https://doi.org/10.31925/farmacia.2020.6.13 **ORIGINAL ARTICLE**

BIOLOGICAL ACTIVITIES AND PHYTOCHEMICAL SCREENING OF ETHANOL EXTRACTS FROM *ADONIS PARYADRICA* (*RANUNCULACEAE*)

OSMAN UCUNCU [1]*, CEMALETTIN BALTACI [2], ZEYNEP AKAR [3], AZER OZAD DUZGUN [3], MUSTAFA CUCE [4], ALI KANDEMIR [5]

[1]*Department of Food Processing, Maçka Vocational School, Karadeniz Technical University, 61750 Trabzon, Turkey*
[2]*Department of Food Engineering, Faculty of Engineering and Natural Sciences, Gümüşhane University, 29100 Gümüşhane, Turkey*
[3]*Department of Genetic and Bioengineering, Faculty of Engineering and Natural Sciences, Gümüşhane University, 29100 Gümüşhane, Turkey*
[4]*Department of Food Technology, Şebinkarahisar School of Applied Sciences, Giresun University, 28400 Giresun, Turkey*
[5]*Department of Biology, Faculty of Arts and Sciences, Erzincan Binali Yıldırım University, 24100 Erzincan, Turkey*

**corresponding author: osmanucuncu@yahoo.com*

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Abstract

In this study, secondary metabolite profiles of flower, leaf and root of *Adonis paryadrica* ethanolic extracts were performed by using a newly developed and validated LC-MS/MS method. For this purpose, the LC-MS/MS system was used for the quantitative and qualitative analysis of 37 phytochemicals. The main component of the flower, leaf and root extracts was the quinic acid (160, 111 and 64 mg/kg, respectively). Antioxidant activities were determined using methods such as DPPH (SC_{50}, mg/mL), FRAP (µM TEAC), CUPRAC (µM TEAC), ABTS (SC_{50}, mg/mL) and TPC (GAE, µg/mL). While the highest antioxidant activity value for FRAP, CUPRAC, ABTS and TPC methods was determined in the leaf part of the plant, this value was determined in the flower part for DPPH. The antibacterial effects of the plant extracts were determined by liquid microdilution and agar diffusion methods. As a result, antimicrobial effect of flowers, leaf and all sections of plant extracts were determined except plant root extracts which did not display any activity against bacteria. Antimicrobial activity of the plant flower extract was found to be higher especially against to *P. vulgaris* with 3.5 mg/mL MIC.

Rezumat

În acest studiu, au fost evaluați metaboliții secundari din extractele etanolice provenite de la florile, frunzele și rădăcinile de *Adonis paryadrica*, utilizând o metodă LC-MS/MS. În acest scop, s-au pus în evidență 37 fitocompuși, componenta principală fiind acidul quinic (160, 111 și respectiv 64 mg/kg). Activitatea antioxidantă a extractelor a fost determinată prin metode precum *DPPH, FRAP, CUPRAC, ABTS* și *TPC*. Extractul de frunze a prezentat activitatea antioxidantă cea mai pronunțată prin metodele FRAP, CUPRAC, ABTS și TPC, iar extractul alcoolic de flori prin metoda DPPH. Efectele antibacteriene ale extractelor au fost determinate prin metoda microdiluției și cea difuzimetrică. Cu excepția extractului de rădăcină, toate celelalte au prezentat activitate antibacteriană. Cea mai mare valoare a CMI pentru *P. vulgaris* a fost obținută pentru extractul de flori.

