Frontiers in Life Sciences and Related Technologies 4(2) (2023) 85-91

Contents lists available at Dergipark

Frontiers in Life Sciences and Related Technologies

Journal homepage: http://dergipark.org.tr/en/pub/flsrt

Research article

Identification of phenolic content, antibacterial and antioxidant activities of *Lonicera caucasica* PALLAS subsp. *orientalis* leaves

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Abstract

Phenolic compounds, which are a prevalent type of secondary metabolite, are responsible for the antioxidant and antimicrobial properties found in the extracts. To contribute new knowledge to scientific literature, it is essential to study the phenolic content and bioactivity of plant parts that have not been previously studied or are unknown. *Lonicera caucasica* PALLAS subsp. *orientalis* is an endemic species that grows in a few cities in Türkiye. The study involved extracting *L. caucasica* leaves using methanol. The resulting fraction was then analyzed using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) to determine the phenolic content of the extract. The quantities of 22 out of the 67 phenolic compounds that were identified were determined. Among these, the top three in terms of quantity are chlorogenic acid, with a concentration of 2.223 ± 0.065 mg g^{-1}, followed by quinic acid at 2.163 ± 0.057 mg g^{-1}, and syringic acid at 1.192 ± 0.036 mg g^{-1}. The antioxidant activities of the extracts were determined using the ABTS•+, DPPH (SC50, mg/mL) and FRAP (µM, TEAC) methods. The values were calculated to be 0.0135 ± 0.0001, 0.0136 ± 0.0001, and 2368.88 ± 2.94, respectively. Furthermore, the extracted substance was subjected to tests against three gram-negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella aerogenes*, and *Escherichia coli*) and three gram-positive bacteria (*Enterococcus faecalis*, *Bacillus megaterium*, and *Streptococcus pyogenes*). The outcomes of the tests were documented. The highest level of antibacterial activity was found to be against *P. aeruginosa*. Based on the findings, it has been determined that the leaves of *L. caucasica* contain a considerable amount of phenolic content, exhibit exceptional antioxidant activity, and display a strong antibacterial effect.

Keywords: *Antimicrobial activity; antioxidant activity; phenolic content; total phenolic content*

1. Introduction

Polyphenols, one of the largest classes of secondary metabolites, include a variety of molecules in the plant kingdom that have at least one aromatic ring replaced by one or more hydroxyl groups (Ferrazzano et al., 2009). Polyphenols have recently attracted the interest of scientists from a variety of disciplines due to the discovery of their extremely advantageous health benefits. Its health benefits are particularly significant in terms of its protective effects against serious diseases such as cardiovascular diseases, diabetes, neurodegenerative diseases, and cancer. Many of these benefits have been partially attributed to polyphenols' antioxidant properties (Petti and Scully, 2009).

Lonicera caucasica PALLAS subsp. *orientalis* (Lam.) Oda. et Long (Caprifoliaceae, MARE 13533) is a member of the Caprifoliaceae family (Dogan and Tuzlaci, 2015). Additionally, *Lonicera* is a genus of the Caprifoliaceae family and includes several endemic species that are classified as being at risk (Iskender et al., 2006). *Lonicera* typically grows in elevated locations over 500-2790 meters, and it is a 15-19 cm tall plant with a 25-35 cm diameter (Palabas et al., 2005; Dogan, 2014; Tunckol et al., 2020). During May and July, it undergoes blooming and is commonly found in both coniferous and deciduous forests, as well as shrub habitats.

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https://doi.org/10.51753/flsrt.1286886 Author contributions
Received 24 April 2023; Accepted 03 June 2023
Available online 26 August 2023

The genus comprises approximately 180 species native to North Africa, Asia, Europe, and North America (Iskender et al., 2006; Tomaszewski et al., 2019). Registered provinces in Türkiye: Trabzon, Giresun, Sivas, Rize, Kayseri, Hatay, Kastamonu, Ankara, Bolu, Amasya, and Tunceli (endemic). Its local name is Ivy (Sarmaşık). It has two locally known uses (Dogan, 2014).

a) The decoction prepared from its leaves is used internally as an expectorant.

b) The decoction prepared from its fruits is used externally against acne.

