit (1.339±0.056 g $100g^{-1}$ kuru ekstre) ve kersetin-3-*O*-glukozit (1.801±0.042 g $100g^{-1}$ kuru ekstre) üzerinden RP-HPLC tekniği ile standardize edilmiştir. Bulgular, organik tarım teknikleriyle yetiştirilen *L. barbarum*'un yaprak infüzyonlarının, hiperkolesterolemi ve obezite kontrolü için doğal ürün geliştirme çalışmalarının kaynağı olabileceğini ve ekstrenin markör olarak klorojenik asit ve kersetin-3-*O*-glukozit kullanılarak standardize edilebileceğini göstermiştir.

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INTRODUCTION

The genus *Lycium* belongs to the Solanaceae family and includes about 100 species, mainly distributed in temperate and subtropical regions of America, Eurasia, South Africa and Australia. There are three known species of *Lycium*; *Lycium barbarum* L., *Lycium chinense* L. and *Lycium ruthenicum* L. (Lei et al., 2021). Goji berry is the name given to the fruits of *L. barbarum* and *L. chinense* (Kulczyński & Gramza-Michałowska, 2016). *L. barbarum* grows in northwestern and central Anatolia (TÜBİVES, 2006). Goji berry is a local food widely consumed in arid or semi-arid regions of China, Korea, Japan, Europe, North America and North America (Lei et al., 2021). Goji berry contains between 39.5% and 46.5% carbohydrates, various essential amino acids, unsaturated fatty acids, vitamin C and mineral elements. In addition, goji berry is known to contain more than 200 different compounds, including carotenoids, phenylpropanoids, flavonoids, polyphenols and polysaccharides (Tian et al., 2019). *L. barbarum* polysaccharides (LBP) are also one of the main bioactive compounds of goji berry and have recently attracted the attention of many researchers. Researchers report that goji berry and LBP supplementation are anti-tumor, hepatoprotective, neuroprotective, antidiabetic and beneficial in cardiovascular diseases and vision improvement (Zhou et al., 2022). The fruit of *L. barbarum* is used in traditional Chinese herbal medicine and as a functional food in daily life. In addition, in China, *L. barbarum*, *L. chinense* and *L. ruthenicum* species are used as a medicinal and functional food due to their anti-aging, antioxidant, antidiabetic, anticancer, cytoprotective, neuroprotective and immunomodulatory effects (Tian et al., 2019).

Diabetes is an endocrine system and metabolism disease characterized by chronic hyperglycemia, which can lead to disorders in carbohydrate, protein and fat metabolism as a result of insufficiency, absence and/or deficiency of the insulin hormone (Yalın et al., 2011). Overweight and obesity are defined by excessive accumulation of adipose tissue that impairs both physical and psychosocial health and well-being. Obesity is considered a health problem in both developed and developing countries. It is stated that both type 2 diabetes and obesity are associated with insulin resistance (Al-Goblan et al., 2014). Oxidative stress has an important role in the development of diabetes-related complications. Existing hyperglycemia in diabetic patients increases the formation of free radicals. Endogenous antioxidants are insufficient to balance toxic reactive oxygen species, and accordingly, an increase in oxidative stress occurs. Since the protective effects of antioxidants have been presented in experimental, clinical and epidemiological studies, it is thought by some researchers that antioxidants may help in the treatment of diabetes and its complications (Hamamcıoğlu, 2017). Dyslipidemia has an important place in the development of coronary artery disease, which is the number one cause of mortality in the world. In addition to dyslipidemia, diabetes and obesity are reported to cause the development of

coronary artery disease in different ways. It is stated that with the control of glycemia level and losing weight in diabetic patients, lipid parameters can also improve and the risk of developing coronary artery disease may decrease (Özdogan et al., 2015). It may be possible to treat these diseases or alleviate their complications by inhibiting key enzymes that play a role in the pathophysiology of diabetes (α-glucosidase and α-amylase enzymes), obesity (pancreatic lipase enzyme), and hyperlipidemia (pancreatic cholesterol esterase enzyme), which is the main subject of the study. Therefore, natural resources have an important role in the research of new drug molecules with antioxidant activity, effective on diabetes, obesity and lipid profile.

Medicinal and aromatic plants, which are the raw materials of herbal medicines and food supplements, are either collected from nature or cultivated. Since these plants, which are collected from nature, are the raw materials for both food and medicines, serious hazards will arise for biodiversity if they are collected uncontrollably. Cultivation of medicinal and aromatic plants with conventional or organic farming techniques is very important for both biodiversity and human health. It is necessary to examine the biological activities and phytochemical contents of these plants, whose production is very limited by organic farming techniques, and to evaluate whether they have the same or superior characteristics with the species collected from nature.

In this study, the extracts of the root, fruit and leaves of *L. barbarum* grown with organic farming were prepared by infusion technique, while the extract of the root part was prepared by decoction technique. The antioxidant (total antioxidant capacity, metal chelating capacity, ferric reducing power, 2,2-diphenyl-1-picrylhydrazil (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\cdot+}$) cation), antidiabetic (α-amylase and α-glucosidase enzymes), antiobesity (pancreatic lipase enzyme), and anticholesteremic (pancreatic cholesterol esterase enzyme) effects of the extracts were evaluated. The total phenolic and total flavonoid content of the extracts were determined by the Folin-Ciocalteu and the aluminum chloride method, respectively. Phytochemical content analysis of the extracts was achieved by Reverse Phase-HPLC (RP-HPLC) method.

MATERIAL and METHODS

Plant Material

L. barbarum grown with organic farming techniques was provided from Beyşehir Road, 3. km Akyokuş Mevki, Konya in 2021 (Certificate no: TR-OT-014-İ-197/01, Temmuz Organik Çiftliği). These species were produced in accordance with the Organic Agriculture Law and Regulation of Turkish Republic and has been certified by Nissert Ltd. Company authorized by the Ministry of Agriculture.

