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ORIGINAL PAPER

Phytochemistry of *Lotus corniculatus* flowers: a new and efficient method for determination of bioactive compounds by LC–ESI–MS/MS and a novel potentiometric PVC membrane sensor for the evaluation of antioxidant activity

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Abstract
Natural products play an essential role in food and pharmaceuticals. Quantification of bioactive compounds in plants is essential for the drug development process. Moreover, the presentation of the antioxidant effects of plants gains great interest for their therapeutic effect. A new and efficient technique was advanced for the determination of bioactive compounds in the methanol extract of *Lotus corniculatus* flowers (MLCF) by Liquid chromatography electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS). A potentiometric polyvinyl chloride (PVC) membrane sensor was progressed for the evaluation of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and total phenolic content of MLCF. Isoquercitrin (200.8 µg/g extract), quercetin-3-*D*-xyloside (189.1), kaempferol-3-glucoside (35.9), coumarin (16.3), salicylic acid (5.3), quercetin (2.51), fisetin (0.81) were detected as major products among the 39 standards. DPPH free radical scavenging effect and total phenolic content of MLCF were determined by potentiometric PVC membrane sensor, and MLCF inhibited the DPPH$^{\bullet}$ with a value of 71.4%. In addition, the total phenolic content was determined to be 228.6 ± 0.05 mg gallic acid eq/g extract. *Lotus corniculatus* leaves could be a significant source of isoquercitrin, and this plant can be accepted as a good antioxidant agent.

Keywords *Lotus corniculatus* · Quantitative analysis · Sensory · Antioxidant activity

Introduction

Plants have been utilized in folk medicine besides food since ancient times (Elmastas et al. 2004; Demirtas et al. 2013). Due to including bioactive compounds, plants exhibit critical biological activities. Hence, they are used in the food and drug development process (Sahin Yaglioglu et al. 2013). After advances in spectroscopy in the nineteenth century, plants began to attract scientific attention for the isolation and identification of secondary metabolites such as terpenoids, phenolics, alkaloids, polyketides, and carbohydrates (Aksit et al. 2014; Bayir et al. 2014; Kaya et al. 2014). Numerous compounds have been isolated and identified from plants that exhibit important biological activities that enable them to be used in the formulation of drugs (Aydin et al. 2016; Elmastas et al. 2016). The isolated compounds have been used as a standard in HPLC or LC–ESI–MS/MS to execute the quantitative analysis. This is a notable method for the determination of the phytochemistry of the plants. Therefore, important information can be obtained about the usage areas of the plant whose phytochemistry is determined (Guzel et al. 2017; Karan and Erenler 2017; Erenler et al. 2018).

Lotus corniculatus, belonging to the Fabaceae family, grows in Eurasia and North Africa. Phytochemical investigation of this plant revealed the identification of flavonoids (kaempferol and quercetin), saponins, fatty acids, and proanthocyanidins. *Lotus corniculatus* L. is a drought-tolerant plant that tolerates low soil fertility. The scientific

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studies reported that kaempferol and quercetin modulate the inducible nitric oxide synthase, cyclooxygenase-2, reactive C-protein, and nuclear factor kappa B. Moreover, the compounds found in this plant revealed anti-inflammatory and antitumoral properties (Koelzer et al. 2009).

Free radicals are highly reactive compounds that have one or more unpaired electrons in their outermost shell (Elmastas et al. 2018). These unpaired electrons make free radicals unstable and highly reactive, which can cause damage to cells and tissues in the body. Free radicals can be produced naturally in the body during metabolic processes, such as cellular respiration and immune system function (Dede et al. 2019). They can also be produced by exposure to environmental toxins, such as cigarette smoke, air pollution, and radiation (Erenler et al. 2019). Free radicals can cause damage to cells and tissues by reacting with proteins, lipids, and DNA. This damage can lead to various health problems, including aging, cancer, and Alzheimer's disease (Genç et al. 2019). Antioxidants can help neutralize free radicals by donating electrons to them, thereby preventing them from reacting with other molecules in the body. A diet rich in fruits, vegetables, beverages, and foods can protect against the harmful effects of free radicals (Koysu et al. 2019; Djermane et al. 2020). Therefore, it is outstanding to carry out antioxidant tests to clarify the natural products that highly exhibit antioxidant properties. One of the most important methods for determining of the antioxidant activity is the DPPH test. The spectroscopic method such as UV–Vis spectroscopy is extensively employed for the evaluation of antioxidant assay (Gecer et al. 2021). The discovery of a new and effective method for determining antioxidant activity is inevitable because the conventional method is costly, time consuming, requires pre-treatment and uses expensive equipment. The potentiometric method developed by our research group (Isildak et al. 2022) is a new and effective that overcomes the mentioned problems. Potentiometric sensors, on the other hand, have many advantages in measurements, including applicability in heterogeneous solutions, simple design, inertness, and robustness, integration into computer systems, fast response, and selectivity.

Herein, bioactive compounds in *Lotus corniculatus* flower extract were quantified by LC–ESI–MS/MS. Moreover, the DPPH antioxidant activity and total phenolic content of this extract were determined by a potentiometric PVC membrane sensor.

