Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

Phytochemical Composition, Antioxidant and Metabolic Enzyme Activity of *Alchemilla vulgaris* Grown in Organic Farming Conditions

Sultan Pekacar[a*], Burçin Özüpek[a], Didem Deliorman Orhan[a]

[a]*Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, 06330, Etiler, Ankara, TÜRKIYE*

ARTICLE INFO
Research Article
Corresponding Author: Sultan Pekacar, E-mail: sultanpkcr94@gmail.com
Received: 2 February 2025 / Revised: 3 June 2025 / Accepted: 23 June 2025 / Online: 20 January 2026

Cite this article
Pekacar S, Özüpek B, Orhan D D (2026). Phytochemical Composition, Antioxidant and Metabolic Enzyme Activity of *Alchemilla vulgaris* Grown in Organic Farming Conditions . *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 32(1):57-65. DOI: 10.15832/ankutbd.1631483

ABSTRACT
The objective of this research was to evaluate the *in vitro* antidiabetic (*α*-glucosidase and *α*-amylase), antihyperlipidemic (pancreatic cholesterol esterase), and antiobesity (pancreatic lipase) activities of an infusion prepared from the aerial parts of *Alchemilla vulgaris*, cultivated using organic farming methods. The phytochemical content of the infusion was determined using UV spectroscopy (total phenol and total flavonoids) and the reverse phase-HPLC method. DPPH and ABTS radical scavenging, metal chelating capacity, and reducing power methods were used to evaluate the antioxidant effect. Regarding the antidiabetic effect, the infusion exhibited the highest activity, with a 61.95 ± 1.57% inhibition rate against *α*-glucosidase at a concentration of 2 mg/mL. While it had 30.81 ± 0.58% inhibition at a concentration of 2 mL on pancreatic cholesterol esterase enzyme, the inhibition value of simvastatin, the reference compound, at the same concentration was determined to be 53.19 ± 3.36%. The *A. vulgaris* infusion demonstrated complete inhibition of ABTS radicals (100%) at concentrations of 1 and 2 mg/mL, and exhibited a 99.41 ± 6.71% metal chelating capacity at a concentration of 2 mg/mL. As a result of reverse phase-HPLC analysis, it was determined that the major compound of the infusion was ellagic acid (2.162 ± 0.010 g/100 g extract), and it also contained quercetin-3-*O*-glucoside and luteolin. The findings suggest that the plant can be evaluated for further studies, including activity-directed isolation, *in vivo* and *in silico* studies, particularly in terms of *α*-glucosidase enzyme inhibitory activity.

Keywords: Organic farming, *α*-amylase, *α*-glucosidase, antioxidant, *Alchemilla vulgaris*, Reverse phase-HPLC

1. Introduction

Diabetes mellitus (DM) is a common disease known for chronic hyperglycemia (Özenoğlu et al. 2020). Diabetes is the disease that causes the most harm and deaths worldwide. According to the International Diabetes Federation, 8.2% of adults aged 20-79 were diabetic in 2014, and this number is expected to exceed 592 million by 2035. Oxidative stress plays a crucial role in the development of complications related to diabetes. In diabetic individuals, high blood glucose levels trigger the generation of free radicals. The body's natural antioxidant defense mechanisms are frequently insufficient to counteract the excess reactive oxygen species, leading to increased oxidative stress (Hamamcıoğlu 2017). In the literature, it has been reported that there is a complex and close relationship between DM and obesity. Additionally, obesity stands out as a risk factor for Type 2 DM. (Hussain et al. 2010). Due to these factors, there is a growing demand for new therapeutics, whether derived from natural or synthetic sources, that can lower blood glucose, mitigate diabetes-induced oxidative stress, and aid in weight management as part of the ongoing battle against this prevalent metabolic disorder.

The genus *Alchemilla* L. (Rosaceae) is a perennial herbaceous plant known as "aslan pençesi" in Turkey. The genus *Alchemilla* is represented by more than 1000 species worldwide (Acet & Özcan 2018). *Alchemilla vulgaris* L. is known as "Lady's mantle" and grows in humid areas, pastures, and wet meadows in Europe, Asia, and America (Jurić et al. 2020). The aerial parts of the plant contain significant amounts of tannins, phenolic acids (including ellagic, gallic, and caffeic acids), flavonoids (such as quercetin), and flavonoid glycosides (like isoquercetin, rutin, and tiliroside). In the literature, *Alchemilla* species have been reported to have various biological activities such as antiviral, antioxidant, antiproliferative, and antibacterial activities, as well as healing effects on skin wounds (Boroja et al. 2018). In addition, *A. vulgaris* is a plant widely used in folk medicine to cure oral infections, nosebleeds, tinnitus, gynecological (menorrhagia and dysmenorrhea), and gastrointestinal disorders (Tadić et al. 2020). Jakimiuk and Tomczyk reported that *A. vulgaris* is used as a folk remedy for diabetes mellitus. As a result of literature review studies, it has been determined that no scientific research parallel to the subject of our study has been conducted on *A. vulgaris*, which grows naturally in Turkey (Jakimiuk & Tomczyk 2024).

Recently, there has been a notable global rise in the demand for organic medicinal plants. These plants can be sourced not only through wild collection but also by employing organic farming methods. The reliance on chemical fertilizers and pesticides in conventional agricultural practices may pose challenges to bio-environmental sustainability. On the other hand, plants collected from nature create problems in terms of protecting biodiversity and optimizing the general quality parameters of plants. The cultivation of medicinal plants through organic farming provides advantages such as (i) protecting biodiversity, (ii) ensuring the quality and standardization of plant products, (iii) obtaining medicinal plants and drugs free from chemical residues, (iv) consistent and optimized metabolite profiles, and (v) providing reliable sources for pharmaceutical research (Yavuz & Erdogan 2019).