Chemicals

In addition to various standard compounds and high purity solvents used in the RP-HPLC study, many compounds such as enzyme substrates used in enzyme and antioxidant studies were obtained from Sigma-Aldrich.

Extraction

Extracts were prepared from the fruit and leaves of the plant by infusion method and from the roots by using both infusion and decoction methods. In the infusion method, the plant parts (10 g) were kept in hot water (200 mL) for 10 minutes in a closed flask. In the decoction method, plant parts (10 g) were boiled in cold water (200 mL) for 30 minutes. Then the extracts were filtered and this process was repeated 3 times. After combining the obtained filtrates, these extracts were frozen and dried in a lyophilizer at -80 °C.

Chemical Composition of Extracts

Total Phenol Content

After adding 100 µL of Folin-Ciocalteu reagent (10% w v-1) to the extracts prepared by infusion or decoction technique, the extracts were incubated at 25°C for 5 minutes. Then, 80 µL of sodium carbonate solution at a concentration of 7.5% was added to the mixture. The extracts were incubated for 30 minutes at room temperature in a dark place. After incubation, the absorbance of the extracts was measured at a wavelength of 735 nm using ELISA (SpectraMax i3x, Molecular Devices, USA) microplate reader. The total phenol content of the extracts was calculated as gallic acid equivalent mg (GAE) g^{-1} extract. The calibration equation was found to be $y= 6.3667x - 0.0118$, and $r^2= 0.9999$ (Zongo et al., 2010).

Total Flavonoid Content

Ethanol, 20 µL sodium acetate and 10% aluminum chloride solutions were added to the extracts, and the mixture was diluted to 1 mL with distilled water. After 30 minutes of incubation at room temperature, the absorbance of the mixture was measured at 415 nm with an ELISA microplate reader. The total flavonoid content of the extracts was expressed as quercetin equivalent mg (QE) g^{-1} extract. Calibration curve equation; $y= 2.1694x - 0.0067$, $r^2= 1$ (Kosalec et al., 2004).

Antioxidant activity

Total Antioxidant Capacity

Distilled water and 1 mL molybdate reagent were added to the extracts and the tubes were vortexed. After 90 minutes of incubation at 90°C, the tubes were

cooled in an ice bath. The absorbances of the samples were measured with an ELISA microplate reader at 695 nm and the results were expressed as ascorbic acid equivalent mg (AAE) g^{-1} extract. The calibration curve equation was found to be y= 1.8309x – 0.1606 and r^2= 0.9981 (Orhan et al., 2017).

DPPH Radical Scavenging Effect

20 µL of 1 mM DPPH (1,1-diphenyl-2-picrylhydrazil) solution was added onto the extracts in 96-well microplates. The mixture was then incubated in the dark for 30 minutes. After this process, the absorbance of the extracts and the reference compound was measured at 520 nm with an ELISA microplate reader. Ascorbic acid was used as the reference compound. In the experiment, DPPH radical scavenging activity was calculated as inhibition $\% = [(Absorbance_{control} - Absorbance_{sample})/Absorbance_{control}] \times 100$ (1). Experiments were made in triplicate repetitions (Jung et al., 2011).

Metal Chelating Capacity

2 mM 10 µL $FeCl_2$ solution was added to the extracts prepared by infusion or decoction technique, whose metal binding capacity was to be evaluated, and incubated for 5 minutes at room temperature. After this process, 5 mM ferrozine solution was added and the mixture was kept at room temperature for 10 minutes. The absorbance values of the extracts and the reference compound were then measured at a wavelength of 562 nm using an ELISA microplate reader. EDTA (Ethylene Diamine Tetra Acetic acid) was used as the reference compound. Metal chelating capacity % of the extracts was calculated as $[(Absorbance_{control} - Absorbance_{sample})/Absorbance_{control}] \times 100$ (2). Experiments were made in triplicate repetitions (Dinis et al., 1994).

Ferric Reducing Power

0.1 mol L^{-1} sodium phosphate buffer (pH= 7.2) was added to the extracts and the reference compound. After this process, 1% (w v^{-1}) 10 µL $K_3Fe(CN)_6$ solution was added and the mixture was left to incubate in an oven at 37°C. After incubation, 10% w v^{-1} trichloroacetic acid solution was added and absorbance values were calculated at 700 nm wavelength using an ELISA microplate reader. After the measurement, 0.1% (w v^{-1}) $FeCl_3$ solution was added to the mixture and the absorbance was measured again, then the difference between the two absorbance measurements was calculated. Quercetin was used as the reference compound. Experiments were made in triplicate repetitions (Orhan et al., 2017).

ABTS Radical Scavenging Activity

1 mL ABTS (7 mM) was dissolved in distilled water and 2.45 mM potassium persulfate solution. The prepared mixture was incubated for 16 hours at 20°C in the dark. pH adjusted ABTS and phosphate buffer solutions were added to the extracts prepared by infusion or decoction technique. After the samples were vortexed, their absorbance was measured at 734 nm using an ELISA microplate reader. Gallic acid was used as the reference compound. In the experiment, ABTS radical scavenging activity was calculated as inhibition $(\%) = [(Absorbance_{control} - Absorbance_{sample})/Absorbance_{control}] \times 100$ (3) (Orhan et al., 2017).

Enzyme Assays

α-Glucosidase Enzyme Inhibitory Activity

The *α*-glucosidase type IV enzyme to be used in the experiment was dissolved in a phosphate buffer solution (0.5 M, pH 6.5). The extracts were prepared at different concentrations using 80% ethanol solution. The enzyme solution and extracts were pre-incubated at 37°C for 15 minutes in a 96-well microplate. Then, 20 mM 10 µL *p*-nitrophenyl-*α*-D-glucopyranoside solution (PNG) was added to the wells. In the microplate, after incubating the mixture for 35 minutes at 37 °C, the increase in absorption at 405 nm due to hydrolysis of PNG by *α*-glucosidase was measured using an ELISA microplate reader. In the experiment, acarbose (Bayer, Turkey) was used as a reference compound. Experiments were made in triplicate repetitions (Orhan et al., 2017).