Keywords: *Adonis paryadrica*, antimicrobial activity, antioxidant activity, LC-MS/MS, phytochemical screening, secondary metabolite

Introduction

Ranunculaceae Juss. family is represented by 2.000 taxa under 50 genera in the world [30]. The family includes 20 genera and 236 taxa in Turkey [8]. The genus *Adonis* L. is one of the genera of *Ranunculaceae* with annual or perennial taxa. Thirty species of *Adonis* are known in Asia, Europe and North America as well as in other areas of the world from Southwest Asia to North Africa and the Mediterranean coast [16, 28]. Turkey has 10 taxa and only one of them is endemic. The endemic *Adonis paryadrica* (Boiss.) Kandemir & Aytaç stat. nova. is the subject of this study. It was collected by Pierre de Tchihatcheff from the subalpine zone in Giresun-Turkey in 1858. No *A. paryadrica* sample has been collected since its identification. The specimens belonging to *A. paryadrica* were recollected within the scope of a project supported by Ministry of Agriculture and Forestry of the Republic of Turkey.

Important biological activities due to their high secondary metabolite contents such as alkaloids, saponins and steroids on the species of the *Ranunculaceae* have been reported by researchers [9, 12]. Furthermore, the family species are included in the group of medicinal and aromatic plants by the same researchers [9, 12]. Members of the *Ranunculaceae* have a rich content

of cardioactive steroids and cardiac glycosides which have been identified as a result of toxic poisoning. It has been reported that the members of the *Adonis* genus are not appropriate for cultivation due to the presence of toxic alkaloids. The extracts and active components of *A. vernalis*, *A. volgensis*, *A. courule* and *A. amurensis* exhibit widespread pharmacological properties (antiinflammatory, cardiovascular, antiviral, antiangiogenic and diuretic effects, and antioxidant, antibacterial and acaricidal activities) [21]. Members of this genus have been reported to cause toxicity due to their adonitoxin and other cardiac glycoside (cardenolides) content [14, 28]. *A. amurensis* is used in traditional medicine to treat various diseases such as cardiac insufficiency and oedema [19, 20].

Although some taxonomic studies have been conducted on the members of the genus [7, 23], there is a limited number of studies on bioactive components of this genus [12]. *A. paryadrica*, an endemic species of Turkey, has not been the subject of a study until now. It was aimed in the present study to explore the secondary metabolite contents, antioxidant and antimicrobial activities of the ethanolic extracts of the *A. paryadrica*.

Materials and Methods

Plant materials

The plant material of *Adonis paryadrica* was collected from the environs of Demirözü village in Şebinkarahisar, Giresun (at a height of *ca.* 1920 m) in Turkey in May 2018. The plant identified by Prof. Ali Kandemir at Erzincan Binali Yıldırım University, Department of Botany, in May 2018. The voucher specimen (No. Cüce, Gültepe & Güzel KTUB795) has been maintained in the the Herbarium of the Department of Biology, Karadeniz Technical University, Trabzon, Turkey.

Chemicals

The reagents; 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet+}$), 2,2-diphenyl-1-picryl-hydrazyl ($DPPH^{\bullet}$), 2,4,6-tripyridyl-s-triazine (TPTZ), neocuproine (2,9-dimethyl-1,10-phenanthroline), potassium persulfate ($K_2S_2O_8$) and standards (Trolox® and gallic acid) were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany). Ammonium acetate, copper (II) chloride, Folin-Ciocalteu reagent, HPLC-grade ethanol (≥ 99.5%) and methanol (≥ 99.9%) were also purchased from Merck Company (Darmstadt, Germany). All other reagents used are of HPLC gradient grade purity and were purchased from Merck Company (Darmstadt, Germany).

Extraction

25.0 g of each shade-dried parts (flower, leaf, root and all sections) of A. *paryadrica* were ground and subjected to extraction with 90% ethanol in a Soxhlet apparatus (4 hours). The ethanol extracts of the plant were evaporated to dryness under reduced pressure on a rotary vacuum evaporator. The dry-crude ethanolic extracts were kept in the dark at -20°C in sealed brown vials until testing.