The information in the literature is very limited and entirely related to the definition of the genre. Other studies on *Lonicera* in the literature are associated with *Lonicera japonica*. This species' various parts were extracted, analysed, and their bioactivities investigated. *L. japonica*'s flowers, leaves, and branches (Caprifoliaceae) Thumb are widely used as traditional teas and medicines in numerous Far Eastern nations. These plant parts have been used to treat various diseases in these regions for centuries. However, studies on the antioxidant activities of even *L. japonica* are still insufficient (Byun et al., 2004a,b). There is no study in the literature regarding the chemical analysis and bioactivity of *L. caucasica*. Giresun Şebinkarahisar region is of great importance in terms of some endemic species. The aim of this study is to obtain more information about the phenolic content, antioxidant activity, total phenolic content, and antimicrobial properties of *L. caucasica*, an endemic species collected from Şebinkarahisar. This is the first study on the chemical composition and biological activity of *L. caucasica* leaves, as no previous research has been conducted on this plant's leaves.

2. Materials and methods

2.1. Chemicals and reagents

Both sodium sulfate and sodium chloride were obtained from Merck in Darmstadt, which is located in Germany. Trolox® and 2,4,6-Tripyridyl-s-Triazine (TPTZ) were provided by Sigma Aldrich in Munich, Germany. HPLC syringe filters made of 0.45 µm polyvinylidene difluoride (PVDF) were provided by ISOLAB (Germany). The standards used in the analysis were all purchased from Sigma Aldrich in Steinheim, Germany. Merck (Darmstadt, Germany) supplied the extraction with HPLC grade methanol.

2.2. Sampling area

Samples of *L. caucasica* PALLAS subsp. *orientalis* plant leaves were collected from the Şebinkarahisar neighborhood in Giresun. Dr. Rena Huseyinoglu was responsible for identifying these species. These plants' leaves were dried in an oven at 40°C for 48 hours.

2.3. Extraction of phenolic compounds

The leaves of *L. caucasica* were extracted using Soxhlet extraction. To do this, each sample was ground into a powder and then put into a Soxhlet cartridge at a mass of 5 grams. The powdered leaves were extracted with 150 mL of methanol. After being filtered, the solvent was evaporated in a rotary evaporator at a temperature of 40°C and a pressure of 175 millibars. Following dissolution in methanol, the samples were kept at a temperature of -20 degrees Celsius for Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) analysis.

2.4. Analysis of phenolic compounds with liquid chromatography-high resolution mass spectrometry

LC-HRMS analyses were performed at Bingöl University (BUMLAM), using a high-resolution MS composite of the DIONEX UltiMate 3000 RS pump, the DIONEX UltiMate 3000 RS autosampler, and the LC system with the DIONEX UltiMate 3000 RS column furnace and Exactive Plus Orbitrap (Thermo Fisher Scientific) with a heated electrospray ionization interface.

The analyses were performed on a Phenomenex® Gemini® 3 µm NX-C18 110 Å (100 mm × 2mm) column. The temperature of the column furnace was maintained at 30°C. For the elution gradient, the mobile A phase was prepared by adding 2% (v/v) glacial acetic acid to ultrapure water obtained through an ultrapure water system (GFL 2004/Human Power 1). For the mobile B phase, LC-MS grade methanol was used that is 99.9% pure from Sigma-Aldrich, Germany. The separation was carried out according to the conditions indicated in Table 1, with the sample injection volume of 20.0 µL and the gradient elution conditions at a flow rate of 0.3 mL/min. The analysis time was set to a total of 20 minutes.

Apigenin standard concentration for phytochemical compounds of the LC-Orbitrap HRMS analysis method 10 - 20 - 40 - 60 - 80 - 100 - 200 - 300 - 400 - 500 µg L^{-1} was prepared and injected three times each.

Table 1
Gradient conditions following elution.