The study assessed the infusion of the aerial parts of *A. vulgaris* cultivated using organic farming methods for its antidiabetic activity (inhibition of α-amylase and α-glucosidase), antiobesity effects (inhibition of pancreatic lipase), and anticholesterolemic potential (inhibition of pancreatic cholesterol esterase) through enzyme models. In addition, the antioxidant properties of the infusion were evaluated through various assays, including metal chelation, ferric reducing power, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and (2,2'–azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) ($ABTS^{\bullet+}$) radical scavenging activity, alongside the determination of total phenol and flavonoid contents. RP-HPLC was employed to standardize the infusion for ellagic acid, quercetin-3-*O*-glucoside, and luteolin

2. Material and Methods

2.1. Plant material

A. vulgaris, cultivated under organic farming practices, was sourced from the Konya region, specifically from the 3rd km of Akyokuş Mevki on Beyşehir Yolu (Certificate no: TR-OT-014-İ-197/02, Temmuz Organic Farm). The plant was grown in compliance with the Organic Agriculture Law and Regulations of the Turkish Republic and has been certified by Nissert Ltd., an organization accredited by the Ministry of Agriculture

2.2. Preparation of aerial part infusion of Alchemilla vulgaris

Ten grams of powdered aerial parts of the plant were extracted with 200 mL of hot water. The extract was then filtered, and this extraction process was repeated three times. After combining the resulting filtrates, they were freeze-dried using a lyophilizer.

2.3. Determination of total phenolic content

To the infusion, 10% (w/v) Folin-Ciocalteu reagent was added and incubated at 25 °C for 5 minutes. Following this, 7.5% (w/v) sodium carbonate solution was introduced to the mixture. The extracts were incubated for an additional 30 minutes at room temperature in the dark. Afterward, absorbance was measured at 735 nm using an ELISA microplate reader (SpectraMax i3x, Molecular Devices, USA). Total phenol content was expressed as milligrams of gallic acid equivalent (GAE) per gram of infusion. In this method, a calibration curve was constructed using five different concentrations (0.01, 0.05, 0.25, 0.5, and 1 mg/mL), and the analysis was performed in triplicate. The calibration equation used was $y = 3.7855x + 0.1735$ with an r^2 value of 0.9931. (Zongo et al. 2010).

2.4. Determination of total flavonoid content

To the infusion, ethanol (96%), sodium acetate (1M), and aluminum chloride (10% w/v) solutions were added, and the mixture was diluted with distilled water to a final volume of 1 mL. After incubating for 30 minutes at room temperature, absorbance was measured at 415 nm using an ELISA microplate reader. The results were reported as quercetin equivalent (QE) in milligrams per gram of extract. In this method, a calibration curve was generated using five concentration levels (0.01, 0.05, 0.25, 0.5, and 1 mg/mL), and all measurements were performed in triplicate. The calibration curve equation was found to be $y = 2.8193x - 0.0996$, with an r^2 value of 0.9977 (Kosalec et al. 2004).

2.5. Antioxidant activity assays

2.5.1. ABTS radical scavenging activity

7 mM ABTS solution and 2.45 mM potassium persulfate were incubated for 16 hours at 20 °C in the dark. ABTS solution and phosphate buffer (pH 7.4) were added to the infusion. The infusion was made at different concentrations (2, 1, and 0.5 mg/mL) using an 80% ethanol solution. After vortexing, absorbance was measured at 750 nm using an ELISA microplate reader. Gallic acid was the reference compound. All experiments were done in triplicate (inhibition %) = $[(Absorbance_{control} - Absorbance_{sample})/Absorbance_{control}] \times 100$ (Orhan et al. 2017).

2.5.2. DPPH radical scavenging activity

DPPH radical scavenging activity was assessed using 96-well microplates. 1 mM DPPH solution (20 µl) was mixed with the infusion and incubated in the dark for 30 minutes. Absorbance was measured at 520 nm using an ELISA microplate reader. The infusion was made at different concentrations (2, 1, and 0.5 mg/mL) using an 80% ethanol solution. Ascorbic acid was used as the reference compound. All experiments were conducted in triplicate (inhibition %) = [($Absorbance_{control}$ - $Absorbance_{sample}$)/$Absorbance_{control}$] × 100 (Jung et al. 2011).

2.5.3. Metal chelating capacity

2 mM $FeCl_2$ solution was added to the infusion and incubated for 5 minutes, followed by the addition of 5 mM ferrozine and incubation for 10 minutes. The infusion was made at different concentrations (2, 1, and 0.5 mg/mL) using an 80% ethanol solution. Absorbance was measured at 562 nm using an ELISA microplate reader. Ethylenediaminetetraacetic acid (EDTA) was used as the reference compound, and metal chelating capacity was calculated as [($Absorbance_{control}$ - $Absorbance_{sample}$) / $Absorbance_{control}$] × 100. All experiments were performed in triplicate (Dinis et al. 1994).

2.5.4. Ferric reducing power

Extracts and reference compounds were mixed with 0.1 mol/L sodium phosphate buffer (pH 7.2), followed by the addition of 1% (w/v) $K_3Fe(CN)_6$ solution. The mixture was incubated at 37 °C for 1 hour. After incubation, 10% trichloroacetic acid was added, and the absorbance was measured at 700 nm. Subsequently, 0.1% $FeCl_3$ was added to the mixture, and absorbance was measured again. The infusion was made at different concentrations (2, 1, and 0.5 mg/mL) using an 80% ethanol solution. The difference between the two readings was calculated. Quercetin was used as the reference compound, and all experiments were performed in triplicate (Orhan et al. 2017).

2.6. Enzyme assays

2.6.1. α-Glucosidase inhibitory activity assay

The *α*-glucosidase type IV enzyme (from *Bacillus stearothermophilus;* 4 U/mL*)* was prepared in a 0.5 M phosphate buffer solution (pH 6.5). The infusion was made at different concentrations (2, 1, and 0.5 mg/mL) using an 80% ethanol solution. Both the enzyme solution and the extracts were pre-incubated at 37 °C for 15 minutes in a 96-well microplate. Following this, a 20 mM solution of p-nitrophenyl-*α*-D-glucopyranoside (PNG) was added to each well. After 35 minutes of incubation at 37 °C, the increase in absorbance at 405 nm, resulting from the hydrolysis of PNG by *α*-glucosidase, was measured using an ELISA microplate reader. Acarbose (Bayer, Turkey) was used as the reference compound. (Orhan et al. 2017).