LC-MS/MS Instrument and Chromatographic Conditions

Quantitative analysis was carried out using an LC-MS/MS system (Shimadzu Nexera model UHPLC coupled to Shimadzu LC-MS 8040 model triple quadrupole mass spectrometer). The separation of thirty-seven phytochemicals was conducted on Inertsil ODS-4 model C18 (100 mm × 2.1 mm, 2µm) column, USA). The mobile phase is comprised of A (10 mM ammonium formate - 0.1% formic acid) and B, (acetonitrile). The applied gradient profiles were optimized as 5% - 20% B (0 - 10 min), 20% B (10 - 22 min), 20% - 50% B (22 - 36 min), 95% B (36 - 40 min), 5% B (40 - 50 min), was 0.25 mL/min and the injection volume was 4 µL. The optimum ESI parameters for the mass spectrometer were determined as: 350ºC interface temperature, 250ºC DL temperature, 400ºC heat block temperature, 3 L/min and 15 L/min were nebulizer and drying gas (N_2) flow rates, respectively [31].

Determination of biological activities

UV-1800 (Shimadzu, Japan) spectrophotometer was used to measure absorbances in all of the antioxidant analyses.

DPPH Radical Scavenging Activity. $DPPH^{\bullet}$ (1,1-diphenyl-2-picrilhydrazil) radical scavenging activity is one of the most frequently used methods in studies carried out for determining the antioxidant activity [5]. It is based on colour phase alternation in purple formed by $DPPH^{\bullet}$ with the interaction of antioxidants and radicals after which absorbance value decreases. Three measurements were performed for each sample and the average values were calculated. As a starting step, 750 µL of 100 µM methanolic DPPH radical solution was added to the sample solutions (750 µL). Afterwards, mixture of DPPH radical solution and sample solutions were stirred for 60 min at room temperature. The absorbance of the mixture solution was determined afterwards in using the spectrophotometer as 517 nm. Different sample concentrations were applied on the DPPH radical and changes in its absorbance were measured. Graphs were plotted for absorbance values with corresponding concentrations. The amount of sample that drops the $DPPH^{\bullet}$ concentration in half was determined as µg/mL (R^2 = 0.9986 for DPPH) for the equation y = ax + b. The results were expressed as SC_{50} with lower values indicating higher radical scavenging potential.

Ferric Reducing Antioxidant Power (FRAP). The method developed by Benzie and Strain was used to determine antioxidant activity of the samples [3]. In the study, Trolox was used as antioxidant standard and it was studied at 6 different concentrations (1000, 500, 250, 125, 62.5 and 31.25 µM and R^2 = 0.999 for FRAP). First, the sample (50 µL) and standard solutions were pipetted to the tubes in triplicates. At the same time 50 µL of methanol were transferred to the reagent

blank tubes as sample solvents. Sample solutions and 1.5 mL of FRAP solvent (60% methanol in distilled water) were then added to the sample blank tubes in volumes of 50 µL respectively. 1.5 mL of freshly prepared FRAP reagents were added to all the tubes excluding the sample blank tubes. In addition, the same volume of FRAP reagent was added to all tubes excluding the sample blank tubes after which all tubes were subjected to vortex. The absorbance values of the samples were read after 20 minutes at 595 nm using a spectrophotometer. The results were compared with standard antioxidant Trolox and calculated as µM TEAC.

Cupric Ion Reducing Capacity (CUPRAC). The method developed by Apak *et al*. was modified and applied to the sample for determining the antioxidant capacity [2]. Initially, Cu (II) chloride solution and neocuproine solution were prepared with 96% ethyl alcohol, ammonium acetate buffer (pH = 7) and analysis solutions were transferred to the test tubes in equal volume respectively. Final solution volumes were adjusted to 4.1 mL. All tubes were incubated for 30 minutes at room temperature and the absorbance values were measured at 450 nm using a spectrophotometer. The Trolox antioxidant standard was used at the same concentrations (1000, 500, 250, 125, 62.5 and 31.25 µM and R^2 = 0.9985 for CUPRAC) as in the FRAP method and calibration graph was drawn for Trolox. The antioxidant capacity of the samples based on the equivalent of Trolox was calculated in µM TEAC.