	Retention Time (min.)	Flow Rate (ml/min.)	Mobile Phase A%	Mobile Phase B%
1	0	0.3	100	0
2	0	0.3	100	0
3	2	0.3	100	0
4	13	0.3	2	98
5	15.9	0.3	2	98
6	16	0.3	100	0
7	19	0.3	100	0

2.5. Methodologies for measuring antioxidant activity

The iron (III) reduction/antioxidant strength (FRAP), ABTS, and DPPH radical scavenging methods, which are widely used in the literature, were used to determine the antioxidant activity in plant extracts. In addition, the total phenolic substance determination in the extracts was determined by the Folin-Ciocalteu method. The analyses were carried out in three replications.

2.5.1. Iron (III) reduction / antioxidant power (FRAP) method

Later-developed absorbance measurement of the TPTZ-Fe(II) complex forms the basis of this study's methodology (Benzie and Strain, 1999). A calibration chart with Trolox concentrations from 31.25 to 1000 µM measured all sample activities as micromolar TEAC (Trolox Equivalent Antioxidant Capacity). The FRAP values for all samples were determined to be 5 mg mL^{-1}. An absorbance reading of 595 nm was obtained following a 20-minute incubation at room temperature with 50 µL of plant extract and 1.5 mL of FRAP reagent (acetate buffer,

TPTZ, and $FeCl_3.6H_20$ solutions) (Karacelik et al., 2021a). Each tube's absorbance was evaluated in comparison to distilled water.

2.5.2. ABTS radical scavenging method

The ABTS•+ radical scavenging technique is based on the method modified by Re et al. (1999). In brief, ABTS was dissolved in a mixture of ethanol and water (5:1) to make a 7 mM stock solution of ABTS. To create ABTS radical cation (ABTS+), the stock solution of ABTS was mixed with 2.45 mM potassium persulfate in a 1:3 ethanol/water mixture. The mixture was then left at room temperature and in dark for 16-20 hours. With 0.70 (±0.02) absorption at 734 nm, a 1:40 dilution of 60-40% ethanol-water was used to dilute a ready-to-use blue-green ABTS•+ radical solution. Each sample's and standard's concentration were run three times in parallel. For each concentration of the sample/standard, a blank was also read. At a wavelength of 734 nm, after 20 minutes incubation at room temperature. Plots of concentrations corresponding to the discovered absorbance were made, and SC_{50} values in mg mL^{-1} were computed (Karacelik et al., 2015).

2.5.3. DPPH radical scavenging activity

DPPH was used to test radical scavenging (Cuendet et al., 1997). All samples were pre-tested to determine the working range, and standards (BHT, Troloks) were prepared at various concentrations. Once samples were pipetted into the necessary tubes, a 750 µL solution of 100 µM methanolic DPPH was added and vortexed. The mixture was then incubated for 50 minutes at room temperature. The absorbances were measured at the end of the period at 517 nm, which is where DPPH• had the highest absorbance. The calculations involved averaging the parallels and then subtracting the blind values. SC_{50} values were calculated in mg mL^{-1} by plotting concentrations against the corresponding absorbances. Lower SC_{50} values indicate greater radical scavenging capacity (Turkucar et al., 2021).

2.5.4. Determination of total phenolic content (TPC)

The total phenolic content of *L. caucasica* extracts was quantified using a modified Slinkard and Singleton (1977) method with Folin-Ciocalteu reagent, gallic acid, and catechin as standards. The analytical concentration for each sample was 5 mg mL^{-1}. To a final volume of 50µL, deionized water (2.5 mL) was added to the sample solution, followed by 250 µL of 0.2 N Folin-Ciocalteu reagent. The mixture was vortexed once more and 750 µL of Na_2CO_3 (7.5%) was added after 3 minutes of incubation and vortexing. In addition to this, each sample as well as the standard concentration had blank samples and reagents analyzed. The absorbance at 765 nm was measured following an incubation period of two hours at room temperature. The total phenolic content is given in gram gallic acid equivalents per mL of sample. This is done by drawing separate calibration graphs for gallic acid and catechin standards in the range of 15.63-1000 g mL^{-1} (Karacelik et al., 2021b).