Materials and methods

Chemicals

All chemicals were analytically pure and used without further purification. DPPH radical (purity 99.9%), gallic acid (99.8%), dibutyl phthalate (DBP), sodium tetraphenyl borate (NaTPB) (99.5%), bis (2-ethylhexyl) adipate (DEHA) (99.0%), polyvinyl chloride (PVC) (99.5%), tetrahydrofuran (THF) (99.8), and powdered graphite (99.8%) were purchased from Merck KGaA, Darmstadt, Germany.

Plant materials

Lotus corniculatus was collected from the natural environment in June 2022 at Igdir University Campus (39.81061°N, 44.07802°E). The plant material was dried in the shade for 10 days, and the flowers were separated for analysis. The plant material was identified by Murat Unal, Ph.D., Van Yuzuncu Yil University, Van, Turkey. A voucher specimen was deposited in the university's herbarium (VANF 20313).

Potential measurement

Potential measurements of the electrodes were accomplished using a potentiometric system (±0.1 mV sensitivity), including a computer controller and high-impedance multichannel. The measurements were achieved against an Ag/AgCl reference electrode (Thermo-Orion) containing 3.0 M KCl aqueous solution saturated with AgCl.

Apparatus

A computer-controlled 4-channel with high input impedance (1.0 Megaohm–10.0 Teraohm) (Medisen Company) was employed for potentiometric measurements. Mettler-Toledo U402-S7/120 (10 402 3311) with a glass pH electrode was used for pH measurements. The Ag/AgCl electrode (Thermo-Orion) was exhausted as the reference electrode. A RADWAG (Model AS 310/X) analytical balance was used for quantity weight.

Preparation of the DPPH$^{\bullet}$-selective PVC membrane biosensor and the FCR-selective PVC membrane biosensor

The development of biosensors was executed in two stages. In the first point, the solid contacts of the sensor were prepared according to the method in our previous study (Isildak et al. 2022). Solid contacts were obtained by coating the ends of copper wires (a diameter of 1.0 mm and length of 10–15 cm) with a certain amount of graphite, epoxy, and hardener. The mixture was prepared by adding 3 mL of THF, graphite (50%, w/w), epoxy (35%, w/w), and hardener (15%, w/w). Later, copper wires were dipped 3–4 times into the solid-contact mixture that reached the appropriate viscosity and coated. After the end surfaces of the copper wires were covered with solid-contact, they were kept in the dark for 24 h to dry. The second step was to prepare the membrane

surface. For this purpose, gallic acid, PVC, and plasticizer were prepared in a watch glass. The mixture was homogenized with 1.0 ml of THF. The homogeneous mixture was then conditioned at rt for 4.0 h. DPPH selective and FCR-selective PVC membrane biosensors were prepared by dipping the surface of the solid contacts prepared the day before into the membrane mixture. These biosensors were dried in the dark and the activities of the plant extracts were measured. The schematic representation of the prepared biosensors and measurements are given below (Fig. 1).

Application of DPPH-selective PVC membrane biosensor to plant extract

DPPH• radical scavenging activity (RSA), which is not quenched in the environment due to the interaction of antioxidant species with the electrodes for which we prepared biosensors, was measured and the DPPH radical scavenging activity was calculated. For this purpose, 10 mL of 500 mg L^{-1} plant extract was taken and 10 mL of 100 µg mL^{-1} DPPH• solution was added to this solution. DPPH-SPMB was directly immersed in these samples and the potential values were measured. Gallic acid was prepared as a standard at different concentrations and subjected to the same procedures. This procedure was repeated 3.0 times for each sample. The DPPH radical scavenging activity of the

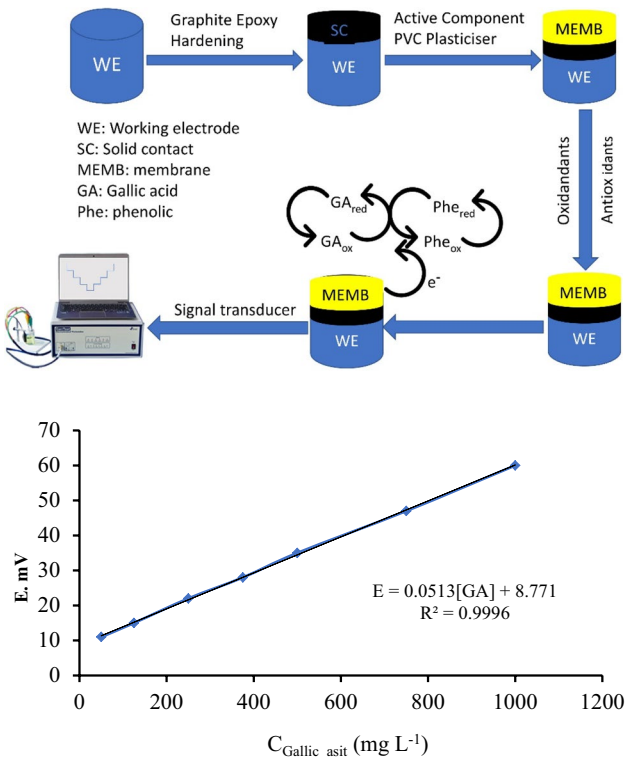


Fig. 1 The measurement scheme of antioxidant activity and Folin Ciocalteu-Gallic acid calibration curve

plant sample was calculated using the following equation invented by our research group (Isildak et al. 2022).

$$\%\mathrm{RSA} = \frac{[(E1 - E0) - (E2 - E0)]}{E1 - E0} \times 100 \quad (1.1)$$

In which E_0 is the potential value of the plant sample. E_1 is the potential value of the standard DPPH solution and E_2 is the potential value of the DPPH• activity remaining in the medium after 30 min reaction.