ABTS Radical Scavenging Activity. ABTS method preferred in the study is one of the commonly used antioxidant capacity determination methods [20]. In this method, 7 mM ABTS stock solution mixture was prepared in a solvent of ethanol:water (5:1) and it was mixed with 2.45 mM potassium persulfate solution dissolved in ethanol:water (1:3). This mixture was stored at room temperature in a dark environment for 18 hours to improve the ABTS radical cation ($ABTS^{\bullet+}$). Finally, it was diluted with 1/40 of ethanol: water (3:2). The absorbance at 734 nm was adjusted to 0.07. The extracts of four different parts of the plant (flower, leaf, root and all parts) and the standard antioxidant Trolox solution used for comparison were pipetted in triplicate. R^2 values were calculated for each test by applying the function y = ax + b and 0.9995 for the ABTS radical. The absorbance values of the reaction mixtures were measured at 734 nm after 20 minutes. The sample quantity that reduces the ABTS concentration in half was then calculated in mg/mL and the results were expressed as SC_{50}.

Total Phenolic Content. The total phenolic content of the flower, leaf, root and all parts of the *Adonis paryadrica* was determined using Folin-Ciocalteu reagent and modified version of the method developed by Slinkard and Singleton [22]. 50 µL of sample solution was diluted with 2.5 mL of distilled water and 250 µL of 0.2 N Folin-Ciocalteu reagent was added to it. Subsequently, 750 µL of Na_2CO_3 (7.5%) was added to the mixture and vortexed. These mixture tubes were incubated at room temperature for 2 hours and the absorbance values were determined at 765 nm. The standard calibration graph was obtained by using gallic acid prepared at six different concentrations (1000, 500, 250, 125, 62.5 and 31.25 µg/mL and R^2 = 0.9993). The amounts of phenolic compounds of the samples were determined as equivalent of the gallic acid (GAE µg/mL) according to the function of line obtained from the graph.

Test Organisms

Bacteria were obtained from the Department of Genetics and Bioengineering, Faculty of Engineering and Natural Sciences, Gumushane University. Three Gram-positive (BS: *Bacillus subtilis* ATCC 6633, SA: *Staphylococcus aureus* ATCC 25923 and SP: *Streptococcus pyogenes* ATCC 19615) and four Gram-negative bacterial strains (EC: *Escherichia coli* ATCC 25922, PA: *Pseudomonas aeruginosa* ATCC 43288, PV: *Proteus vulgaris* ATCC 13315, YP: *Yersinia pseudotuberculosis* ATCC 911) were used in this study.

Agar Diffusion Assay

The agar diffusion test was performed in accordance with Irshad *et al*., [10]. Holes were punched using sterile hole openers (6 mm in diameter) and the sample was loaded in each hole (50 µL). 50µL of ampicillin was loaded to the central hole. Ampicillin was used as the positive control. The prepared Petri dishes were kept at room temperature for 2 hours which were then placed face up in the incubator to be subject to incubation at 37°C for 24 hours, and the inhibition zone diameters were measured with scale (± 1.28 standard error calculated by SigmaPlot commercial software).

Determination of Minimum Inhibition Concentration

The minimum inhibitory concentrations (MIC) of the plant extracts against to three Gram (+) and four Gram (-) bacterial strains were determined using the liquid microdilution method. DMSO solutions of the dried ethanol extracts of the plant were prepared for antimicrobial studies. The concentrations of extracts are 100 mg/mL (serial two fold dilution was performed) obtained from the flower, leaf, root and all section of the plant. Ampicillin used as a control. MIC value of the DMSO flower, leaf, root and all section extracts were determined in the 96-well plates and triplicate (±3.5 standard error calculated by SigmaPlot commercial software).