2.6. Determination of antibacterial activity of the extracts

L. caucasica subsp. *orientalis* was tested for three Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC27853), *Enterobacter aerogenes* (ATCC 3048), and *Escherichia coli* (ATCC 25922). Also, *L. caucasica* was tested on three Gram-positive bacteria: *Enterococcus faecalis* (ATCC 29212), *Bacillus megaterium* (ATCC 6633), and *Streptococcus pyogenes* (ATCC 19615). The bacteria were supplied by the Molecular Biology and Genetics Laboratory at Erzurum Technical University. The bacteria were aerobically grown on Mueller Hinton Agar (MHA, Merck 1.05437) at 37°C. Disc diffusion and minimum dilution techniques were used to measure antimicrobial activity and minimum inhibitory concentrations (MIC) utilizing the technique described by Seker et al. (2021). The extract was diluted to a concentration of 39-4.8 mg mL^{-1} with 20% DMSO. Ofloxacin (10 g $disc^{-1}$), netilmicin (30 g $disc^{-1}$), and sulbactam (SCF) were positive and were used as positive controls for disc diffusion test and Maxipim (Bristol-Myers Squibb, New York, NY, USA) at concentration of 7.81 to 500 g μL^{-1} were used as positive controls for minimum dilution method. 10 µL of 20% DMSO was the negative control for both tests. MIC values were checked by the minimum dilution test. All of the experiments were conducted for three times.

3. Results and discussion

The leaves of *L. caucasica* were screened for eighty-two phenolic compounds, and the presence of fifty-six of them was determined. All substances sought and identified are listed in Table 2. Table 3 lists the determined concentrations of seventeen of these compounds.

Table 2
Phenolic compounds, retention times and mass load ratios used in the determination of phenolic compounds.

Phenolic Compounds	RT (min.)	Quan Peak
Benzoic acid	6.86	121.02950 mz
4-Hydroxybenzoic acid	6.21	137.02442 mz
Salicylic acid	9.95	137.02442 mz
3-hydroxybenzoic acid (3-HBA)	7.17	137.02442 mz
3-hydroxyphenylacetic acid (3-HPA)	**<LOD**	107.05053 mz
Syringic acid	7.78	197.04555 mz
Gallic acid	0.78	169.01425 mz
Protocatechuic acid	4.30	153.01933 mz
Protocatechuic acid ethyl ester	9.19	181.05063 mz
Protocatechuic aldehyde	5.66	137.02442 mz
2,4-dihydroxybenzoic acid (beta-Resorcylic acid)	7.33	153.01933 mz
Vanillic acid	7.30	167.03498 mz
Homovanillic acid	7.11	181.05063 mz
Vanillin	7.66	151.04007 mz
Gentisic acid	6.43	153.01933 mz
3,4-Dihydroxyphenylacetic acid (DOPAC, Homoprotocatechuic acid)	7.3	167.03498 mz
trans Cinnamic acid	10.33	147.04515 mz
Coumaric acid (trans-3-Hydroxycinnamic acid)	8.29	163.04007 mz
Caffeic acid	7.24	179.03498 mz
Caffeic acid phenhyl ester (CAPE)	12.08	283.09758 mz
Ferulic acid	8.54	193.05063 mz
Sinapic acid	8.69	223.06120 mz
Chlorogenic acid	7.07	353.08781 mz
Quinic acid	1.14	191.05611 mz
3-(4-Hydroxyphenyl) propionic acid	**<LOD**	165.05572 mz