2.6.2. α-Amylase inhibitory activity assay

α-Amylase type I-A (EC 3.2.1.1, Sigma) (from porcine pancreas; 0.25 U/mL) was dissolved in buffer solution. 2.5% potato starch solution in phosphate buffer (pH 6.9) served as the substrate. Infusions at concentrations of 2, 1, and 0.5 mg/mL were prepared with 80% ethanol. After enzyme addition, mixtures were incubated at room temperature for 5 minutes, followed by the addition of the substrate and incubation at 37 °C for 15 minutes. 3,5-dinitrosalicylic acid (DNS) reagent (5.31 M sodium potassium tartrate in 96 mM DNS and 2 M NaOH) was added, and the mixture was heated at 80 °C for 40 minutes. After cooling, absorbance was measured at 540 nm using an ELISA reader. Maltose production was quantified using a standard curve ($y = 0.6762x - 0.0404$, $r^2 = 0.9966$). Acarbose was used as the reference compound. All experiments were performed in triplicate (Orhan et al. 2017).

2.6.3. Pancreatic lipase inhibitory activity assay

Pancreatic lipase type II (from porcine pancreas) was dissolved in a buffer containing 10 mM 4-morpholine propane sulfonic acid and 1 mM EDTA (pH 6.8). Infusions were prepared at 2, 1, and 0.5 mg/mL concentrations using 80% ethanol. The enzyme and extracts were pre-incubated for 15 minutes at 37 °C in Tris buffer (100 mM Tris-HCl, 5 mM $CaCl_2$, pH 7.0). After adding 4-nitrophenyl butyrate, the mixture was incubated at 37 °C for 30 minutes. Absorbance at 405 nm, resulting from hydrolysis by lipase, was measured using an ELISA microplate reader. Orlistat was the reference compound. All experiments were conducted in triplicate (Lee et al. 2012).

2.6.4. Pancreatic cholesterol esterase inhibitory activity assay

Porcine pancreatic cholesterol esterase enzyme was dissolved in a 100 mM buffer with 100 mM NaCl (pH 7). Infusions at three concentrations were added to 50 µL of phosphate buffer. After the addition of taurocholic acid (12 mM) and 5 mM 4-nitrophenylbutyrate, the mixture was incubated at room temperature for 5 minutes. The enzyme (0.1 µg/mL) was then added,

and kinetic readings were taken at 405 nm for 15 minutes using an ELISA microplate reader. Simvastatin served as the reference compound. All experiments were conducted in triplicate (Ngamukote et al. 2011).

2.6.5. Standardization of the infusion by using the RP-HPLC method

The HP Agilent 1260 series LC system, equipped with an ACE 5 C18 column (5 µm, 150 mm × 4.6 mm), was used for HPLC analysis. The system also included an HP Agilent 1260 series autosampler unit. The column temperature was maintained at 25 °C during the analysis. For the qualitative and quantitative determination of phenolic compounds and flavonoids in the infusion, standard mixtures of the following compounds were used. 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. Flavonoid mixture: umbelliferone, rutin, naringenin, hesperidin, quercetin-3-*O*-glucoside, apigenin-7-*O*-glucoside, myrcetin, quercetin, luteolin, apigenin. Standard compounds were sourced from Sigma-Aldrich. These three standard compounds were prepared at five different concentrations in a 25% acetonitrile and water mixture, and the extract was prepared at 10 mg/mL and filtered using a 0.45 µM filter. For analysis, a gradient flow system was employed, starting with 5% solvent A (acetonitrile:water:formic acid, 50:50:0.5) and 95% solvent B (water:formic acid, 100:0.5). The total run time was 58 minutes, with a 20 µL injection volume. Measurements were taken at four wavelengths (260, 280, 320, and 350 nm) using a DAD detector. Calibration curves for ellagic acid, quercetin-3-*O*-glucoside, and luteolin were established using standard solutions at concentrations of 5, 10, 20, and 100 ppm. A calibration curve was constructed by plotting peak areas (y) against concentration values (x, in ppm), and the amounts of the compounds in the extracts were calculated accordingly (Gök et al. 2021).

2.7. Statistical analysis

All analyses were conducted in triplicate, and the results were presented as the mean ± standard deviation (S.D.). Statistical analyses, including linear regression, were performed using Microsoft Excel and GraphPad Instat software. Differences were considered statistically significant at $P<0.05$. ($^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$).

3. Results and Discussion

The yield of the *A. vulgaris* extract obtained by the infusion technique was found to be 11.02% (w/w). The total phenolic content of the extract was determined to be 237.27 ± 8.94 mg GAE/g extract, and the total flavonoid content was determined to be 61.69 ± 2.17 mg QE/g extract. Four distinct methods were employed to assess the antioxidant activity of the *A. vulgaris* infusion. The ABTS radical scavenging activity exhibited a concentration-dependent increase, with 1 and 2 mg/mL concentrations showing significant antioxidant activity and higher inhibition values compared to the reference compound, gallic acid. In the DPPH radical scavenging assay, the inhibition values of the extracts decreased as the concentration increased. The highest inhibition value (81.05 ± 2.78%) was observed at a concentration of 0.5 mg/mL, while for the reference compound ascorbic acid, this value was calculated as 89.52 ± 3.07% at the same concentration. When evaluating the metal chelating capacity, we found that the infusion exhibited an inhibition value of 99.41 ± 6.71% at a concentration of 2 mg/mL, which was higher than that of the reference compound, EDTA (95.75 ± 0.63%), at the same concentration. Similarly, it was determined that the infusion exhibited high reducing power, with an absorbance value (3.560 ± 0.020) comparable to that of the reference compound quercetin (3.568 ± 0.020) at a concentration of 2 mg/mL.