Statistical analysis

Data were analysed using Microsoft Excel software with XLSTAT (14-Day Free Trial & Free Version). All the experimental results were presented as mean values ± SD of triplicate measurements. The data were evaluated by using the analysis of variance (ANOVA). Significant differences in groups were indicated at $p < 0.05$.

Results and Discussion

LC-MS/MS results

Secondary metabolite profiles of ethanol extracts of flower, leaf and root of *A. paryadrica* were identified using the LC-MS/MS system. For this purpose, qualitative and quantitative analyses were performed on a total of 37 standard chemicals (quinic, malic, fumaric, gallic, protocatechuic, chlorogenic, 4-OH-benzoic, vanillic, caffeic, syringic, salicylic, ferulic, *p*-coumaric, sinapinic, rosmarinic, *o*-coumaric and cinnamic acids; rhoifolin, quercitrin, apigetrin, fisetin, coumarin, myricetin, liquiritigenin, quercetin, luteolin, naringenin, apigenin, hesperetin, kaempferol, vanillin, rutin, hesperidin, nicotiflorin, isoquercitrin, pyrocatechol and chrysin) to determine the secondary metabolite content of *A. paryadrica*. The phytochemicals (organic acids (quinic acid, protocatechuic acid, salicylic acid and *p*-coumaric acid) flavon glycosides (isoquercitrin and apigetrin), and flavonoids (quercetin, luteolin, naringenin and apigenin)) were detected in different parts of the plant through LC-MS/MS system (Table I).

Table I

Determined quantitative results for organic acid, phenolic and flavonoid analysis of extracts by LC-MS/MS (mg/kg extracts)

		Conc. (mg/kg extract)			
		Flower	**Leaf**	**Root**	***Class***
1	Quinic acid	160.02 ^{a} ± 12.23	111.09 ^{b} ± 16.02	65.21 ^{c} ± 4.17	Organic acid
2	Protocatechuic acid	24.03 ^{a} ± 2.12	40.33 ^{b} ± 4.21	55.02 ^{c} ± 3.55	Organic acid
3	Salicylic acid	N.D.	N.D.	12.08 ^{c} ± 0.98	Organic acid
4	*p*-Coumaric acid	N.D.	68.04 ^{b} ± 4.11	N.D.	Organic acid
5	Isoquercitrin	21.43 ^{a} ± 1.22	N.D.	N.D.	Flavon glycoside
6	Apigetrin	10.32 ^{a} ± 0.93	23.12 ^{b} ± 3.02	N.D.	Flavon glycoside
7	Quercetin	15.11 ^{a} ± 1.21	N.D.	N.D.	Flavonoid
8	Luteolin	30.67 ^{a} ± 5.65	15.32 ^{b} ± 1.12	N.D.	Flavonoid
9	Naringenin	6.04 ^{a} ± 0.43	3.05 ^{b} ± 0.43	N.D.	Flavonoid
10	Apigenin	4.02 ^{a} ± 0.16	4.33 ^{a} ± 0.32	N.D.	Flavonoid

N.D. = Not detected; results are presented as means; ± standard deviations; all phenolic and flavanoid compounds were expressed as mg/kg; different letters (a - c) in the same horizontal lines are significantly different at the 5% level ($p < 0.05$)

According to the literature survey conducted, more than 120 phytochemicals such as cymarin, adonitoxin, alepposide A-D, amurensioside A-K, luteolin, orientin, apigenin, kaempferol, scopoletin, isoquercitrin, adoligose A-E and linolenic acid have been reported from the genus *Adonis* with wide pharmacological activities [21]. In our study, different amounts of isoquercitrin, luteolin and apigenin were determined in accordance with the literature.