α-Amylase Enzyme Inhibitory Activity

The enzyme *α*-amylase type I-A (EC 3.2.1.1, Sigma) to be used in the experiment was dissolved in the buffer solution. 15 µL Potato starch (2.5%, w v^{-1}) prepared in phosphate buffer solution (pH 6.9) was used as substrate solution in the experiment. The extracts were prepared at different concentrations using 80% ethanol solution. After the enzyme solution was added, the mixtures were incubated at room temperature for 5 minutes, then substrate solution was added. The mixtures were then allowed to a new incubation at 37°C for 15 minutes. The mixture in the microplate to which 3,5-dinitrosalicylic acid (DNSA) color reagent solution (5.31 M sodium potassium tartrate in 96 mM DNS, 2 M NaOH) was added, was placed in an oven at 80°C. After 40 minutes, distilled water was added. The absorbances of the mixtures were measured at 540 nm using an ELISA microplate reader. Acarbose was used as reference compound. Then, the standard maltose calibration chart was prepared, the amount of maltose produced was calculated using the standard maltose calibration chart (y = 0.6762x - 0.0044 and r^2= 0.9966) and the net absorbance values were obtained. Experiments were made in triplicate repetitions (Orhan et al., 2017).

Pancreatic Lipase Enzyme Inhibitory Activity

The pancreatic lipase enzyme type II solution to be used in the experiment was prepared in 10 mM 4-morpholinepropanesulfonic acid and 1 mM EDTA buffer solution (pH 6.8). The extracts were prepared at different concentrations using 80% ethanol solution. The enzyme solution and extracts were pre-incubated for 15 minutes at 37°C in 150 µL Tris buffer (100 mM Tris-HCl and 5 mM $CaCl_2$, pH 7.0) in a 96-well microplate. After this process, 4-nitrophenyl butyrate solution, which was used as a substrate, was added to the wells. The mixture on the microplate was reincubated at 37°C for 30 minutes. The increase in absorption at 405 nm as a result of hydrolysis of 4-nitrophenylbutyrate with the pancreatic lipase enzyme was measured using an ELISA microplate reader. Orlistat was used as the reference compound. Experiments were made in triplicate repetitions (Lee et al., 2012).

Pancreatic Cholesterol Esterase Enzyme Inhibitory Activity

The porcine pancreatic cholesterol enzyme was dissolved in 100 mM buffer solution containing 100 mM NaCl (pH 7). 20 µL extracts prepared at different concentrations were added to 50 µL of phosphate buffer. After adding taurocholic acid (12 mM) and its substrate, this mixture was left to incubate at room temperature for 5 minutes. After incubation, porcine pancreatic cholesterol esterase enzyme (0.1 µg mL^{-1}) was added to the mixture and a kinetic reading was performed at 405 nm for 15 minutes using an ELISA microplate reader. Simvastatin was used as a reference compound in the experiment (Ngamukote et al., 2011).

Standardization of *L. barbarum* Leaf Infusion using RP-HPLC Method

HP Agilent 1260 series LC System and ACE 5 C18 (5 µm, 150 mm x 4.6 mm) column were used in the HPLC system used for phytochemical analysis. The device also has an HP Agilent 1260 series Autosampler unit. The column temperature was kept constant at 25°C throughout the analysis. The following standard copmoun mixtures were used for the qualitative and quantitative analysis of the phenolic compounds and flavonoids in the extract. Phenolic compound mixture; gallic acid, protocatechic acid, chlorogenic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, ellagic acid, caffeic acid, *trans*-cinnamic acid, rosmarinic acid, epicatechin, catechin. The flavonoid mixture; rutin, naringenin, hesperidin, quercetin-3-*O*-glucoside, apigenin-7-*O*-glucoside, myrcetin, quercetin, luteolin, apigenin. All of the standard compounds were purchased from Sigma-Aldrich. The gradient flow was initiated with a mobile phase system containing 5% solvent A (acetonitrile: water: formic acid, 50:50:0.5) and 95% solvent B (water: formic acid, 100:0.5). Total analysis time is 55 minutes. The injection volume is 20 µl. Analyzes were carried out at 4 different wavelengths, 260, 280, 320 and 350 nm, using the DAD detector. The extract was prepared at a concentration of 10 mg mL^{-1} using 25% v v^{-1} acetonitrile solution. All sample solutions were filtered through a 0.45 µm membrane filter. Then, qualitative and quantitative analysis of the compounds in the extract was carried out (Gök et al., 2021). The calibration equations and correlation coefficients determined for chlorogenic acid (Rt= 15.36 min) and quercetin-3-*O*-glucoside (Rt= 29.52 min) were [y= 114.77x + 25.057] (r^2= 0.998) and [y= 65.002x – 40.251] (r^2= 0.998), respectively.

Statistical analysis

All analyses were repeated in triplicate and the results were averaged. All values are expressed as mean ± standard deviation (S.D.). Linear regression analyses and calculations were made using Microsoft Excel and GraphPad Instat software. A difference in $p<0.05$ values was considered statistically significant (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

RESULTS and DISCUSSION

It was determined that the infusion prepared from *L. barbarum* fruits had the highest extract yield among the extracts prepared by infusion and decoction techniques from various parts of the *L. barbarum* grown with organic farming. It was found that *L. barbarum* leaf infusion had the highest total phenol (72.53±9.13 mg GAE g^{-1} extract) and total flavonoid (14.92±0.53 mg QE g^{-1} extract) content. It was determined that the extract with the lowest total phenol (9.03±0.77 mg GAE g^{-1} extract) and flavonoid (1.24±0.80 content QE g^{-1} extract) was *L. barbarum* fruit infusion (Table 1).