Table 1- ABTS, DPPH radical scavenging activity, metal chelating capacity, and reducing power results of *A. vulgaris* infusion

Sample Name	***Concentration (mg/mL)***	***ABTS radical scavenging activity Inhibition% ± S.D.***	***DPPH radical scavenging activity Inhibition% ± S.D.***	***Metal Chelating Activity% ± S.D.***	***Reducing Power Absorbance ± S.D.***
A. vulgaris	0.5	$44.95 \pm 0.47^{***}$	$81.05 \pm 2.78^{***}$	8.19 ± 1.96^{ns}	$1.670 \pm 0.11^{***}$
	1	100^{***}	$73.92 \pm 0.71^{***}$	$65.27 \pm 9.14^{***}$	$2.903 \pm 0.05^{***}$
	2	100^{***}	$45.16 \pm 0.99^{***}$	$99.41 \pm 6.71^{***}$	$3.560 \pm 0.02^{***}$
References	GA/AA/EDTA/QE 0.5	$99.55 \pm 1.04^{a***}$	$89.52 \pm 3.07^{b***}$	$99.84 \pm 0.39^{c***}$	$3.256 \pm 0.38^{d***}$
	GA/AA/EDTA/QE 1	$98.96 \pm 0.26^{a***}$	$90.72 \pm 0.65^{b***}$	$99.88 \pm 0.26^{c***}$	$3.550 \pm 0.02^{d***}$
	GA/AA/EDTA/QE 2	$98.12 \pm 0.80^{a***}$	$90.38 \pm 0.86^{b***}$	$95.75 \pm 0.63^{c***}$	$3.568 \pm 0.02^{d***}$

-: No activity, SD: Standard Deviation, ^{ns}: Statistically insignificant, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, CI: ^{a}Gallic acid, AA: ^{b}Ascorbic acid, ^{c}EDTA: Ethylenediamine tetraacetic acid, ^{d}QE: Quercetin

To investigate the potential effects of *A. vulgaris* infusion on metabolic disorders, its inhibitory activity against key enzymes, including α-glucosidase, α-amylase, pancreatic lipase, and pancreatic cholesterol esterase, was evaluated. The infusion exhibited a concentration-dependent inhibition of *α*-glucosidase, with statistically significant activity observed at all tested concentrations ($P<0.001$). The highest inhibition was detected at 2 mg/mL (61.95 ± 1.57%), whereas the reference compound, acarbose, showed 99.37 ± 0.22% inhibition at the same concentration. A mild inhibitory effect on *α*-amylase was observed only at 1 mg/mL (19.55

± 5.54%), which was not statistically significant (P>0.05). The infusion did not exhibit any inhibitory activity on pancreatic lipase at any concentration tested. However, it significantly inhibited pancreatic cholesterol esterase at 2 mg/mL (30.81 ± 0.58%, P<0.001), while the reference compound, simvastatin, demonstrated 53.18 ± 3.36% inhibition.

Table 2- Inhibition results of *A. vulgaris* infusion on *α*-glucosidase, *α*-amylase, pancreatic lipase, and pancreatic cholesterol esterase enzyme

Sample Name	*Concentration (mg/mL)*	*Inhibition% ± S.D.*			
		α-Glucosidase	*α-Amylase*	*Pancreatic lipase*	*Pancreatic cholesterol esterase*
A. vulgaris	0.5	$24.64 \pm 0.93^{*}$	-	-	$22.26 \pm 4.82^{***}$
	1	$29.01 \pm 4.57^{**}$	19.55 ± 5.54^{ns}	-	$20.42 \pm 3.33^{***}$
	2	$61.95 \pm 1.57^{***}$	-	-	$30.81 \pm 0.58^{***}$
References	AKA/OR/SIM 0.5	$98.89 \pm 0.21^{a***}$	$94.75 \pm 0.60^{a***}$	$53.16 \pm 0.00^{b***}$	$47.78 \pm 5.11^{c***}$
	AKA/OR/SIM 1	$99.45 \pm 0.13^{a***}$	$98.48 \pm 0.50^{a***}$	$69.55 \pm 4.19^{b***}$	$52.25 \pm 0.12^{c***}$
	AKA/OR/SIM 2	$99.37 \pm 0.22^{a***}$	$95.27 \pm 2.60^{a***}$	$62.54 \pm 1.76^{b***}$	$53.18 \pm 3.36^{c***}$

-: No activity, SD: Standard Deviation, ns: Not statistically significant *P<0.05, **P<0.01, ***P<0.001, [a]AKA: Acarbose, [b]OR: Orlistat, [c]SIM: Simvastatin

To elucidate the phytochemical content of the infusion prepared from the aerial parts of *A. vulgaris*, various phenolic compounds and flavonoids were analyzed qualitatively and quantitatively using reverse-phase HPLC. The analysis revealed that the infusion contained ellagic acid, quercetin-3-*O*-glucoside, and luteolin (Figure 1–5). The analysis revealed that the infusion contained the highest amounts of ellagic acid (2.162% ± 0.010 g/100 g extract) (LOD: 0.611 ppm, LOQ: 1.852 ppm) and quercetin-3-*O*-glucoside (1.745% ± 0.007 g/100 g extract) (LOD: 0.061 ppm, LOQ: 0.184 ppm), while luteolin was present in the lowest amount (0.206% ± 0.000 g/100 g extract) (LOD: 0.008 ppm, LOQ: 0.025 ppm) (Table 3).

Table 3- The amounts of ellagic acid, quercetin-3-*O*-glucoside, and luteolin (g/100g dry infusion) in *A. vulgaris* infusion and retention times (Rt), standard curve, and r^2 values of phenolic compounds

Sample	*Compounds*	*Rt (min)*	*(g/100 g dry extract)*	*Standard curve*	R^2
Infusion	Ellagic acid	30.09	2.162 ± 0.010	y = 187.77x – 556.66	0.9984
	Quercetin-3-*O*-glucoside	30.63	1.745 ± 0.007	y = 65.006x – 40.123	0.9998
	Luteolin	39.09	0.206 ± 0.000	y = 27.688x – 43.08	0.9993