α-Cyano-4-hydroxycinnamic acid	9.23	188.03532 mz
Catechin (Cianidanol)-p	**<LOD**	289.07176 mz
Epigallocatechin	**<LOD**	305.06668 mz
Epigallocatechin gallate	**<LOD**	457.07763 mz
Chrysin	12.25	253.05063 mz
Apigenin	11.39	269.04555 mz
Acacetin	12.48	283.06120 mz
Rhoifolin (Apigenin 7-O-neohesperidoside)	9.49	577.15692 mz
Vicenin 2	**<LOD**	593.15119 mz
Apigenin 7-glucuronide	10.42	445.07763 mz
Apigenin 7-glucoside	9.64	431.09837 mz
Genkwanin (4',5-Dihydroxy-7-metthoxyflavone, Apigenin 7-O-methyl ether)	12.48	283.06120 mz
Apiin (Apigenin-7-(2-O-apiosylglucoside)	9.38	563.14075 mz
Vitexin (Apigenin 8-C-glucoside)	**<LOD**	431.09837 mz
Schaftoside	8.40	563.14063 mz
Rutin trihydrate M-3H2O	9.24	609.14611 mz
Rutin hydrate M-OH2	9.24	609.14611 mz
Luteolin	10.85	285.04046 mz
Luteolin-7-O-glucuronide	9.80	461.07255 mz
Diosmetin (Luteolin 4′-methyl ether)	11.4	299.05611 mz
Orientin	**<LOD**	447.09328 mz
Isoorientin	**<LOD**	447.09328 mz
Luteoloside (Luteolin 7-glucoside)	9.03	449.10784 mz
Luteolin 7-rutinoside	9.03	595.16364 mz
Galangin	12.41	269.04555 mz
Quercetin	10.54	301.03538 mz
Isoquercitrin (Quercetin 3-glucoside)	9.30	463.08820 mz
Narcissin (Narcissoside, Isorhamnetin 3-rutinoside)	9.85	623.16176mz
Quercetin 3-rutinoside 7-glucoside	**<LOD**	771.20374 mz
Isorhamnetin (Quercetin 3′-methyl ether)	11.33	315.05103 mz
Hyperoside (Quercetin 3-D-galactoside)	9.30	463.08820 mz
Kaempferol	10.32	287.05350 mz
Afzelin (Kaempferol 3-rhamnoside)	10.40	431.09837 mz
Kaempferide	**<LOD**	299.05611 mz
Kaempferitrin	9.50	579.17083 mz
Nicotiflorin (Kaempferol 3-rutinoside, Kaempferol 3-O-β –rutinoside)	9.78	593.15106mz
Astragalin (Kaempferol 3-glucoside)	9.81	447.09328 mz
Tiliroside	10.67	595.14264 mz
Leucoside (Kaempferol 3-sambubioside)	9.84	289.06924 mz
Myricetin	**<LOD**	319.04291 mz
Fisetin hydrate	9.80	285.04046 mz
Naringin	**<LOD**	609.18390mz
Naringenin	10.13	271.06120 mz
Sakuranetin (Naringenin 7-O-methyl ether)	11.91	285.07685 mz
Narirutin (Narirutinsa, Naringenin rutinoside)	**<LOD**	579.17193 mz
Taxifolin	**<LOD**	305.06558 mz
Taxifolin M+3H	**<LOD**	308.09012 mz
Hesperidin	**<LOD**	609.18249 mz
Neohesperidin	**<LOD**	609.18249 mz
Eriodictyol (3,4,5,7-Tetrahydroxyflavanone)	**<LOD**	287.05501 mz
Eriocitrin	8.33	595.16684 mz
Liquiritigenin	9.88	255.06628 mz
Liquiritin (4′,7-Dihydroxyflavanone 4′-glucoside)	**<LOD**	417.11911 mz
Genistein	10.95	269.04555 mz
Daidzin	8.24	255.06433 mz
Formononetin (Neochanin)	11.47	267.06628 mz
Kuromanine (Cyanidin 3-glucoside chloride)	9.16	447.09328 mz
Ellagic acid	9.49	300.99899 mz
Esculin hydrate	6.24	339.07216 mz
Phloridzin	9.40	435.12967 mz
Rosmarinic acid	**<LOD**	359.07724 mz
Glabridin	**<LOD**	323.12888 mz
Arbutin	**<LOD**	271.08233 mz
Emodin	13.48	269.04555 mz
Etoposide	**<LOD**	383.11053 mz
Procyanidin B2	**<LOD**	577.13515 mz
Doxorubicin Hydrchloride	**<LOD**	544.18134 mz
Ethylgallate	7.78	197.04555 mz

RT: Retention Time, mz: mass divided by charge number, LOD: Limit of Dedection

Table 3
The amount of some phenolic compounds in the leaves of *L. caucasica* and the detection limits of the instrument.