Five different methods were used to evaluate the antioxidant activities of the extracts prepared from various parts (root, leaves, and fruit) of *L. barbarum* using the infusion or decoction technique. In the antioxidant capacity evaluation method, which is one of these methods, *L. barbarum* fruit infusion (170.19±11.56 mg AAE g^{-1} extract) was found to be the extract with the highest total antioxidant capacity. It was concluded that the extracts obtained from the roots of the plant by both decoction and infusion techniques had the lowest total antioxidant capacity (Table 1). ABTS radical scavenging activities of all samples, except *L. barbarum* root infusion, increased in a dose-dependent manner. Especially *L. barbarum* root decoction (81.46±3.71%) showed inhibitory activity close to gallic acid (98.15±0.80%) used as reference compound. Interestingly, in the method in which DPPH radical scavenging activity was evaluated, no activity was observed at 2 mg mL^{-1}

concentrations of other extracts except *L. barbarum* fruit infusion, while *L. barbarum* root decoction and leaf infusion had the highest radical scavenging activities at both 0.5 (74.15±0.36% and 76.29±1.28%, respectively) and 1 mg mL^{-1} concentrations (79.19±3.39% and 62.84±5.81%, respectively). When the metal chelating capacity of the extracts was evaluated, it was determined that the metal chelating capacity of all extracts was the same as that of EDTA (100%), except for *L. barbarum* fruit infusion (57.13±0.75%) at a concentration of 2 mg mL^{-1}. While the absorbance values of the extracts increased depending on the concentration in the ferric reducing power method, it was found that *L. barbarum* root decoction was the most effective extract with the highest absorbance value (3.220±0.31) as in the DPHH radical scavenging method (Table 2).

Table 1. Results of the yields (w w-1), total phenol, total flavonoid contents and total antioxidant capacity of the extracts

Çizelge 1. Ekstrelerin verim (a a^{-1}), toplam fenol, toplam flavonoid içeriği ve toplam antioksidan kapasitesi sonuçları

Extracts	Yield% (w w^{-1})	Total Phenol Content mg (GAE) g^{-1} extract±SD	Total Flavonoid Content mg (QE) g^{-1} extract±SD	Total Antioxidant Capacity mg (AAE) g^{-1} extract±SD
***L. barbarum* root infusion**	7.82	40.28±3.80	4.78±1.41	98.28±5.84
***L. barbarum* fruit infusion**	27.17	9.03±0.77	1.24±0.80	170.19±11.56
***L. barbarum* leaf infusion**	11.05	72.53±9.13	14.92±0.53	121.76±11.54
***L. barbarum* root decoction**	5.96	62.48±6.12	11.85±1.38	50.39±3.01

GAE: Gallic Acid Equivalent, QE: Quercetin Equivalent, AAE: Ascorbic Acid Equivalent, SD: Standard Deviation

Table 2. ABTS, DPPH radical scavenging activity, metal chelating capacity and ferric reducing power results of the extracts

Çizelge 2. Ekstrelerin ABTS, DPPH radikal süpürücü aktivitesi, metal bağlama kapasitesi ve demir indirgeme gücü sonuçları

Samples	Concentration (mg mL^{-1})	ABTS Radical Scavenging Effect (Inhibition%±SD)	DPPH Radical Scavenging Activity (Inhibition%±SD)	Metal Chelating Capacity %±SD	Ferric Reduction Power (Absorbance±SD)
***L. barbarum* root infusion**	**0.5**	64.28±5.81***	15.26±8.33**	67.35±5.58***	0.282±0.02**
	1	28.10±6.39***	21.69±5.12**	90.92±3.72***	0.365±0.03**
	2	32.88±0.91***	-	100***	1.001±0.04***
***L. barbarum* fruit infusion**	**0.5**	14.87±0.72**	10.76±2.78*	58.44±1.81***	0.100±0.01*
	1	17.56±1.71**	17.02±1.33**	32.96±0.71***	0.185±0.01*
	2	21.52±2.59**	25.22±3.63***	57.13±0.75***	0.455±0.01**
***L. barbarum* leaf infusion**	**0.5**	25.86±0.72***	76.29±1.28***	78.01±2.46***	0.486±0.02***
	1	25.11±1.79***	62.84±5.81***	99.46±2.11***	1.096±0.00***
	2	44.84±2.16***	-	100***	2.439±0.27***
***L. barbarum* root decoction**	**0.5**	26.08±1.62***	74.15±0.36***	89.31±0.79***	0.509±0.03***
	1	30.87±0.93***	79.19±3.39***	80.85±2.78***	0.904±0.01***
	2	81.46±3.71***	-	100***	3.220±0.31***
References	**GA/AA/EDTA/QE 0.5**	99.55±1.04[a]***	89.40±0.61[b]***	100[c]***	3.930±0.00[d]***
	GA/AA/EDTA/QE 1	98.92±0.26[a]***	89.63±0.35[b]***	100[c]***	3.932±0.00[d]***
	GA/AA/EDTA/QE 2	98.15±0.80[a]***	90.48±0.59[b]***	100[c]***	3.845±0.14[d]***

-: No activity. SD: Standard Deviation. ns: Not statistically significant. *p<0.05. **p<0.01. ***p<0.001. GA: [a]Gallic acid. AA: [b]Ascorbic acid. [c]EDTA: Ethylenediamine tetraacetic acid. [d]QE: Quercetin

The inhibitory effects of extracts prepared from various parts of *L. barbarum* by infusion or decoction method on four different enzyme systems (α-glucosidase, α-amylase, pancreatic lipase and pancreatic cholesterol esterase enzyme) were evaluated. None of the extracts showed an inhibitory effect on the α-glucosidase enzyme, which was used to evaluate their antidiabetic effect potential. All of the extracts showed an extremely weak inhibitory effect (1.21±0.15-19.82±0.58%) on the α-amylase enzyme

when compared with the reference compound acarbose (98.40±0.50%). *L. barbarum* root and fruit infusion displayed no inhibitory effect on pancreatic lipase enzyme. *L. barbarum* leaf infusion at a concentration of 2 mg mL^{-1} among all extracts was determined as the extract with the highest effect with an inhibition value of 62.16±3.33%, while this inhibition value was found to be the same as the reference compound orlistat (62.80±1.76%). While all of the extracts inhibited pancreatic cholesterol esterase enzyme, *L. barbarum* leaf infusion had the highest inhibition with a value of 97.35±0.82% at a concentration of 1 mg mL^{-1}. It was determined that this value was considerably higher than that of the reference compound simvastatin (53.20±3.26%) at the same concentration. It was determined that the extract with the weakest effect on pancreatic cholesterol esterase enzyme was *L. barbarum* fruit infusion (23.29±0.50-37.35±1.30%). (Table 3).