Application of FCR-selective PVC membrane biosensor to plant extract

In the total phenolic test, a GA-FCR calibration curve was established to determine the gallic acid equivalent corresponding to the amount of FCR reduced by the antioxidant species. GA solutions were prepared at concentrations of 1.0–0.75–0.50–0.375–0.375–0.250–0.125–0.050–0.025 mg mL^{-1} from the stock GA solution. The potentiometric responses of these FCR solutions were measured and potential values were obtained. To determine the total phenolic content of the plant extracts using the calibration graph, plant solutions were prepared in volumes of 0.25 mg mL^{-1} and 50.0 mL. 5.0 mL of each of the prepared *Lotus Corniculatus* solutions were taken, 40.0 mL deionized water and 5.0 mL 0.5 mmol L^{-1} FCR solution were added and vortexed. After the reduction reaction, the potential values of the plant solutions were measured. The total phenolic content of the plant was calculated as GA equivalent (FCR-GAE) by substituting the measured potential values into the line equation obtained from the GA-FCR calibration curve. The total amount of phenolic compounds was calculated as FCR-gallic acid equivalent (FCR-GAE) using the line equation ($E = 0.0513$ [GA] + 8.771) $R^2 = 0.9996$ obtained from the FCR-gallic acid calibration graph (Fig. 2).

Extraction

Lotus Corniculatus (5.0 g) was macerated with methanol (50 mL) at room temperature for 24 h. After removing of solvent under reduced pressure, the crude extract was acquired (0.4 g).

Quantification of compounds by LC–ESI–MS/MS

Lotus Corniculatus flower extract (50 mg) was discovered in methanol (1.0 mL) in Eppendorf and hexane (1.0 mL) was added. After the mixing in the ultrasonic bath, the solution was centrifuged at 9000 rpm for 10 min. A 100 µL aliquot of the methanol phase was taken and diluted with 450 µL each of water and methanol. The resulting mixture was filtered (0.45 µm) and then transferred to the vial. The quantification