Phenolic Compound	Detected amount of dry extract (mg g^{-1})	LOD (µg kg^{-1})	LOQ (µg kg^{-1})
Gallic Acid	0.011 ± 0.0003	0.04	0.13
Benzoic acid	0.468 ± 0.014	0.7	2.7
Syringic Acid	1.192 ± 0.036	0.6	1.8
Protocatechuic Acid	0.072 ± 0.0020	0.3	0.9
Protocatechuic Aldehyde	0.029 ± 0.0012	1.5	5.1
Sesamol	0.009 ± 0.0003	0.5	1.6
Vannilin	0.003 ± 0.0001	1.0	3.3
p_coumaric acid	0.013 ± 0.0003	0.3	0.9
Caffeic Acid	0.044 ± 0.0013	0.2	0.6
Luteolin	0.088 ± 0.0024	1.3	4.4
Chlorogenic acid	2.223 ± 0.065	1.2	3.9
Quinic acid	2.163 ± 0.057	0.3	0.9
Rutin	0.041 ± 0.001	0.5	1.7
Luteoloside	0.440 ± 0.011	4.4	14.7
Luteolin 7-rutinoside	0.315 ± 0.010	0.5	1.6
Kaempferitrin	0.160 ± 0.005	0.7	2.2
Apigenin	0.019±0.0004	0.9	2.9
Rhoifolin	0.026±0.0005	3.8	12.7
Apigenin 7-glucoside	0.074±0.0013	0.5	1.5
Tiliroside	0.037±0.0008	0.5	1.6
Hyperoside (Quercetin 3-D-galactoside)	0.060±0.0012	0.2	0.8
Kuromanine (Cyanidin 3-glucoside chloride)	0.874 ± 0.019	0.5	1.6

LOQ: Limit of Quantitation

When the results are examined, benzoic acid (0,468 ± 0,014), syringic acid (1.192 ± 0,036), chlorogenic acid (2,223 ± 0,065), quinic acid (2,163 ± 0,057), luteoloside (0,440 ± 0,011),

luteolin 7-rutinoside (0,315 ± 0,010) and kuromanine (0,874 ± 0,019) amounts are higher than the other phenolic compounds found in the leaves. Since the results are presented in milligrams per gram, they are usually considered high. The extract contains significant amounts of chlorogenic acid and quinic acid.

Studies in the literature are generally related to different species such as *L. japonica* Thunb., *Lonicera maackii* and *Lonicera nummularifolia*. In the study conducted with *L. nummularifolia* in Iran, the total phenolic, antioxidant and antibacterial activities of this species were examined, but detailed chemical analyses were not performed. (Farboodniay Jahromi et al., 2020). In a study performed in Ohio, United States, the chemical content of *L. maackii* leaves was investigated, and the presence of quinic acid, coumaric acid hexoside, chlorogenic acid, eriodictyol hexoside, luteolin diglycoside, luteolin-7-glucoside, apigenin-7-glucoside, luteolin, and apigenin was detected. Among them, the amount of chlorogenic acid was determined as 0.737 mg g^{-1}, Apigenin as 0.109 mg g^{-1}, Apigenin-7-Glucoside as 0.652 mg g^{-1}, Luteolin as 1.044 mg g^{-1} and Luteolin-7-Glucoside as 7.036 mg g^{-1}. In this study, it was found that Luteoloside (also known as Luteolin 7-glucoside) has a value of 0.440 ± 0.01, and Luteolin has a value of 0.088 ± 0.0024 mg g^{-1}. These values are lower compared to those obtained from *L. maackii*. The amount of Luteolin discovered is considerably lower when compared to *L. maackii*. Interestingly, the leaves of *L. caucasica* contain a significantly higher amount of chlorogenic acid (2,223 ± 0.065 mg g^{-1}) compared to the leaves of *L. maackii.* Research conducted in the United States detected the presence of quinic acid, but the concentration was not determined (Cipollini et al., 2008). However, further research indicates that the value is 2,163 ± 0.057 mg g^{-1}.