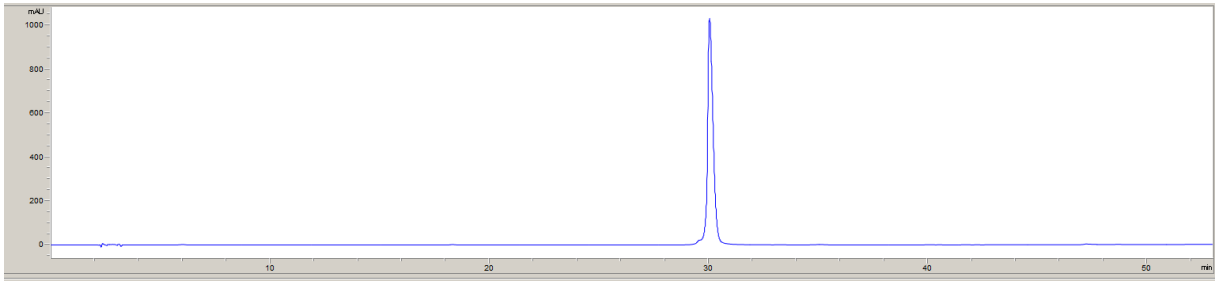

Figure 1- RP-HPLC chromatogram of ellagic acid

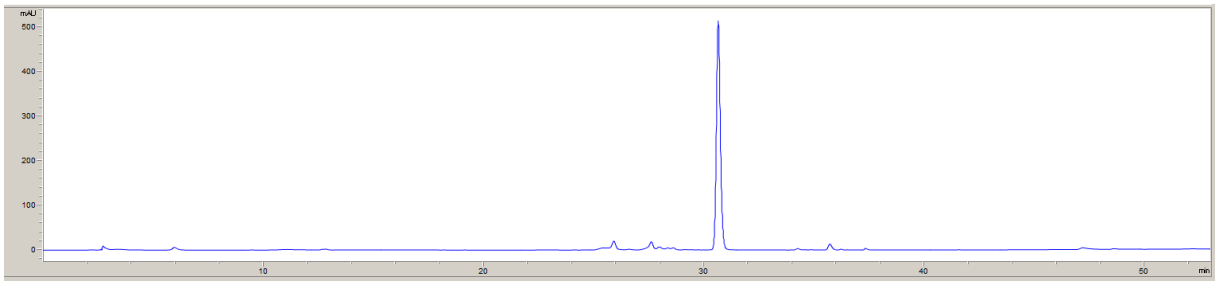

Figure 2- RP-HPLC chromatogram of quercetin-3-*O*-glucoside

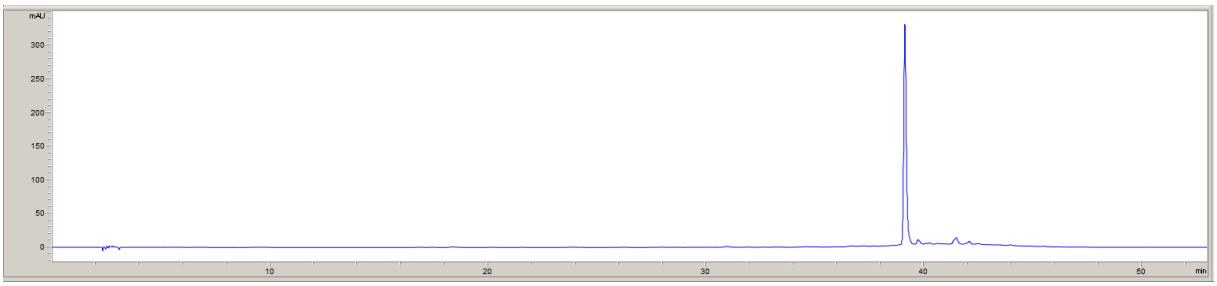

Figure 3- RP-HPLC chromatogram of luteolin

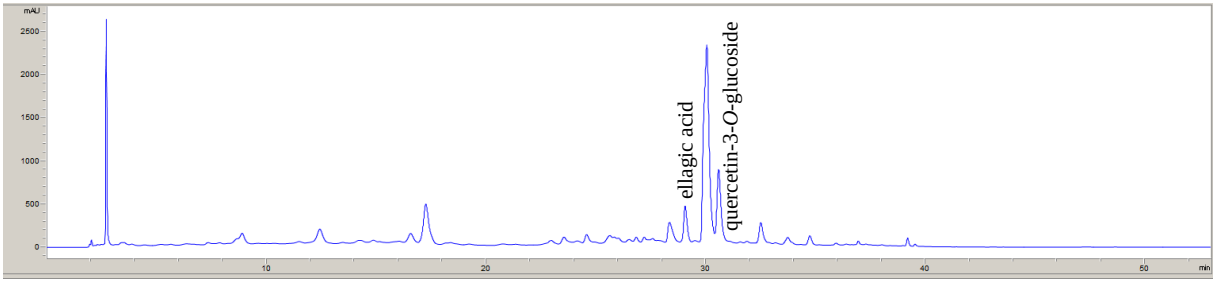

Figure 4- RP-HPLC chromatogram of *A. vulgaris* at 260 nm

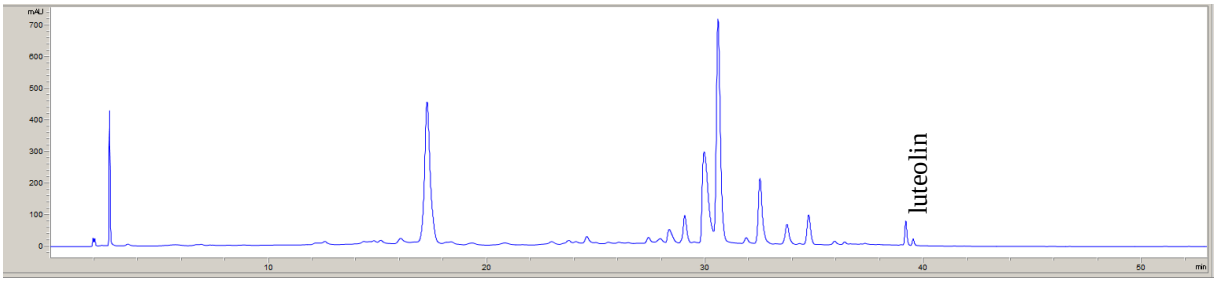

Figure 5- RP-HPLC chromatogram of *A. vulgaris* at 350 nm

Kiselova et al. investigated the antioxidant capacities of infusions prepared from 23 different species, including *A. vulgaris*, using the ABTS radical scavenging activity method. The findings revealed that the *A. vulgaris* infusion exhibited the highest antioxidant potential among 23 species (4.79 ± 0.1 Trolox equivalents) and had a total phenol content of 946.59 ± 2.26 µM quercetin equivalents (Kiselova et al. 2006).