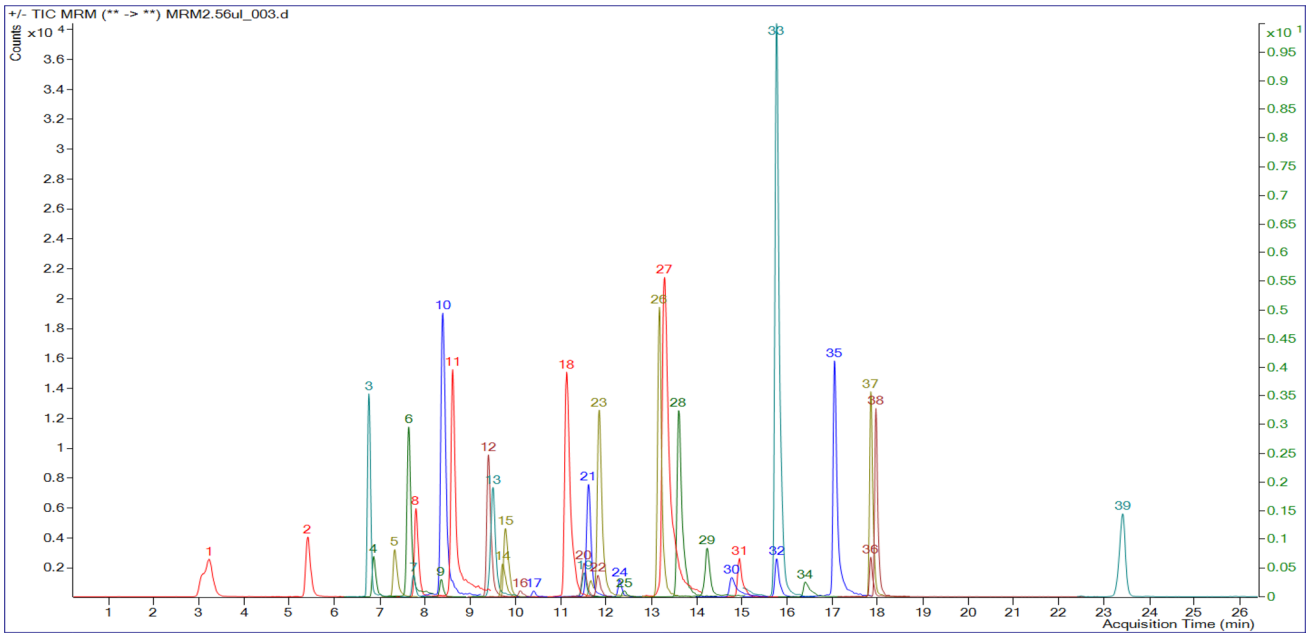


Fig. 2 The MRM chromatogram of 39 standard compounds by using LC–MS/MS (1-Gallic acid, 2-Protocatechuic acid, 3-Epigallocatechin, 4-Catechin, 5-Chlorogenic acid, 6–*4*-Hydroxybenzaldehyde, 7-Vanillic acid, 8-Caffeic acid, 9-Syringic acid, 10-Caffeine, 11-Vanillin, 12-*p*-Coumaric acid, 13-Salicylic acid, 14-Taxifolin, 15-Resveratrol, 16-*trans*-ferulic acid, 17-Sinapic acid, 18-Scutellarin, 19-*o*-Coumaric acid, 20-Coumarin,, 21-Protocatechuic ethyl ester, 22-Rutin, 23-Isoquercitrin, 24-Hesperidin, 25-Quercetin-*3*-*D*-xyloside, 26-Kaempferol-*3*-glucoside, 27-Fisetin, 28-Baicalin, 29-*trans*-Cinnamic acid, 30-Quercetin, 31-Naringenin, 32-Hesperetin 33-Morin, 34-Kaempferol, 35-Baicalein, 36-Luteolin, 37-Biochanin A, 38-Chrysin, 39-Diosgenin)

of natural compounds in methanol extract of *L. Corniculatus* flowers was carried out using LC–ESI–MS/MS (An Agilent Technologies 1260 Infinity II, jointed 6460 Triple Quad mass spectrometer). The reverse phase column was employed (Poroshell 120 EC-C18, 4.6 × 150 mm 2.7 µm). An Electrospray ionization (ESI) source was employed with positive and negative ionization modes to detect the mass-to-ion ration (m/z) of the compounds. The column temperature was set at 40 °C and injection volume was kept at 4.0 µL. The mobile phase was formed as water including formic acid (0.1%), ammonium formate (5.0 mM) A, and methanol consisting of formic acid (0.1%), and ammonium formate (5.0 mM) B. The gradient program was adjusted as 75% for 0–3 min, 50% for 4–12 min, 10% for 13–16 min, and 10% for 17–21 min, 22–24 min 97.5 was applied in the A mobile phase. The flow rate was adjusted to 0.4 mL/min, the nebulizing gas (N_2) flow was 11 L/min, the voltage of the capillary was 4000 V and the gas temperature was 300 °C. Both precursor and product ions, cleavage voltage, and collision energies of each compound were detected for quantitative analysis as multiple reaction monitoring.

Statistical analysis

GraphPad Prism (8.0.1) was employed for statistical analysis. One-way ANOVA with Tukey's multiple comparison test was carried out for comparison of groups. The results were indicated as mean ± standard deviation (SDs) of three independent assays. The statistical significance level was accepted at $p < 0.05$.

Results and discussion

Antioxidant activity

DPPH radical scavenging activity was measured by a novel potentiometric PVC membrane sensor (Table 1). Trolox and Gallic acid were used as standards. Hence, it was determined that the total phenolic and DPPH radical scavenging activity

Table 1 Antioxidant activity

Sample	DPPH$^{\bullet}$ scavenging effect (%)	Total phenolic mg GA/g plant extract)
Lotus corniculatus	71.43 ± 0.1^{c}	228.635 ± 0.05^{a}
25 ppm Gallic Acid	92.44 ± 0.1^{e}	555.625 ± 0.05^{c}
12.5 ppm Gallic Acid	81.43 ± 0.07^{d}	325.625 ± 0.05^{b}
10 ppm Trolox	51.35 ± 0.08^{b}	–
5.0 ppm Trolox	18.47 ± 0.78^{a}	–

GA: Gallic acid. Different letters indicate the significantly different

was coherent. Previous studies on the antioxidant activity of natural products have revealed that high total phenolic content exhibits a high antioxidant activity (Erenler et al. 2014; Elmastaş et al. 2015). DPPH activity and Total phenolic content, respectively. *L. corniculatus* was found to have excellent antioxidant activity with values of 71.43 ± 0.1%) in comparison to Trolox 5.0 ppm (18.47 ± 0.78%), Trolox 10.0 ppm (51.35 ± 0.08%). In total phenolic content analysis, *L. corniculatus* was determined to include total phenolic with the value of 228.64 ± 0.05 (mg GA/g plant extract) (Table 1).

Quantitative analysis of bioactive compounds

A new method was developed to determine the quantitative analysis of bioactive compounds in the methanol extract of *L. corniculatus* using LC–ESI–MS/MS analysis (Fig. 3). Retention time (RT), Ion transition (m/z), Ion mode, coefficient of determination (R^2), Limit of quantification (LOQ), Limit of detection (LOD), Collision energy (CE), Range (µg/L) and Precision (%CV) were shown. (Table 2, 3).

Isoquercitrin (200.8 µg/g extract) was found to be a major compound. Quercetin-3-*D*-xyloside (189.1 µg/g extract) was measured as a second chief product. In addition, kaempferol-3-glucoside (35.9 µg/g extract), coumarin (16.3 µg/g extract), salicylic acid (5.3 µg/g extract), quercetin (2.51 µg/g extract), fisetin (0.81 µg/g extract) were detected as concentrated compounds in methanol extract of the corresponding plant (Table 3). The chemical structure of major compounds was also shown (Fig. 3).