Table 3. The Inhibitory effects of extracts on α-glucosidase, α-amylase, pancreatic lipase and pancreatic cholesterol esterase enzyme

Çizelge 3. Ekstrelerin α-glukozidaz, α-amilaz, pankreatik lipaz ve pankreatik kolesterol esteraz enzimi üzerindeki inhibitör etkileri

Samples	Concentration (mg mL^{-1})	Inhibition%±SD			
		α-Glucosidase	α-Amylase	Pancreatic Lipase	Pancreatic Cholesterol Esterase
L. barbarum root infusion	0.5	-	3.53±0.06^{ns}	-	42.47±3.93***
	1	-	3.77±2.05^{ns}	-	44.26±2.03***
	2	-	10.15±4.69*	-	45.87±1.65***
L. barbarum fruit infusion	0.5	-	1.21±0.15^{ns}	-	37.35±1.30***
	1	-	10.28±1.81*	-	23.29±0.50***
	2	-	7.77±2.65*	-	23.80±3.81***
L. barbarum leaf infusion	0.5	-	19.82±0.58**	46.01±7.26***	88.27±4.05***
	1	-	6.44±1.67*	57.91±1.67***	97.35±0.82***
	2	-	13.62±5.82**	62.16±3.33***	93.98±0.54***
L. barbarum root decoction	0.5	-	9.07±2.86*	34.05±3.97***	74.25±0.84***
	1	-	12.40±6.78**	32.22±1.78***	27.94±2.27**
	2	-	4.41±1.50*	25.20±2.08**	96.46±0.43***
References	ACA/OR/SIM 0.5	99.10±0.12^{a***}	94.86±0.60^{a***}	52.17±0.00^{b***}	47.89±5.11^{c***}
	ACA/OR/SIM 1	99.88±0.09^{a***}	98.40±0.50^{a***}	69.55±4.19^{b***}	52.25±0.12^{c***}
	ACA/OR/SIM 2	99.75±0.05^{a***}	95.25±2.60^{a***}	62.80±1.76^{b***}	53.20±3.26^{c***}

-: No activity, SD: Standard Deviation, ns: Not statistically significant *p<0.05, **p<0.01, ***p<0.001, ^{a}ACA: Acarbose, ^{b}OR: Orlistat, ^{c}SIM: Simvastatin

When the enzyme inhibitory activity findings of the extracts were evaluated, it was thought that this extract should be standardized with the RP-HPLC technique, since *L. barbarum* leaf infusion showed a strong inhibitory effect especially on pancreatic lipase and pancreatic cholesterol esterase enzymes. Leaf infusion was screened with diode array detector using RP-HPLC technique for 22 standard phenolic compounds and standardization was made by choosing chlorogenic acid (1.339±0.056 g $100g^{-1}$ dry extract) and quercetin-3-*O*-glucoside (1.801±0.042 g $100g^{-1}$ dry extract) as marker compounds in leaf infusion (Fig 1-6).

Figure 1. RP-HPLC chromatogram of chlorogenic acid

Şekil 1. Klorojenik asidin RP-HPLC kromatogramı

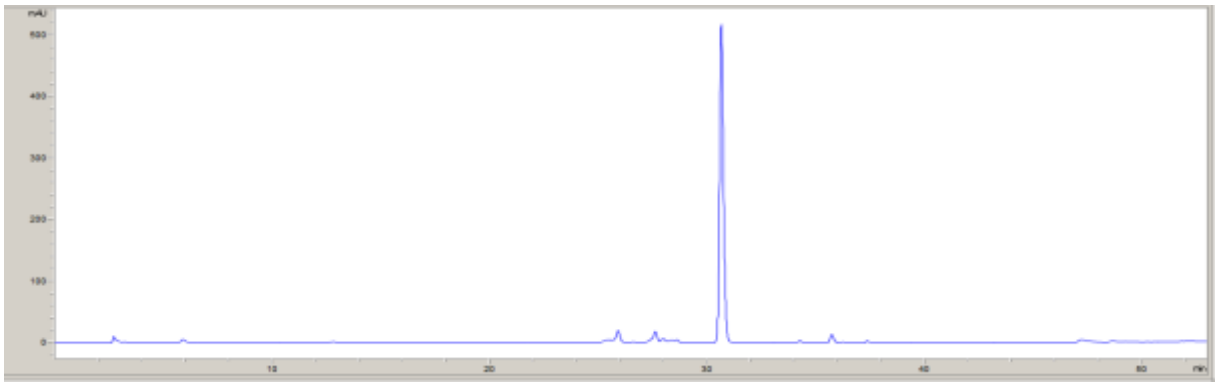

Figure 2. RP-HPLC chromatogram of quercetin-3-*O*-glucoside
Şekil 2. Kersetin-3-O-glukozitin RP-HPLC kromatogramı