Boroja et al. evaluated the *in vitro* antioxidant, antibacterial, antifungal, and anti-inflammatory potential of methanol extracts prepared from the root and aerial parts of *A. vulgaris* collected in Serbia. They also analyzed the phytochemical contents of the extracts using the HPLC-PDA technique. The total phenol content of the methanol extract was calculated as 58.27 mg GAE/g extract, while the total flavonoid content was 13.30 ± 1.69 mg rutin equivalent/g extract. The DPPH radical scavenging activity of the extract was determined with an IC_{50} value of 5.96 ± 0.21 µg/mL, whereas the IC_{50} value of the reference compound, butylated hydroxytoluene (BHT), was reported as 26.25 ± 1.90 µg/mL. For ABTS radical scavenging activity, the IC_{50} value of the extract was 4.80 ± 2.15 µg/mL, compared to 44.67 ± 3.00 µg/mL for BHT. The reducing power of the extract was reported as 632.99 ± 10.26 mg Trolox equivalent/g extract. HPLC analysis revealed the presence of ellagic acid in the aerial part and catechin in the root part (Boroja et al. 2018).

Neagu et al. calculated the total polyphenol content of *A. vulgaris* aqueous and ethanol extracts as 94.66 ± 8.12 µg gallic acid equivalent/mL and 112.33 ± 6.17 µg gallic acid equivalent/mL, respectively. They evaluated the *in vitro* antioxidant potential of the extracts using DPPH radical scavenging activity and reducing power assays and analyzed their phytochemical contents through HPLC. The ethanol extract demonstrated the highest DPPH radical scavenging activity and reducing power capacity. Qualitative and quantitative analyses of ellagic acid, quercetin-3-*β*-glucoside, and luteolin in the extracts were conducted using HPLC. Ellagic acid was identified as the main compound in the extracts, with its amount calculated as 996.6 µg/mL in the ethanol extract and 15.1 µg/mL in the aqueous extract. Additionally, the amount of quercetin-3-*β*-glucoside in the ethanol extract (20.2 µg/mL) was significantly higher than in the aqueous extract (1.8 µg/mL). Luteolin (4.2 µg/mL) was detected only in the ethanol extract (Neagu et al. 2015).

In a study involving HPLC-DAD and LC-ESI-MS/MS analyses of aqueous acetone extracts prepared from various parts of *A. vulgaris* and *A. mollis* (Buser) Rothm species, 24 compounds were identified in *A. vulgaris* and 27 compounds in *A. mollis*. The major compounds in the phenolic fractions of both species were reported to be monomeric and oligomeric ellagitannins. Additionally, the presence of gallic acid and chlorogenic acid was confirmed in both species. In the flavonoid fraction of the extracts, several quercetin glycosides were identified, with quercetin glucuronide being reported as the main compound (Duckstein et al. 2012). Furthermore, D'Agostino et al. isolated four pure flavonoids, including quercetin-3-*O*-*β*-D-glucopyranoside, from the crude methanol extract of *A. vulgaris* (D'Agostino et al. 1998).

Vlaisavljević et al. investigated the antioxidant and α-amylase inhibitory activities of methanol, ethanol, ethyl acetate, and aqueous extracts of *A. vulgaris* samples collected from Serbia. It was observed that the ethyl acetate extract (0.41 ± 0.03 mmol/g acarbose extract equivalent) had the highest *α*-amylase inhibitory activity among the extracts. The *in vitro* antioxidant capacities of the extracts were evaluated, and it was determined that the ethyl acetate extract showed the highest DPPH and ABTS radical scavenging activity (502.56 ± 0.01 and 283.16 ± 12.36 mg trolox/g extract equivalent, respectively). It was concluded that the methanol extract had the highest metal chelating activity (42.58 ± 0.26 mg EDTA/g extract equivalent), and the ethyl acetate extract had the highest reducing power capacity (8745.31 ± 0.04 mg ascorbic acid/g extract equivalent). The amount of twenty-six phenolic compounds in methanol, ethanol, ethyl acetate, and aqueous extracts was determined by the researchers using the HPLC method, and it was determined that the ethyl acetate extract had high gallic acid, caffeic acid, catechin, and quercetin content (Vlaisavljević et al. 2019). Various researchers have reported that ellagic acid inhibits the *α*-glucosidase enzyme, which plays a role in breaking down complex carbohydrates, such as starch and glycogen, into monomers (You et al. 2012; Yin et al. 2018).

Kurt et al. investigated the effects of a commercial formulation called F13, comprising 13 plant extracts, including *A. vulgaris* extract, on diabetic rats in comparison to acarbose. The study demonstrated that both acarbose and the F13 formulation exhibited comparable ameliorative effects on diabetic complications in streptozotocin-nicotinamide-induced diabetic animals and significantly reduced malondialdehyde levels (Kurt et al. 2012).

Samah et al. investigated the inhibitory effects of methanol, 70% methanol, ethanol, 70% ethanol, hexane, and chloroform extracts from the leaf and flower parts of *A. vulgaris*, *Sophora japonica* L., and *Crataegus azarolus* L. on pancreatic lipase and *α*-amylase enzymes. The researchers found that extracts prepared with polar solvents generally exhibited strong inhibitory activity. Specifically, methanol extracts showed the highest inhibition against pancreatic lipase, while 70% methanol extracts displayed the strongest inhibitory effect on *α*-amylase activity. Conversely, extracts prepared with non-polar solvents such as hexane and chloroform showed weaker inhibition. Additionally, it was reported that *A. vulgaris* leaf and flower extracts had stronger effects on these enzymes compared to those from *S. japonica* and *C. azarolus* (Samah et al. 2018).