Isoquercitrin (quercetin-3-*O*-*β*-d-glucopyranoside) is the medicinally significant flavonoid found in aromatic and medicinal plants and plant-derived foods. Since isoquercitrin is the monoglucoside of quercetin, the most common naturally occurring flavonoid, it makes sense to believe that it is widely dispersed across the kingdom of plants. For example, one of the bioactive ingredients of the medicinal plant, *Hypericum perforatum* is isoquercitrin, which is frequently present in fruits, vegetables, cereals, and drinks made from plants, including tea. Despite being widely available, isoquercitrin is in extremely low quantity in plant material making it difficult to attain significant amounts in a pure form for the food and pharmaceutical industries. The selective approach to yield isoquercitrin is the removal of rhamnose from the rutin molecule. The primary mechanisms by which flavonoids contribute to human health are their anti-inflammatory and antioxidant properties. They can directly scavenge reactive oxygen and nitrogen species, block prooxidant enzymes, or induce antioxidant enzymes to mediate their antioxidant and antiradical effects. Isoquercitrin can scavenge reactive oxygen and nitrogen species, including superoxide anion radicals, hydroxyl radicals, and peroxynitrite.

Quercetin is a significant natural compound existing in various plants, vegetables, fruits, and seeds, known as an excellent antioxidant. Consuming a diet high in flavonoids decreases the chance of developing chronic illnesses linked to oxidative stress, including diabetes, heart disease, and stroke. Quercetin has strong anti-inflammatory capacity as well. Many studies confirmed that flavonoids can inhibit LPS-induced cytokine production. Additionally, quercetin was reported to reveal antiproliferative, antiatherogenic,

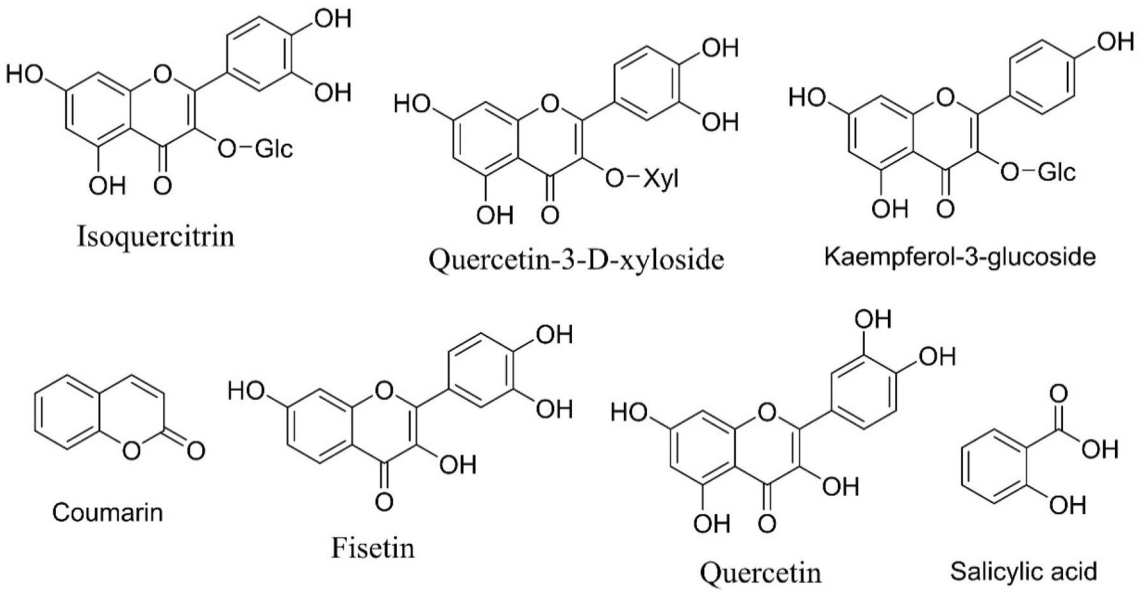


Fig. 3 The natural compounds found in *Lotus corniculatus*

Table 2 Qualitative and quantitative analysis of phytochemical in *L. corniculatus* methanol extract