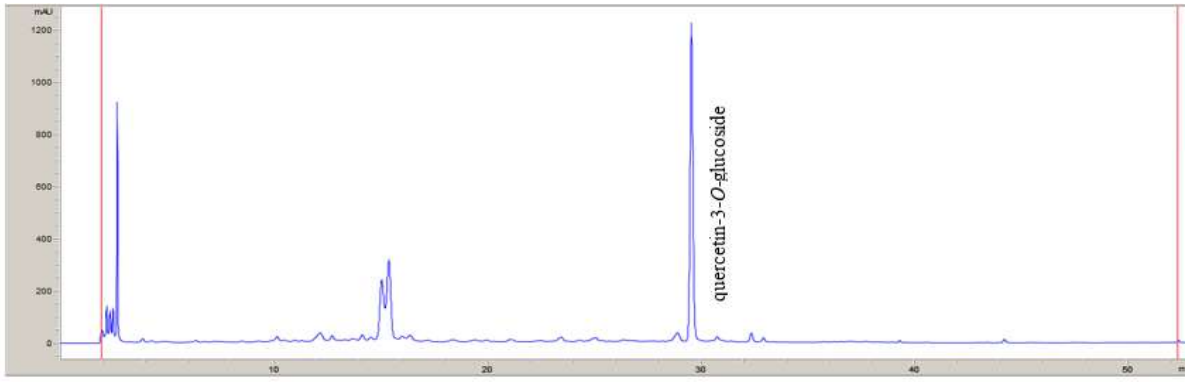

Figure 3. RP-HPLC chromatogram of *L. barbarum* leaf infusion at 260 nm
Şekil 3. L. barbarum yaprak infüzyonunun 260 nm'deki RP-HPLC kromatogramı

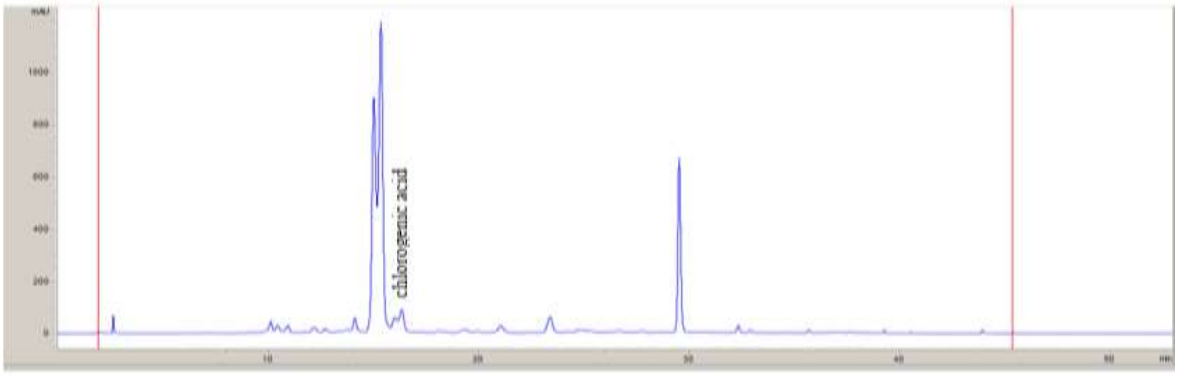

Figure 4. RP-HPLC chromatogram of *L. barbarum* leaf infusion at 320 nm
Şekil 4. L. barbarum yaprak infüzyonunun 320 nm'deki RP-HPLC kromatogramı

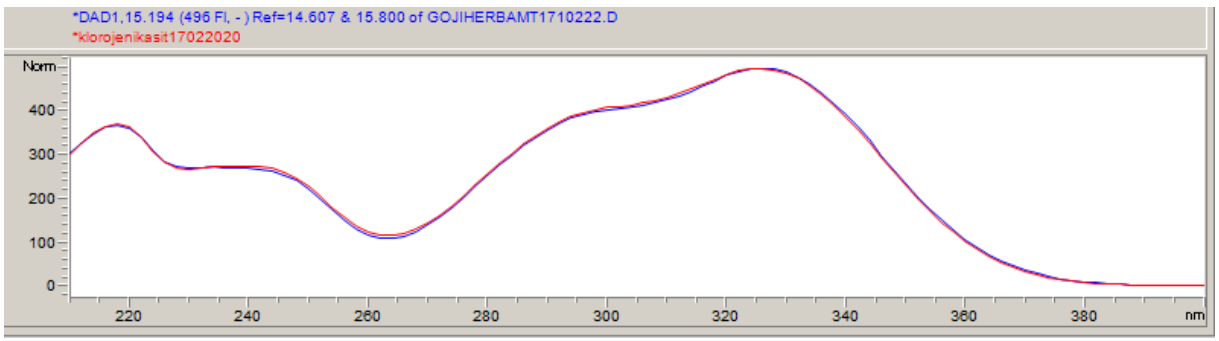

Figure 5. The overlaid UV spectra of standard chlorogenic acid and chlorogenic acid in the extract
Şekil 5. Ekstredeki klorojenik asit ve standart klorojenik asidin çakıştırılmış UV spektrumları

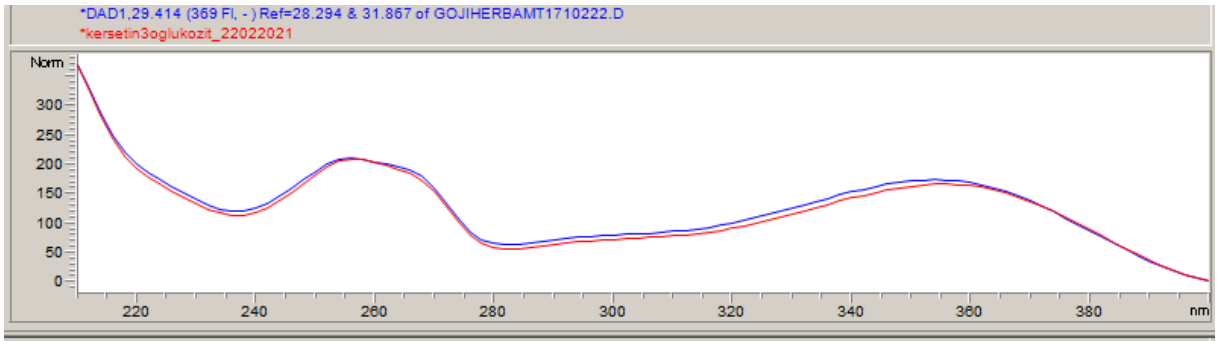

Figure 6. The overlaid UV spectra of standard quercetin-3-*O*-glucoside and quercetin-3-*O*-glucoside in the extract
Şekil 6. Ekstredeki kersetin-3-O-glukozit ve standart kersetin-3-O-glukozitin çakıştırılmış UV spektrumları

This is the first study to examine the antioxidant, antidiabetic, antiobesity and anticholesterolemic effect potential and phytochemical contents of the extracts prepared by using infusion and decoction techniques from the root, fruit and leaves of *L. barbarum* grown in organic farming techniques in Turkey.