Swanston-Flatt et al. investigated the impact of *A. vulgaris* on glucose regulation in both normal and streptozotocin-induced diabetic mice. Their findings revealed that the extract did not influence food and fluid consumption, body weight gain, or plasma glucose and insulin levels in healthy mice. Furthermore, the extract had no effect on the onset of hyperphagia, polydipsia, weight loss, hyperglycemia, or hypoinsulinemia in diabetic mice (Swanston-Flatt et al. 1990).

Said et al. conducted a study to assess the impact of a four-herb extract mixture, known as Weighlevel, which included *A. vulgaris* leaf extract, on weight loss in both animal and human subjects. In the animal model, chickens administered the mixture once a week for 4 weeks exhibited significant weight loss compared to the control group. In a clinical trial involving 66 volunteers with a body mass index (BMI) of 30.67 ± 2.14 kg/m² over a 3-month period, the mixture resulted in significant weight loss without any reported adverse effects. Notably, participants with a BMI between 25 and 30 kg/m² (overweight) showed more pronounced weight loss than those with a BMI greater than 30 kg/m² (obese). After 3 months, the BMI of the overweight group decreased from 28.5 ± 1.2 kg/m² to 24.5 ± 1.4 kg/m², while the obese group experienced a reduction from 32.1 ± 1.8 kg/m² to 27.5 ± 2.2 kg/m². Based on these results, the researchers concluded that the Weighlevel mixture was both safe and effective for weight loss (Said et al. 2011).

4. Conclusions

This study underscores the significant potential of *A. vulgaris*, cultivated by organic farming techniques, as a natural therapeutic agent. Notably, the infusion showed superior ABTS radical scavenging and metal chelating activities compared to standard reference compounds, demonstrating its strong antioxidant potential. In enzyme inhibition studies, the infusion exhibited significant inhibition of the *α*-glucosidase enzyme in a concentration-dependent manner, making it a promising candidate for managing postprandial hyperglycemia. However, it showed moderate inhibition of pancreatic cholesterol esterase and limited activity against the *α*-amylase enzyme. The lack of pancreatic lipase inhibition suggests the infusion may not be suitable for lipid absorption modulation. Phytochemical analysis revealed that the major bioactive compounds in *A. vulgaris* infusion are ellagic acid, quercetin-3-*O*-glucoside, and luteolin. Among these, ellagic acid was identified as the most abundant compound, aligning with its known role in antioxidant and *α*-glucosidase inhibitory activities. While *in vitro* results are promising, additional *in vivo* and clinical studies are required to confirm the efficacy and safety of *A. vulgaris* infusion, particularly in glucose regulation, lipid metabolism, and weight management. Given its strong antioxidant potential and enzyme inhibitory activities, *A. vulgaris*

infusion could be explored as a functional food ingredient or nutraceutical for managing oxidative stress-related and metabolic disorders. Organic farming practices enhance the safety, quality, and standardization of plant materials, thereby promoting the sustainable use of medicinal plants while also playing a crucial role in biodiversity conservation

Acknowledgments

We would like to express our gratitude to Temmuz Organic Farm for supplying the plants cultivated through organic farming practices.

Author Declarations

Authors' contributions: Concept: S.P., B.Ö., D.D.O., Design: S.P., B.Ö., D.D.O., Data collection: S.P., B.Ö., D.D.O., Analysis: S.P., B.Ö., D.D.O., Writing: S.P., B.Ö., D.D.O.

Conflict of interest/Competing interests: The author(s) declare no conflict of interest.

Ethics approval/declarations: No need/ Not applicable.

Funding: This research received no external funding.

Consent to participate: No need/ Not applicable.

Consent for publication: No need/ Not applicable.

Data availability: Data are available on request due to privacy or other restrictions.

References

Acet T & Özcan K (2018). Determination of antioxidant and antimicrobial properties of *Alchemilla ellenbergiana* extracts. *Gümüşhane Üniversitesi Fen Bilimleri Enstitüsü Dergisi* 8(1): 113-121. doi: 10.17714/gumusfenbil.308430 (In Turkish)

Boroja T, Mihailović V, Katanić J, Pan S P, Nikles S, Imbimbo P & Bauer R (2018). The biological activities of roots and aerial parts of *Alchemilla vulgaris* L. *South African Journal of Botany* 116: 175-184. doi: 10.1016/j.sajb.2018.03.007

D'Agostino M, Dini I, Ramundo E & Senatore F (1998). Flavonoid glycosides of *Alchemilla vulgaris* L. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 12(S1): S162-S163. doi: 10.1002/(SICI)1099-1573(1998)12:1+<S162::AID-PTR284>3.0.CO;2-P

Dinis T C P, Maderia V M C & Almeida L M (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics* 315(1): 161-169. doi: 10.1006/abbi.1994.1485

Duckstein S M, Lotter E M, Meyer U, Lindequist U & Stintzing F C (2012). Phenolic constituents from *Alchemilla vulgaris* L. and *Alchemilla mollis* (Buser) Rothm. at different dates of harvest. *Zeitschrift für Naturforschung C* 67(11-12): 529-540.