S. no	Compounds	RT	Ion transition (m/z)	CE	Ion Mode	R^2	LOQ (ug/L)	LOD (ug/L)	Range (ug/L)	Precision (%CV)
1	Gallic acid	3.218	169.0–> 125.1	12	Neg	0.999	18.586	7.167	31.25–500	0.560
2	Protocatechuic acid	5.449	153.0–> 109.0	14	Neg	0.997	13.173	3.156	15.625–250	0.633
3	Epigallocatechin	6.796	307.0–> 139.0	8	Pos	0.999	3.875	2.090	12.5–200	0.266
4	Catechin	6.904	288.9–> 245.1	12	Neg	0.995	7.501	1.705	343.750–5500	1.185
5	Chlorogenic acid	7.378	353.0–> 191.0	12	Neg	0.998	25.902	11.589	31.25–500	0.735
6	*4*-Hydroxybenzaldehyde	7.679	121.0–> 92.0	24	Neg	0.999	12.865	4.974	15.625–250	0.376
7	Vanillic acid	7.791	167.0–> 151.8	12	Neg	0.996	914..213	219.042	1250–20000	0.398
8	Caffeic acid	7.847	178.9–> 135.1	14	Neg	0.999	24.162	6.921	31.25–500	0.646
9	Syringic acid	8.401	197.1–> 181.8	12	Neg	0.999	857..339	358.500	1250–20000	0.978
10	Caffeine	8.431	195.0–> 137.9	18	Pos	0.999	15.496	6.810	18.75–300	0.450
11	Vanillin	8.649	153.0–> 125.0	6	Pos	0.995	23.541	14.588	31.25–500	0.596
12	*p*-coumaric acid	9.441	163.0–> 119.1	10	Neg	0.999	7.997	4.016	15.625–500	0.423
13	Salicylic acid	9.539	137.0–> 93.1	18	Neg	0.998	82.965	47.669	112.5–1800	0.075
14	Taxifolin	9.743	304.8–> 258.9	12	Pos	0.998	82.965	47.669	112.5–1800	1.146
15	Resveratrol	9.791	229.0–> 107.0	19	Pos	0.991	13.557	4.581	18.75–300	0.095
16	*trans*-ferulic acid	10.132	193.1–> 133.9	14	Neg	0.995	11.528	1.761	31.25–1000	1.001
17	Sinapic acid	10.414	223.1–> 208.0	10	Neg	0.997	4.965	1..944	125–2000	2.269
18	Scutellarin	11.151	462.8–> 286.8	16	Pos	0.998	4.001	3.135	9.375–300	0.645
19	*o*-coumaric acid	11.502	163.0–> 119.0	10	Neg	0.999	17.542	3.535	31.25–500	0.694
20	Coumarin	11..531	*147.1–> 103.2*	*22*	Pos	0.997	20.703	6.008	62.5–1000	1.378
21	Protocatehuic ethyl ester	11.622	181.0–> 107.9	20	Neg	0.999	24.920	14.561	31.25–1000	0.710
22	Rutin	12.293	611.0–> 302.8	9	Pos	0.998	204.672	59.560	250–4000	1.486
23	Isoquercitrin	11.867	464.9–> 302.8	8	Pos	0.998	11.268	9.938	18.75–300	0.438
24	Hesperidin	11.842	611.0–> 302.9	14	Pos	0.996	17.675	4.140	31.25–500	0.899
25	Quercetin-3-D-xyloside	12.433	432.7–> 299.5	24	Neg	0.988	69.406	18.713	125–2000	0.824
26	Kaempferol-3-glucoside	12.887	448.8–> 286.9	10	Pos	0.999	4.524	1.161	7.8125–125	0.514
27	Fisetin	13.327	287.0–> 137.0	32	Pos	0.995	8.366	4.896	15.625–250	0.336
28	Baicalin	13.653	446.8–> 270.9	16	Pos	0.999	3.099	0.528	15.625–250	0.554
29	*trans*-cinnamic acid	14.272	149.0–> 131.1	6	Pos	0.999	22.028	11.185	31.25–500	0.768
30	Quercetin	14.821	300.8–> 151.0	22	Neg	0.996	16.913	4.656	27.5–440	3.311
31	Naringenin	14.999	270.9–> 119.1	24	Neg	0.996	4.575	1.369	31.25–500	0.662
32	Hesperetin	15.815	300.9–> 164.0	20	Neg	0.997	0.650	0.301	31.25–500	0.513
33	Morin	15.819	302.8–> 153.0	30	Pos	0.998	0.528	0.125	1.5625–50	0.432
34	Kaempferol	16.431	284.9–> 116.9	44	Neg	0.999	54.004	18.683	312.5–5000	0.652
35	Baicalein	17.084	271.0–> 123.0	36	Pos	0.999	0.963	0.596	1.5625–25	0.473
36	Luteolin	17.909	285.0–> 133.1	36	Pos	0.996	21.453	6.262	31.25–500	1.822
37	Biochanin A	17.910	284.9–> 151.9	28	Pos	0.996	0.733	0.148	1.5625–25	0.896
38	Chrysin	14.230	254.9–> 153.0	32	Pos	0.999	0.134	0.074	1.5625–25	0.281
39	Diosgenin	23.542	415.0–> 271.0	18	Pos	0.998	14.652	2.933	15.625–250	2.207

anticoagulant, antifibrotic, and antihypertensive properties. Furthermore, through its interaction with estrogenic binding sites, quercetin inhibited cell growth and directly altered the gene expression of enzymes involved in biotransformation (Boots et al. 2008).

Coumarin (benzopyran-2-one, or chromen-2-one) is a natural compound displaying interesting pharmacological properties. Innovative synthetic approaches have been employed to generate several compounds based on the coumarin ring structure. Numerous pharmacological characteristics, including anti-inflammatory, antibacterial, antiviral, antioxidant, antithrombotic, anti-Alzheimer, and anticancer effects, are possessed by coumarin and its derivatives (Venugopala et al. 2013).