Mocan et al. (2014) investigated the total phenol (61.59±1.68 mg GAE g^{-1} plant material) and total flavonoid (43.73±1.43 rutin mg g^{-1} plant material) content and *in vitro* antioxidant activity (DPPH radical scavenging activity and antioxidant capacity) of the 70% ethanolic extract of *L. barbarum* leaves. The DPPH radical scavenging activity of *L. barbarum* extract was calculated as 29.30±4.34 quercetin µg mg^{-1} plant material equivalent, and the antioxidant capacity was calculated as 35.72±6.29 trolox µg mg^{-1} plant material equivalent. It was determined that the DPPH radical scavenging activity of *L. barbarum* extract (29.30±4.34 quercetin µg mg^{-1} plant material equivalent) was lower than its antioxidant capacity (35.72±6.29 trolox µg mg^{-1} plant material equivalent) (Mocan et al., 2014).

Islam et al. (2017) compared the total phenol and flavonoid profiles and antioxidant capacities of extracts prepared with acetone:water:acetic acid mixture from red (*L. barbarum)* and black (*L. ruthenicum)* goji berries collected from different regions of China. The total phenol and flavonoid contents of black goji berry extracts were higher than red goji berry extracts. Likewise, considering the results of the three antioxidant activity methods (ABTS and DPPH radical scavenging activity, FRAP) used in the study, it was observed that black goji berry extracts had stronger antioxidant effects than red goji berry extracts (Islam et al., 2017).

It has been reported that the total polyphenolic content of the extracts obtained by ultrasound extraction technique using water at different temperatures from the fruits of cultivated *L. barbarum* and *L. chinensis* in Greece varies between 234.3-394.3 mg GAE L^{-1}. The antioxidant effects (DPPH and ABTS radical scavenging, peroxyl radical-induced DNA plasmid strand cleavage assay) of these extracts, which were prepared using different temperatures, were evaluated by comparing them with each other (Skenderidis et al. 2018).

Total phenol contents of the extracts prepared by using ultrasound extraction with 70% methanol from two cultivars (cv. Erma and cv. Biglifeberry) grown in Romania with organic farming were calculated as 11.6 mg GAE g^{-1} dw for cv. Erma and 15.7 mg GAE g^{-1} dw for cv. Biglifeberry. The antioxidant activity of both extracts was tested with six different methods. While cv. Biglifeberry had a high antioxidant capacity, both cultivars showed strong antioxidant activities in the CUPRAC method. Both methanol extracts moderately inhibited both α-glucosidase and α-amylase enzymes. The highest α-amylase inhibition was calculated as 0.24±0.01 acarbose mmol g^{-1} extract equivalent for cv. Biglifeberry and the highest α-glucosidase inhibition was calculated as 0.22±0.02 acarbose mmol g^{-1} extract equivalent for cv. Erma (Mocan et al., 2018).

Among the methanol extracts of red, yellow and black goji berries cultivated in Serbia, it was found that black fruits (295.7±18.8 mg GAE $100g^{-1}$) had the highest total phenol content and yellow fruits (335.5±27.1 mg hyperoside equivalent $100g^{-1}$) had the highest total flavonoid content. According to the antioxidant compound index obtained using the results of FRAP, CUPRAC, DPPH, ABTS and beta-carotene bleaching experiments, it was determined that black goji berries had the highest antioxidant activity (Ilić et al., 2020).

It was determined that the total phenol content of the methanol and digested methanol extracts prepared from the cultivated *L. ruthenicum* and *L. barbarum* fruits varied between 18.5±0.4 and 64.9±0.4 mg GAE g^{-1} of extract, and the total flavonoid content varied between 17.9±2.6 and 93.9±1.0 mg QE g^{-1} of extract. In the study, four methods (DPPH radical and nitric oxide scavenging assay, ferric reducing antioxidant power and iron chelating activities) were used to evaluate the antioxidant activity of the extracts and the results were expressed in terms of EC_{50} value, while no reference compound was used for comparison (Magalhães et al., 2022).

When the previous studies on *L. barbarum* samples grown with organic or conventional farming

techniques were evaluated, it was found that the total phenol and total flavonoid content of *L. barbarum* leaves in this study were quite high, while the total phenol and flavonoid content of the fruits were found to be quite low. It was thought that the differences in the total phenol and flavonoid results reported in previous studies and the total phenol and flavonoid content of *L. barbarum* may be caused by factors such as soil, climate factor, organic farming techniques and harvest time.