Gök H N, Orhan N, Özüpek B, Pekacar S, Selvi Ş N & Orhan D D (2021). Standardization of *Juniperus macrocarpa* Sibt. & Sm. and *Juniperus excelsa* M. Bieb. extracts with carbohydrate digestive enzyme inhibitory and antioxidant activities. *Iranian Journal of Pharmaceutical Research: IJPR* 20(3): 441. doi: 10.22037/ijpr.2021.114838.15055

Hamamcıoğlu A C (2017). Oxidative stress in diabetes and the role of antioxidants. *Türkiye Diyabet ve Obezite Dergisi* 1(1): 7-13. (In Turkish)

Hussain A M Z I, Hydrie M Z I, Claussen B & Asghar S (2010). Type 2 Diabetes and obesity: A review. *Journal of Diabetology 2*(1): 1-7. doi:10.4103/2078-7685.197547

Jakimiuk K & Tomczyk M (2024). A review of the traditional uses, phytochemistry, pharmacology, and clinical evidence for the use of the genus *Alchemilla* (Rosaceae). *Journal of Ethnopharmacology* 320: 117439. doi: 10.1016/j.jep.2023.117439

Jung H A, Jin S E, Choi R J, Manh H T, Kim Y S, Min B S, Son Y K, Ahn B R, Kim B W, Sohn H S & Choi J S (2011). Anti-tumorigenic activity of sophoflavescenol against Lewis lung carcinoma *in vitro* and *in vivo*. *Archives of Pharmacal Research* 34(12): 2087-2099. doi: 10.1007/s12272-011-1212-y

Jurić T, Stanković J S K, Rosić G, Selaković D, Joksimović J, Mišić D & Mihailović V (2020). Protective effects of *Alchemilla vulgaris* L. extracts against cisplatin-induced toxicological alterations in rats. *South African Journal of Botany* 128: 141-151. doi: 10.1016/j.sajb.2019.09.010

Kiselova Y, Ivanova D, Chervenkov T, Gerova D, Galunska B & Yankova T (2006). Correlation between the *in vitro* antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 20(11): 961-965. doi: 10.1002/ptr.1985

Kosalec I, Bakmaz M, Pepeljnjak S & Vladimir-Knezevic S (2004). Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta Pharmaceutica* 54(1): 65-72

Kurt H, Ozbayer C, Degirmenci I, Ustuner M C, Ozden H, Civi K & Gunes H V (2012). Comparative therapeutic potentials of acarbose and a formulated herbal extract on type 2 diabetic rats. *African Journal of Pharmacy and Pharmacology* 6(29): 2194-2204. doi: 10.5897/ajpp12.296

Lee Y M, Kim Y S, Lee Y, Kim J, Sun H, Kim J H & Kim J S (2012). Inhibitory activities of pancreatic lipase and phosphodiesterase from Korean medicinal plant extracts. *Phytotherapy Research* 26(5): 778-782. doi: 10.1002/ptr.3644

Neagu E, Paun G, Albu C & Radu G L (2015). Assessment of acetylcholinesterase and tyrosinase inhibitory and antioxidant activity of *Alchemilla vulgaris* and *Filipendula ulmaria* extracts. *Journal of the Taiwan Institute of Chemical Engineers* 52: 1-6. doi: 10.1016/j.jtice.2015.01.026

Ngamukote S, Mäkynen K, Thilawech T & Adisakwattana S (2011). Cholesterol-lowering activity of the major polyphenols in grape seed. *Molecules* 16(6): 5054-5061. doi: 10.3390/molecules16065054

Orhan N, Deliorman Orhan D, Gökbulut A, Aslan M & Ergun F (2017). Comparative analysis of chemical profile, antioxidant, *in-vitro* and *in-vivo* antidiabetic activities of *Juniperus foetidissima* Willd. and *Juniperus sabina* L. *Iranian Journal of Pharmaceutical Research* 16(Special Issue): 64-74

Özenoğlu S, Turan İ, Özaçmak H S & Özaçmak V H (2020). Investigation of the effect of melatonin on Nrf2 expression and oxidative stress in heart and skeletal muscle of experimentally induced diabetic rats. *Türkiye Diyabet ve Obezite Dergisi* 4(1): 46-53. doi: 10.25048/tudod.595479 (In Turkish)

Said O, Saad B, Fulder S, Khalil K & Kassis E (2011). Weight loss in animals and humans treated with "weigh level," a combination of four medicinal plants used in traditional Arabic and Islamic medicine. *Evidence-Based Complementary and Alternative Medicine 2011*.

Samah S, Abdullah K & Ream N (2018). Phytochemical screening of *Alchemilla vulgaris, Sophora Japonica, Crataegus azarolus*, and their inhibitory activity on lipase and *α*-amylase. *International Journal of Academic Scientific Research* 6: 1-21

Swanston-Flatt S K, Day C, Bailey C J & Flatt P R (1990). Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. *Diabetologia* 33(8): 462-464. doi: 10.1007/BF00405106

Tadić V M, Krgović N & Žugić A (2020). Lady's mantle (*Alchemilla vulgaris* L., Rosaceae): A review of traditional uses, phytochemical profile, and biological properties. *Lekovite Sirovine* 40: 66-74. doi: 10.5937/leksir2040066T

Vlaisavljević S, Jelača S, Zengin G, Mimica-Dukić N, Berežni S, Miljić M & Stevanović Z D (2019). *Alchemilla vulgaris* agg. (Lady's mantle) from central Balkan: antioxidant, anticancer and enzyme inhibition properties. RSC Advances9(64): 37474-37483. doi: 10.1039/C9RA08231J

Yavuz A & Erdoğan Ü (2019). Production quantity and evaluation of organic medicinal and aromatic plants in Turkey. *Bayburt Üniversitesi Fen Bilimleri Dergisi* 2(1): 124-130. (In Turkish)

Yin P, Yang L, Xue Q, Yu M, Yao F, Sun L & Liu Y (2018). Identification and inhibitory activities of ellagic acid-and kaempferol-derivatives from Mongolian oak cups against *α*-glucosidase, *α*-amylase and protein glycation linked to type II diabetes and its complications and their influence on HepG2 cells' viability. *Arabian Journal of Chemistry* 11(8): 1247-1259. doi: 10.1016/j.arabjc.2017.10.002

You Q, Chen F, Wang X, Jiang Y & Lin S (2012). Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase. *LWT-Food Science and Technology* 46(1): 164-168. doi: 10.1016/j.lwt.2011.10.01

Zongo C, Savadogo A, Ouattara L, Bassole I H N, Ouattara C A T, Ouattara, A S, Barro N, Koudou J & Traore A S (2010). Polyphenols content, antioxidant and antimicrobial activities of *Ampelocissus grantii* (Baker) Planch. (Vitaceae): a medicinal plant from Burkina Faso, *International Journal of Pharmacology* 6(6): 880-887. doi: 10.3923/ijp.2010.880.887