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Lonicera iberica M. Bieb.: Investigation Antioxidant Activity and Bioactive Chemicals

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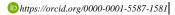
ABSTRACT

Research Article

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Keywords: Lonicera iberica DPPH FRAP Phenolic substance Flavonoid substance In this study, it was investigated the total amounts of phenolic and flavonoid substances and antioxidant activities in different solvent extracts of Lonicera iberica M. Bieb. wild fruit. Total phenolic contents of the extracts were determined as equivalent to gallic acid using Folin-Ciocalteu reagent, and total flavonoid contents as equivalent to quercetin by aluminium nitrate method. In addition, the antioxidant properties of the extracts were determined using free radical scavenging (DPPH) and reducing power (FRAP) methods. The amount of total phenolic substance of L. iberica fruits in hexane and methanol extracts was calculated as 30.96 ± 0.67 mg of GAE / g and $23.70 \pm$ 1.56 mg of GAE / g, respectively. In addition, the amount of total flavonoid substance was calculated as 46.50 ± 8.54 mg of QE / g and 42.09 ± 2.58 mg of QE / g, respectively. It was determined that DPPH radical scavenging activity correlated with total phenolic and flavonoid amount of substance, and L. iberica had a strong antioxidant effect. It is predicted that our study will shed light on new researches, since it is the first study done with L. Iberica fruits in this field.







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Introduction

Forests are among the most important elements of nature. In addition, they are natural areas where many living things keep living vital activities. In terms of health, forests have a beneficial effect on many health problems such as hypertension, stress, depression and anxiety (Jarmer et al., 2021). Forests are rich environments that contain macro and micronutrients that are important for human nutrition, depending on the plant diversity (Rowland et al., 2017). Edible wild plants in forests are classified as vegetables, greens, fruits and grains, seeds and nuts (Abbasi et al., 2013; Sevindik et al., 2017; Mohammed et al., 2018). In addition to their nutritional value, these plants also have medicinal values (Turner et al., 2011; Mohammed et al., 2020). Recently, the increasing interest in natural nutrition has increased the tendency to these edible plants and has led to the formation of a new sector accordingly (Mahapatra and Panda, 2012).

Honeysuckle, which is a forest plant, is a shrub species belonging to the Caprifoliaceae family. In general, parts of edible honeysuckle as fruit, flower, branch, leaf and shell have been used in folk medicine for many years in many countries. Especially due to its antiseptic properties, its leaves are used in the treatment of stomach, throat and eye, while its branches are used as diuretic and its fruits as a general strengthener (Tunde et al., 2012). Plekhanova (2000) reported that flowers of honeysuckle species are rich in ascorbic acid, contain bioactive flavonoids, and plants and fruits are highly resistant to cold. There are many types of honeysuckle. The fruits of Lonicera caerulea L. (blue honeysuckle), one of these species, are rich in phenolic compounds and is a widely used herbal medicine as an antiinflammatory (Sandigawad, 2015). Lonicera japonica Thunb. is another type of honeysuckle reported to have antimicrobial, antioxidative, antiviral and antiinflammatory effects (Shang, 2011).

L. iberica (Dadaş honeysuckle, lili, lilik), is another species of honeysuckle that grow in 800-2500 m in Turkey, Caucasia and in Northern Iran (Eminağaoğlu et al., 2014). In Turkey, it spreads over a wide area in North East Anatolia, especially in Artvin, Erzurum and Kars. The stem part of the

plant is used in making music instrument sticks, and the fibrous parts are used in making bath fiber. *L. iberica*, which is among the edible forest fruits, has a berry-like, red, compound and very small seeded fruit. In this species, flowering occurs in June-July and reaches fruit maturity in October (Eminağaoğlu et al., 2014). This type is called "lilik" among people in Eastern Anatolia. It is among the indispensable fruits of the shepherds and hunters living in the region. Fruits are sweet but leave a slightly bitter taste in the mouth after eating (figure 1).

The species belonging to the Caprifoliaceae family, which grow spontaneously without any intervention in nature, have been the subject of many studies. However, there is no study on *L. iberica* specie. In this study, the total phenolic content of *L. iberica* fruit was determined equivalent to gallic acid by using Folin-Ciocalteu reagent. Total flavonoid content was calculated as equivalent to quercetin by aluminum nitrate method. In addition, antioxidant capacity was determined using DPPH and FRAP methods. By comparing the results of the study with other species and forest fruits in the literature, it was contributed to the emergence of the bioactive power of *L. iberica*.