Wojdylo et al. (2018) investigated the inhibitory effects of *α*-amylase and *α*-glucosidase enzymes of 80% methanol extract obtained from the fruits of 21 different *L. barbarum* samples collected from Poland. As a result of the study, it was reported that while the inhibition rates of 21 different extracts on the *α*-glucosidase enzyme ranged from 5.7±0.3% to 15.3±2.1%, the inhibition rates of these extracts on the *α*-amylase enzyme ranged from 9.6±0.5% to 89.0±3.1%. Researchers emphasized that Goji berries can be used for medicinal or cosmetic purposes, as well as functional foods (Wojdyło et al., 2018). Nikolava et al. (2018) investigated the antidiabetic activity of Goji berry fruit extracts and their corresponding polyphenols. Researchers found a 50% inhibitory concentration on the α-glucosidase enzyme of the extract as IC_{50}=91.7 µg GAE g^{-1} fruit. As a result of the study, it was stated that some polyphenols in the examined extracts showed competitive properties against the enzyme (Nikolava et al., 2018). When we evaluated the findings in the above-mentioned two literature, it was determined that the *α*-amylase and *α*-glucosidase inhibitory activities of goji berries did not have strong inhibitory effects like the goji berries grown with organic farming used in this study. Pollini et al. (2020) examined the antioxidant capacity and total phenol content of the methanol extract of *L. barbarum* leaves collected from Italy. As a result of the study, it was determined that the total phenol content of the extract was 7.75 GAE mg g^{-1} dry leaves. The DPPH radical, ABTS radical scavenging potential and ferric reducing the power of the extract was found to be 9.39, 11.28 and 8.25 Trolox equivalent mg g^{-1} dry leaves, respectively. In the study, it was determined that the extract showed an inhibitory effect against the *α*-amylase enzyme with an IC_{50} value of 25.4 mg mL^{-1}, while this value was found to be 0.1 mg mL^{-1} for the reference compound acarbose. The findings of the enzyme inhibition assay indicated that the leaf extract of *L. barbarum* caused a concentration-dependent inhibition of the *α*-amylase enzyme (Pollini et al., 2020).

Donno et al. state that goji berry fruits contain various bioactive compounds such as various cinnamic acid and benzoic acid derivative compounds, hyperoside, catechins, various monoterpenes, organic acids, and vitamin C (Donno et al., 2015). Mocan et al. (2014) detected the presence of rutin, chlorogenic acid, gentisic acid, caffeic acid, *p*-coumaric acid, ferulic acid, quercetin-3-*O*-glucoside (25.08±0.72 µg g^{-1}), quercitrin and quercetin in 70% ethanol extracts of cultivated *L. barbarum* leaves collected from Romania by HPLC-MS technique. Chlorogenic acid (12045.96±9.25 µg g^{-1}) and rutin (5646.66±3.32 µg g^{-1}) as major phenolic compounds were detected in the extract (Mocan et al., 2014). Pollini et al. (2020) analyzed the phenolic content of the methanol extract of *L. barbarum* leaves harvested in Italy by QTOF-LC/MS method. It was stated that the most abundant phenolic acids in *L. barbarum* leaf extract were chlorogenic (358.34±0.004 µg g^{-1}) and salicylic (239.02±0.005 µg g^{-1}) acids. In this study, quercetin-3-*O*-glucoside could not be detected in the leaves (Pollini et al., 2020). In this study, it was determined that the amount of chlorogenic acid in leaf infusions was lower than the amount of chlorogenic acid found in leaf extracts of *L. barbarum* grown with organic farming in Romania, while the amount of quercetin-3-*O*-glucoside, on the contrary, was lower. In addition, *p*-coumaric acid, ferulic acid, caffeic acid, rutin, and quercetin could not be detected in the leaf samples in this study.

There are no phytochemical analysis and bioactivity studies on *L. barbarum* roots to date. And root decoction showed even higher effects on pancreatic cholesterol esterase enzyme than simvastatin at 0.5 and 2 mg mL^{-1} concentrations. While it was seen in previous studies that *L. barbarum* leaf and fruit extracts had *α*-glucosidase enzyme inhibitory activity, albeit weak, in this study infusions and decoctions obtained from all parts of the plant were found to have no *α*-glucosidase inhibitory activity. In this study, it has been reported that chlorogenic acid, which was found to be in leaf infusion by HPLC analysis, has a strong effect on pancreatic lipase enzyme, and has lipid-lowering and anticardiovascular effects *in vivo* (Miao et al., 2020; Ahmad et al., 2021). Docking studies on the pancreatic lipase enzyme of quercetin-3-*O*-glucoside, which is used for the standardization of leaf infusion, have been reported in the literature. Chen et al. (2020) revealed that quercetin 3-*O*-glucoside has a potential binding ability to pancreatic lipase enzyme with high energy scores (-30.02 kcal mol^{-1}) (Chen et al., 2020).

CONCLUSION and RECOMMENDATIONS

Supporting medicinal and aromatic plants with organic agriculture can be a solution to many problems such as protecting biodiversity and growing healthy and high-quality species. On the other hand, it is necessary to examine whether these species grown with organic agriculture have biological activities similar to or superior to those of wild species in terms of health and changes in their phytochemical contents. In this study, the phytochemical contents, antioxidant activity and effects on some metabolic enzymes (*α*-glucosidase, *α*-amylase, pancreatic lipase and

cholesterol esterase enzymes) of all parts of the *L. barbarum* plant grown with organic farming techniques in Turkey were evaluated for the first time. In terms of antioxidant activity, *L. barbarum* leaf infusion had a higher effect and this was also correlated with total phenol and flavonoid content. It has been determined that the effect of *L. barbarum* leaf infusion on pancreatic lipase enzyme, which is effective in obesity control, is close to that of orlistat. On the other hand, more *in vitro* and *in vivo* studies should be conducted on this extract so that it can be considered as a source for natural product development studies for obesity control. For the first time in this study, the determination that *L. barbarum* root decoction and leaf infusion has strong cholesterol esterase inhibitory activity showed that further antihyperlipidemic effect studies on the roots and leaves of the plant should be performed. Because of the promising antioxidant, antihyperlipidemic and antiobesity activity potential of the infusion prepared from the leaves among all plant parts tested, this extract was standardized by RP-HPLC technique using two marker compounds (chlorogenic acid and quercetin-3-*O*-glucoside). As a result, it was concluded that both roots and leaves of *L. barbarum* grown with organic farming techniques can be a good source for developing natural products.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The authors of the article declare that there is no conflict of interest between them.

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