In a research project that was carried out in Korea using the leaves of *L. japonica*, the presence of 25 different types of phenolic compounds was discovered (Seo et al., 2012). The derivatives of caffeoylquinic acid appear to be more prevalent than the other phenolics. No common phenolic compounds were analyzed for comparison between the two studies. Recently, research has been conducted on the potential benefits of using *L. japonica* Thunb, a plant from the Caprifoliaceae family, as a supplement to enhance the antioxidant power of cookies. This is due to the plant's high polyphenolic content, as reported by Cao et al. (2022). Phenolic content was investigated in the berries of some *L. caerulea* cultivars collected from Canada (Yemis et al., 2022). Based on the findings, the concentration of chlorogenic acid ranged from 2.77-3.07 mg per gram, while routine was detected between 1.51-1.96 mg per gram. Quinic acid was also present at a concentration of 9.7-13 mg per gram. From these results, the levels of routine and quinic acid are higher than our previous findings, but the amount of chlorogenic acid is almost identical to what was discovered before. However, it should be noted that this study was conducted on a different species and fruit of *Lonicera*. A recent study by Orsavová et al. (2022) examined the presence of phenolic compounds in the culture fruits of *L. caerulea* L. var. *kamtschatica* collected from various regions. Based on the findings, the levels of gallic acid fall within the range of 0.011-0.038 mg g^{-1}, rutin falls within 0.02-0.150 mg g^{-1}, syringic acid falls within 0.001-0.009 mg g^{-1}, protocatechuic acid falls within 0.022-0.196 mg g^{-1}, caffeic acid falls within 0.022-0.226 mg g^{-1}, and chlorogenic acid falls within 2.12-4.77 mg g^{-1}.Since there is no study to compare the content of L. *caucasica* leaves, a comparison can be made with the leaf of a different species with high phenolic content collected from the Şebinkarahisar region. Comparing the phenolic compounds found in the leaves of *L. caucasica* with those found in the leaves of *Geranium ibericum* subsp. *jubatum*, another endemic species collected around Şebinkarahisar, it is notable that *Geranium* has higher levels of gallic, protocatechuic, and ellagic acid (Seker et al., 2021). On the contrary, *Lonicera* leaves have higher amounts of chlorogenic acid, coumaric acid, and rutin.

Aside from analyzing the chemicals found in *L. caucasica* leaf extracts, its TPC and AA content was also evaluated. The results are presented in Table 4. The results show that *L. caucasica* leaf extracts have strong antioxidant activity. Based on the results, the ABTS•+ analysis of the leaf extract displayed lower antioxidant effects when compared to Trolox and BHT. However, DPPH• results showed different results. Particularly, DPPH• tests demonstrated that leaf extracts possess greater antioxidant activity than BHT. ABTS•+ analysis results also indicate that leaf extracts have good antioxidant activity, albeit less than the antioxidant standards BHT and Trooloks. These outcomes were roughly 4.3 times stronger than the antioxidant

Table 4
The antioxidant activity and total phenolic content of a phenolic mixture isolated from *L. caucasica* leaves.

Sample	ABTS•+ (SC_{50}, mg/mL)	DPPH• (SC_{50}, mg/mL)	FRAP (µM, TEAC)	Total phenolic content (TPC) (GAE, µg/mL)
L. caucasica leaves	0.0135±0.0001	0.0136±0.0001	2368.88±2.94	808.34±4.40
BHT	0.0005±0.0001	0.0146±0.0004	ND	ND
Troloks	0.0032± 0.0002	0.0029±0.0001	ND	ND

N.D.: not detected.
Triple measurements are represented as means ±SD (standard deviation).

Table 5
The inhibition effects of methanol extract of *L. caucasica* subsp. *orientalis* on various bacteria.