Materials and Methods

Supply of Plant Samples

The samples to be used in this study were collected from plants (L. iberica) spontaneously grown in their natural environment at coordinates of 40°49'36 "N 42°05'47" E (1521 m). The collected samples were washed first with tap water and then with distilled water in order to be purified from physical contamination. L. iberica fruits were dried in the shade and stored at +4°C until use.

Preparation of L. Iberica Fruit Extracts

Methanol and hexane extraction of L. iberica fruit was carried out according to literature (Gulcin et al., 2005). 15 g of dried plant specimens was ground in a grinder and placed in a 1 liter closed flask. Then solvent (300 mL) twenty times the amount of the sample was added and stirred in the magnetic stirrer. The extracts obtained was filtered. This procedure was repeated three times at regular intervals. The filtered extracts were combined. These procedures were done separately for hexane and methanol. Methanol was removed at 45°C and hexane 40°C in the evaporator. The extracts were stored at + 4°C for the studies.

Determination of Total Phenolic Substances

Determination of total phenolic substance in extracts obtained from L.iberica fruit was made according to Folin-Ciocaltaeu method (Slinkard and Singleton, 1977). Gallic acid was used as standard and standard graphic was prepared. Solutions of plant methanol and hexane extracts at a concentration of 1000 ppm were prepared. 50 μL was taken from stock solutions, completed to 1840 μL with distilled water. 40 μL of Folin-Ciocalteu reagent (FCR) was added to the mixture and incubated for 3 minutes at room temperature. Then, 120 μL of 2% (w / v) Na₂CO₃ solution was added. The mixture was kept in room temperature for 2 hours. The absorbance of the samples was measured against a blank at 760 nm. Three parallel studies were performed for measurements. The total phenolic contents of the extracts

were found as equivalent to gallic acid by using the equation obtained from the standard graph (mg GAE/g).

Determination of Total Flavonoid Substance

The total flavonoid contents of the extracts prepared were determined by aluminum nitrate method as equivalent to quercetin (Moreno et al., 2000). Quercetin was used as standard and standard graphic was prepared. 1000 ppm solution was prepared from methanol and hexane extracts obtained from *L. iberica* fruit. 50 μ L of this stock solution was taken and the volumes were completed to 1920 μ L with methanol. 40 μ L of 1 M potassium acetate was added and after one minute 40 μ L of 10% aluminum nitrate was added. It was incubated for 40 minutes. Absorbance at 415 nm against a blank prepared with pure water was measured. Three parallel studies were performed for measurements. The total flavonoid contents of the extracts were found as equivalent to quercetin using the equation obtained from the standard graph (mg QE/g).

Determination of DPPH• Free Radical Scavenging Activity

Free radical scavenging l activities of the extracts were found by using Blois method (Blois, 1958). 1,1-Diphenyl-2picrylhydrazyl (DPPH•) solution was used as free radical. 1000 ppm stock solution was prepared from hexane and methanol extracts of L. iberica fruit and 2,6-di-t-butyl-1hydroxytoluene (BHT) which was used as standard. 20, 40, 80 ve 100 µL were taken from these stock solutions and their volumes were completed to 400 µL with methanol. Then, 1600 μL DPPH• solution (0,1 mM) was added. The prepared solutions were incubated for 30 minutes at room temperature in the dark. The absorbance changes at 517 nm were measured against methanol. Control solution was prepared under the same conditions using methanol instead of sample and standard material. Decreasing absorbances yielded the amount of free DPPH• solution remaining, i.e., radical scavenging activity.

% DPPH• radical scavenging activity was calculated by the following formula:

 $% DPPH \bullet = [(A_0 - A_1)/A_0] \times 100$

A₀: Absorbance of control reaction

A₁: Absorbance of fruit extracts and standard solutions



Figure 1. Lonicera iberica fruits.

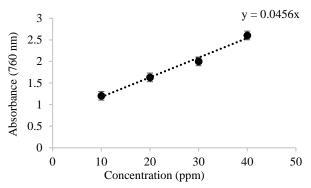


Figure 2. Calibration curve graph of gallic acid

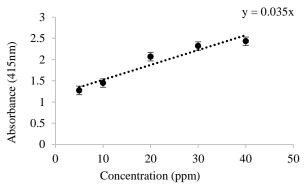


Figure 3. Calibration curve graph of quercetin

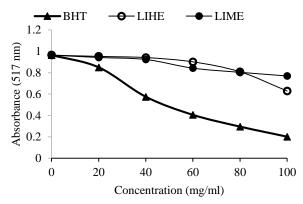


Figure 4. The DPPH• scavenging effect of hexane and methanol extracts and BHT at different concentrations (BHT: Butylated hydroxytoluene, LIHE: *L. iberica* hexane extracts, LIME: *L.iberica* methanol extracts).