Numerous preclinical investigations have demonstrated the diverse pharmacological properties of kaempferol and

Table 3 Quantitative analysis of phenolic compounds in methanol extract of *L. corniculatus* by LC–ESI–MS/MS (µg/g extract)

No	Compound	RT	Conc
1	Gallic acid	3.221	nd
2	Protocatechuic acid	5.467	0.3410
3	Epigallocatechin	6.815	nd
4	Catechin	6.904	nd
5	Chlorogenic acid	7.443	nd
6	Hydroxybenzaldeyde	7.697	0.3430
7	Vanillic acid	7.829	nd
8	Caffeic Acid	7.891	0.2118
9	Syringic acid	7.945	nd
10	Caffein	8.498	0.1189
11	Vanillin	8.678	0.4069
12	o-coumaric acid	9.495	0.5466
13	Salicylic Acid	9.871	5.3031
14	Taxifolin	9.670	nd
15	Resveratrol	9.874	0.0612
16	Trans-ferulic acid	10.182	nd
17	Sinapic acid	10.447	nd
18	Scutellarin	11.148	nd
19	p-coumaric acid	11.607	nd
20	Coumarin	11.173	16.2675
21	Protocatehuic ethyl ester	11.676	nd
22	Hesperidin	11.906	nd
23	Isoquercitrin	11.735	200.8117
24	Rutin	12.375	nd
25	Quercetin-3-D-xyloside	12.770	189.1413
26	Kaempferol-3-glucoside	13.282	35.8989
27	Fisetin	13.293	0.8082
28	Baicalin	13.923	nd
29	Chrysin	14.255	nd
30	Trans-cinnamic acid	14.340	0.5184
31	Quercetin	14.931	2.5126
32	Naringenin	15.041	nd
33	Hesperetin	15.848	nd
34	Morin	15.802	nd
35	Kaempferol	16.523	nd
36	Baicalein	17.101	nd
37	Luteolin	17.901	0.5891
38	Biochanin A	17.909	0.2771
39	Diosgenin	23.533	nd

nd: not detected

its glycosides, encompassing anti-inflammatory, anti-tumor, neuroprotective, antioxidant, and antidiabetic effects (Calderon-Montano et al. 2011).

Salicylic acid is an important natural compound found in plants that plays a vital role in protecting plants against pathogens. It is the important active component of acetylsalicylic acid, which is an anti-inflammatory drug widely used in clinical practice. It has been determined that taking low doses of acetylsalicylic acid may be effective in preventing cardiovascular disease and colorectal carcinoma (Randjelović et al. 2015).

Conclusion

Isoquercitrin, quercetin-3-*D*-xyloside, kaempferol-3-glucoside, coumarin, salicylic acid, quercetin, fisetin were detected as major products. Therefore, this plant can be considered as a source of corresponding compounds. The developed sensory technique for determination of antioxidant is easy, fast, and sensitive that can be applied to large scale production. Due to the revealing of good antioxidant activity, the bioactive compounds responsible for the activity should be isolated and identified.

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Author contributions IY Methodology, validation. MNA Methodology, data curation. RE Conceptualization, Writing—Review and Editing. ID Formal analysis, Methodology. IH Methodology, data curation. MHA Supervision.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability Data will be provided upon request.

References

Aksit H, Çelik SM, Sen Ö, Erenler R, Demirtas I, Telci I, Elmastas M (2014) Complete isolation and characterization of polar portion of *Mentha dumetorum* water extract. Rec Nat Prod 8:277–280

Aydin A, Erenler R, Yılmaz B, Tekin Ş (2016) Antiproliferative effect of *Cherry laurel*. J Turk Chem Soc Sec A Chem 3:217–228. https://doi.org/10.18596/jotcsa.21204

Bayir B, Gündüz H, Usta T, Şahin E, Özdemir Z, Kayır Ö, Sen Ö, Akşit H, Elmastaş M, Erenler R (2014) Chemical composition of essential oil from *Marrubium Vulgare* L. Leaves J New Results Sci 6:44–50

Boots AW, Haenen GRMM, Bast A (2008) Health effects of quercetin: from antioxidant to nutraceutical. Eur J Pharmacol 585:325–337. https://doi.org/10.1016/j.ejphar.2008.03.008

Dede E, Genc N, Elmastas M, Aksit H, Erenler R (2019) Chemical constituents isolated from *Rhododendron ungernii* with antioxidant profile. Nat Prod J 9:238–243. https://doi.org/10.2174/2210315508666181024114812

Demirtas I, Erenler R, Elmastas M, Goktasoglu A (2013) Studies on the antioxidant potential of flavones of *Allium vineale* isolated from its water-soluble fraction. Food Chem 136:34–40. https://doi.org/10.1016/j.foodchem.2012.07.086

Djermane N, Gali L, Arhab R, Gherraf N, Bensouici C, Erenler R, Gok M, Abdessamed A (2020) Chemical composition and in vitro

evaluation of antioxidant, antimicrobial, and enzyme inhibitory activities of *Erucaria uncata* and *Thymeleae hirsuta*. Biocatal Agric Biotechnol 29:101834. https://doi.org/10.1016/j.bcab.2020.101834

Elmastas M, Ozturk L, Gokce I, Erenler R, Aboul-Enein HY (2004) Determination of antioxidant activity of marshmallow flower (*Althaea officinalis* L.). Anal Lett 37:1859–1869. https://doi.org/10.1081/AL-120039431