Bacterium	39.0 mg mL^{-1}	19.5 mg mL^{-1}	9.75 mg mL^{-1}	4.8 mg mL^{-1}	NET 30 µg $disc^{-1}$	MIC **Con/*DD	DMSO (%10)
P. aeruginosa	19.33±0.33^{a}	10±1.52^{b}	8.33±0.33^{b}	-	17.3±0.5^{b}	**9.75**	-
E. aerogenes	8.33±0.33^{a}	7.33±0.33^{a}	9.66±0.33^{b}	8.33±0.33^{a}	20.6±0.5^{b}	**4.8**	-
E. coli	8.66±0.33^{a}	18.66±0.33^{d}	17±0.57^{c}	11.66±0.33^{b}	18.2±^{b}1	**4.8**	-
E. faecalis	13±0.57^{b}	16±0.57^{c}	13±0.57^{b}	11±0.57^{a}	19.3±0.5^{b}	**4.8**	-
B. megaterium	6.33±0.33^{a}	8.66±0.33^{b}	6.66±0.33^{a}	-	15.4±0.5^{b}	**9.75**	-
S. pyogenes	-	8.66±0.33^{a}	8.66±0.33^{b}	-	18 ±0.5^{b}	**9.75**	-

Duncan's multiple range test shows that, at the *p* 0.05 significance level, the means of each line that ends with the same letter are not significantly different from each other.
DD, Diameter of the inhibition zone (mm/sensitive strains), **Con: Concentration

activity of *L. japonica* leaves (Dung et al., 2011).

According to the results, the methanol extract of *L. caucasica* has shown antibacterial effects against both Gram-positive and Gram-negative bacteria. The most antibacterial activity was observed against *P. aeruginosa* and the least antibacterial activity was observed against *B. megaterium* and *S. pyogenes*. Although there was a decrease in the zones due to the decreasing dose for *P. aeruginosa*, this effect was not observed in the other bacteria that were tested. In contrast, dose-independent zones were observed for all other bacteria. It was determined that the extracts caused significant statistical differences compared to the positive control of the netilmicin antibiotic. Netilmicin was chosen because of its inhibitory activity against both Gram-positive and Gram-negative bacteria. Only significant differences were detected between all doses against *E. coli*. According to the obtained results, it can be concluded that the Gram-negative bacteria were more sensitive than the Gram-negative bacteria against the methanol extract of *L. caucasica* subsp. *orientalis*. The results are given in Table 5. There are just a few studies about the various parts of honeysuckle. According to the literature, the plant is traditionally used for colds, stomach problems (Sakinoglu-Oruc and Oruc, 2017), kidney pains (Mehrnia et al., 2021), and as a disinfectant or wound-washing solution (Asaadi, 2021). Examining the literature, it was discovered that the number of studies on this species was limited, and the majority of the existing studies were the plant's taxonomic, systematic, and ethnobotanical studies. This is the first antibacterial record of *L. caucasica* subsp. *orientalis.* Compared to the ethanol extract of *L. nummularifolia* leaves, the antibacterial effects of the methanol extract of *L. caucasica* leaves appear to be nearly identical (Farboodniay Jahromi et al., 2020). The antibacterial activity of the plant could be associated with its chemical compounds with have various biological activities.

4. Conclusion

In this investigation, chemical analyses of *L. caucasica* leaves were performed for the first time. Phenolic content, total phenolic content amounts, antioxidant and antibacterial effects were measured. Consequently, 67 phenolic compounds were identified in the leaf extracts obtained via methanolic extraction, and the amounts of 22 of these compounds were determined. It has been determined that the extract has strong antibacterial and antioxidant properties. Considering that this plant is used to treat a number of human diseases, it is possible that these treatments result from the leaves' phenolic compounds, antioxidant properties, and bacteria-killing ability. Further exploration of this plant, which can be found in multiple cities across Türkiye, can enhance the general comprehension of the plant and provide scientists with insights into the regional diversity of its chemical composition.

Acknowledgment: This study was supported in 2022 by Giresun University's Commission for Scientific Research Projects, in accordance with the project number FEN-BAP-A-240222-44.

Conflict of interest: The author declares that he has no conflict of interests.

Informed consent: The author declares that this manuscript did not involve human or animal participants and informed consent was not collected.

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Cite as: Seker, M. E. (2023). Identification of phenolic content, antibacterial and antioxidant activities of *Lonicera caucasica* PALLAS subsp. *orientalis* leaves. *Front Life Sci RT*, *4*(2), 85-91.