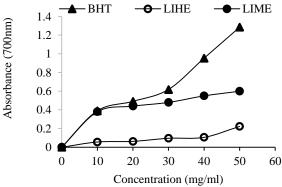


Figure 5. Comparison of Fe³⁺-Fe²⁺reducing power activities of extracts of *L. iberica* fruits with BHT (20-50 mg/mL) (BHT: Butylated hydroxytoluene, LIHE: *L. iberica* hexane extracts, LIME: *L. iberica* methanol extracts)

Determination of Ferric Reducing Power (FRAP)

The determination of Fe³⁺ reducing power was made according to Oyaizu (1986). 1000 ppm stock solution was prepared from hexane and methanol extracts. These stock solutions were taken into the tube at that the concentration was 10, 20, 30, 40 and 50 mg / mL). Distilled water was added so that the total volume was 1.0 mL.2.5 mL of phosphate buffer (0.2 M pH 6.6) and potassium ferricyanide (1%) solution were added to these solutions and kept in a water bath at 50°C for 20 minutes. Then 2.5 mL of 10% trichloroacetic acid (TCA) was added and vortexed. 2.5 mL ultrapure water and 0.5 mL iron (III) chloride (0.1%) were added to 2.5 mL samples taken from vortexed tubes and the absorbance at 700 nm measured against a blank. BHT was used as standard and the same process was applied to the prepared standard solution of 1000 ppm.

Result and Discussion

Plants, which grow spontaneously without human intervention and called wild fruits, have been the subject of many studies because they are eaten by humans and animals. Wild fruits are important in terms of the nutrients they contain, especially antioxidants. Recently, due to the increasing interest in natural nutrition, the trend towards edible wild fruits, which are seen as a source of bioactive components, has increased (Mohammed et al., 2021).

In this study, total phenolic and flavanoid content, DPPH• radical scavenging activities and Fe³⁺ reducing power capacity were determined in extracts of *L. iberica* fruit that grown in North Anatolia Turkey. The activity results obtained were compared with standard.

The yields of hexane and methanol extracts of *L. iberica* fruits were calculated as 26.01% and 46.06%, respectively, and are shown in table 1. Folin-Ciocaltaeu method was used to determine the amount of phenolic substance. Total amount of phenolic substance was calculated as equivalent to gallic acid by using gallic acid standard graph given in figure 2.

It was determined that there is relatively more phenolic substance in hexane extracts of *L. iberica* fruits compared to methanol extracts. Total phenolic matter was calculated as 30.96 ± 0.67 mg GAE/g in hexane extracts and 23.70 ± 1.56 mg GAE/g in methanol extracts (Table 1.)

The values found are similar to the total phenolic substance amounts found by Dung et al., (2010) in ethanol extracts of L. japonica's dried flowers, leaves and branches. In another study, the total amount of phenolic substance of L. japonica was determined as 27.36 ± 0.29 mg GAE/g in the flowers and 7.81 ± 0.39 mg GAE/g in the stem (Li et al., 2008). While the values we found in the hexane and methanol extracts of L. iberica are close to the values determined in the flowers of L. japonica, they are higher than the value found in the stem extracts of L. japonica. In addition, the values we found are lower than the total amount of phenolic substances found in L. caerulea (Polikova et al., 2008).

The total amount of flavonoids in our study was calculated as equivalent to quercetin using the standard graph of quercetin. The standard graphic is given in Figure 3. The total amount of flavonoid was determined as 46.50 \pm 8.54 mg QE/g in the hexane extract and 42.09 \pm 2.58 mg QE/g in the methanol extract.

Table 1. Total phenolic and flavonoid substance amount and yield of L. iberica fruit extracts.

Exstract	Yield (%)	Total phenolics (mg of GAE/g)	Total flavonoids (mg of QE/g)
L.iberica hexane extract	26.01	30.96±0,67	46.50±8.54
L.iberica methanol extract	46.06	$23.70\pm1,56$	$42.09\pm2,58$

Table 3. DPPH•% radical scavenging activities of *L. iberica* fruit extracts and BHT (BHT: Butylated hydroxytoluene, LIHE: *L. iberica* hexane extracts, LIME: *L. iberica* methanol extracts)

Extracts / standard	20 mg/mL	40 mg/mL	60 mg/mL	80 mg/mL	100 mg/L
LIHE	1.2	2.3	6.5	16	34.8
LIME	2.2	4.2	12.6	16.7	20.22
BHT	15.04	41.49	56.95	69.91	77.69