Elmastaş M, Telci İ, Akşit H, Erenler R (2015) Comparison of total phenolic contents and antioxidant capacities in mint genotypes used as spices/Baharat olarak kullanılan nane genotiplerinin toplam fenolik içerikleri ve antioksidan kapasitelerinin karşılaştırılması. Turk J Biochem 40:456–462. https://doi.org/10.1515/tjb-2015-0034

Elmastas M, Erenler R, Isnac B, Aksit H, Sen O, Genc N, Demirtas I (2016) Isolation and identification of a new neo-clerodane diterpenoid from *Teucrium chamaedrys* L. Nat Prod Res 30:299–304. https://doi.org/10.1080/14786419.2015.1057583

Elmastas M, Celik SM, Genc N, Aksit H, Erenler R, Gulcin İ (2018) Antioxidant activity of an anatolian herbal tea—*Origanum minutiflorum*: isolation and characterization of its secondary metabolites. Int J Food Prop 21:374–384. https://doi.org/10.1080/10942912.2017.1416399

Erenler R, Yilmaz S, Aksit H, Sen O, Genc N, Elmastas M, Demirtas I (2014) Antioxidant activities of chemical constituents isolated from *Echinops orientalis* Trauv. Rec Nat Prod 8:32–36

Erenler R, Demirtas I, Karan T, Gul F, Kayir O, Karakoc OC (2018) Chemical constituents, quantitative analysis and insecticidal activities of plant extract and essential oil from *Origanum onites* L. Trends Phytochem Res 2:91–96

Erenler R, Nusret G, Elmastaş M, Eminağaoğlu Ö (2019) Evaluation of antioxidant capacity with total phenolic content of *Galanthus krasnovii* (Amaryllidaceae). Turk J Biodiv 2:13–17

Gecer EN, Erenler R, Temiz C, Genc N, Yildiz I (2021) Green synthesis of silver nanoparticles from *Echinacea purpurea* (L.) Moench with antioxidant profile. Particul Sci Technol 40:50–57. https://doi.org/10.1080/02726351.2021.1904309

Genç N, Yıldız İ, Karan T, Eminağaoğlu Ö, Erenler R (2019) Antioxidant activity and total phenolic contents of *Galanthus woronowii* (Amaryllidaceae). Turk J Biodiv 2:1–5

Guzel A, Aksit H, Elmastas M, Erenler R (2017) Bioassay-guided isolation and identification of antioxidant flavonoids from Cyclotrichium origanifolium (Labill.) Manden. and Scheng. Pharmacogn Mag 13:316. https://doi.org/10.4103/0973-1296.204556

Isildak Ö, Yildiz I, Genc N (2022) A new potentiometric PVC membrane sensor for the determination of DPPH radical scavenging activity of plant extracts. Food Chem 373:131420. https://doi.org/10.1016/j.foodchem.2021.131420

Karan T, Erenler R (2017) Screening of norharmane from seven cyanobacteria by high-performance liquid chromatography. Pharmacogn Mag 13:723–725. https://doi.org/10.4103/pm.pm_214_17

Kaya G, Karakaya R, Tilgel E, Sandikci M, Yucel E, Cicek G, Kayir O, Aksit H, Telci I, Guzel A (2014) Essential oil constituents of *Thuja orientalis* berries. J New Results Sci 7:1–6

Koelzer J, Pereira DA, Dalmarco JB, Pizzolatti MG, Fröde TS (2009) Evaluation of the anti-inflammatory efficacy of Lotus corniculatus. Food Chem 117:444–450. https://doi.org/10.1016/j.foodchem.2009.04.044

Koysu P, Genc N, Elmastas M, Aksit H, Erenler R (2019) Isolation, identification of secondary metabolites from *Salvia absconditiflora* and evaluation of their antioxidative properties. Nat Prod Res 33:3592–3595. https://doi.org/10.21448/ijsm.40411410.1080/14786419.2018.1488700

Calderon-Montano MJ, Burgos-Moron E, Perez-Guerrero C, Lopez-Lazaro M (2011) A review on the dietary flavonoid kaempferol. Mini-Rev Med Chem 11:298–344. https://doi.org/10.2174/138955711795305335

Randjelović P, Veljković S, Stojiljković N, Sokolović D, Ilić I, Laketić D, Randjelović D, Randjelović N (2015) The beneficial biological properties of salicylic acid. Acta Fac Med Naissensis 32:259–265. https://doi.org/10.1515/afmnai-2015-0026

Sahin Yaglioglu A, Akdulum B, Erenler R, Demirtas I, Telci I, Tekin S (2013) Antiproliferative activity of pentadeca-(8E, 13Z) dien-11-yn-2-one and (E)-1,8-pentadecadiene from *Echinacea pallida* (Nutt.) Nutt. roots. Med Chem Res 22:2946–2953. https://doi.org/10.1007/s00044-012-0297-2

Venugopala KN, Rashmi V, Odhav B (2013) Review on natural coumarin lead compounds for their pharmacological activity. Biomed Res Int. https://doi.org/10.1155/2013/963248

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