Table 4. *L. iberica* extracts and of BHT IC₅₀ values (BHT: Butylated hydroxytoluene, LIHE: *L. iberica* hexane extracts, LIME: *L. iberica* methanol extracts)

Extracts and standard	IC_{50} (mg/mL)
LIHE	155.54
LIME	192,09
BHT	57,20

When the total phenolic substance amounts we determined in *L. iberica* fruit extracts (Table 1) were compared with similar wild fruit studies; it is seen that they are similar to *Vibirnum opulus* L. (Demirkol et al., 2018), Indian gooseberry (Rahman et al., 2016) and *Punica granatum* (Abdolahi, 2018), and higher than *Lycium barbarum* L. (Engin, 2019). In addition, the total amount of flavonoids found in the study is higher than the values determined in the study conducted on edible wild fruits (*Hippophae rhamnoides L., Crataegus monogyna* Jacq., *Rubus fruticosus L. Cornus mas* L., *Prunus spinosa* L., *Rosa canina* L., *Prunus padus* L.) (Cosmulescu et al., 2017; Pehlivan et al., 2018).

DPPH• free radical was used to determine the antioxidant activity of *L. iberica* fruit extracts. Phenolic compounds in plants give a hydrogen atom to the DPPH• radical, forming DPPH₂. Thus, they prevent the negative effects of free radicals. DPPH• method for determining free radical scavenging activity is used to determine radical scavenging activities, especially in plant extracts. In this study, the absorbance decrease of DPPH• radical at 517 nm was measured, and the amount of free DPPH• solution remaining, i.e., free radical scavenging activity, was determined. BHT was used as a standard in activity studies. Parallel to the increase in the concentration of hexane and methanol extracts (20-100 μg / mL), an increase in DPPH• radical scavenging activities was observed (figure 4).

DPPH• radical scavenging activities of the extracts and BHT were calculated (Table 3). In the antioxidant analysis, when the DPPH• radical scavenging activities were compared at 100 μ g / mL concentration, it was observed that although the activity of hexane extracts was lower than the standard used, it was higher than the methanol extracts. Jahromi et al. found DPPH• radical scavenging activity at 100 mg/mL in the ethanol extract of *Lonicera nummularifolia* as 12.28% \pm 1.34 (Jahromi, 2020). It is seen that this value is lower than the values we found.

The concentration of extract and standard substance that provides inhibition of 50% of DPPH radical scavenging was determined as IC50. This value was calculated using graph of DPPH• % radical scavenging activity values versus studied concentrations

There is an inverse proportion between the IC₅₀ value and the DPPH radical scavenging activity. Accordingly, the activity ranking is BHT> LIHE> LIME. It was determined that methanol and hexane extracts could not decrease below 50% inhibition value. It is seen that the hexane extract can achieve 50% inhibition at a concentration of 155.54 mg/mL, and the methanol extract at a concentration of 192.09 mg/mL. Dung et al., in their study with L. japonica in 2010, found the best result in flowers as 19.45 ± 2.74 mg/mL. In another study, DPPH• radical scavenging activity in L. japonica was determined as 204.26 ± 1.79 mmol/g (Zhenga et al., 2018). In a study conducted on five different Lonicera flowers, IC50 values were found to range between 235.27 ± 1.21 mg/mL-905.23 \pm 1.02 mg/mL (Zhou et al., 2020). When the DPPH• radical scavenging activity results found in L. iberica fruits are examined, although there was a difference between the species, it was determined that it showed high activity.

Fe³⁺-Fe²⁺ reduction capacity of *L.iberica* fruit extracts and BHT was determined by FRAP method. The antioxidant effect of reducing agents in plants is based on the principle of giving a hydrogen atom and breaking the radical chain. In Fe³⁺-Fe²⁺ reduction capacity measurements, absorbances at 700 nm were determined and the graph was obtained by placing the absorbance values against the concentration (Figure 5).

In this graph, increasing absorbance values indicate the reducing power capacity. Reducing power increased in response to increasing concentration in *L. iberica* hexane and methanol extract. With increasing concentration in methanol extracts, the increase in reducing power was much greater. The reducing power activity ranking is BHT> LIME> LIHE. At a concentration of 50 mg/mL reducing power capacity of methanol extract reached the value in 30 mg/mL of BHT, a synthetic antioxidant.

Conclusion

The results of this study show that *L. iberica* fruits have DPPH radical scavenging and reducing power activity, and can be used as a natural antioxidant source and alternative food supplement. However, due to the lack of previous

research on *L. iberica*, more studies are needed to identify its antioxidant components.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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