The main component of *A. paryadrica* was found as quinic acid from among 37 chemical standarts. Quinic acid amounts of flower, leaf and root of *A. paryadrica* were 160, 111 and 65 mg/kg, respectively. Quinic acid has been shown to possess radioprotection, antiHIV, antineuroinflammatory, antioxidant activities, inhibition of mutagenesis and carcinogenesis and astringent effect [11, 13]. Significant differences ($p < 0.05$) were found among the flower, leaf and root in the analysis in terms of phenolic and flavonoid substances.

Antioxidant activities

Antioxidant activities of flower, leaf, root and all parts of *A. paryadrica* were studied at 40 mg/mL concentration. Five methods used in the study are the most common preferred antioxidant methods among all antioxidant activity determination methods in literature. There are many studies conducted to determine the activity of natural and synthetic compounds using these selected activity methods [1, 27].

According to the results of the antioxidant activity tests, it was found that all parts of the plant display antioxidant activity (Table II). The results of the four other activity methods except DPPH$^{\bullet}$ were similar with an overall ranking as Leaf > All > Root > Flower. However, the order for the DPPH$^{\bullet}$ test was Flower > All > Leaf > Root. The reaction mechanisms, reaction rates and interactions of the antioxidant activity methods are different. Therefore, activity differences between methods can be seen among the same samples [4]. To date, no study has been carried out on antioxidant activity of *A. paryadrica*. However, a few studies have been conducted on some species of *Adonis* genus with regard to biological activity and chemical composition of essential oils [24, 29]. In these studies, antioxidant activities of plant extracts prepared with different solvents were determined with using different methods. The results of these different methods displayed significant levels of antioxidant activity of the plant extracts [6, 15]. As an example, the antioxidant activities of the aqueous and ethanolic extracts of *A. palaestina* were determined using ABTS and total phenolic methods and it was reported that the extracts exhibit activities in both methods [25]. In addition, significant results were obtained during the activity analyses of 90% ethanolic extract of *A. wolgensis* by way of various antioxidant methods [17].

Table II

Values of antioxidant activity of different parts of *Adonis paryadrica*

Extracts	DPPH	FRAP	CUPRAC	ABTS	TPC
Flower	0.144 [a] ± 0.02	945.24 [a] ± 0.05	2.052 [a] ± 0.03	0.630 [a] ± 0.05	333.26 [a] ± 0.06
Leaf	0.216 [b] ± 0.07	4078.57 [b] ± 0.11	5.781 [b] ± 0.08	0.350 [b] ± 0.01	465.56 [b] ± 0.08
Root	0.312 [c] ± 0.02	1976.19 [c] ± 0.07	2.270 [a] ± 0.04	0.395 [b] ± 0.04	392.49 [c] ± 0.05
All sections	0.173 [d] ± 0.01	2345.24 [d] ± 0.09	3.048 [d] ± 0.08	0.413 [d] ± 0.02	414.28 [c] ± 0.07

Means ± standard deviations; antioxidant activities of samples were expressed: as SC_{50} (mg/mL) for DPPH Radical Scavenging Activity, as µM TEAC for FRAP, as µM TEAC for DPPH, as SC_{50} (mg/mL) for ABTS, as GAE µg/mL for TPC; different letters (a-d) in the same columns are significantly different at the 5% level ($p < 0.05$); TPC = Total phenolic capacity; TAC = Total Antioxidant capacity, DPPH = Scavenging Activity; FRAP = Ferric reducing antioxidant power; CUPRAC = Cupric reducing antioxidant capacity; ABTS = Radical Scavenging Activity

Antimicrobial activities

According to the results of the agar well diffusion test, bacteria were inhibited by all parts including the flower and leaf extract, excluding the DMSO extract of the plant root which did not display antimicrobial activity against to bacteria. *P. aeruginosa* and *S. pyogenes* were not inhibited by plant extracts. The inhibition zones against the tested bacteria ranged from 6 to 14 mm. The highest inhibition zone (14 mm) against to *Y. pseudotuberculosis* was the flower extract. The inhibition zone of leaf extracts for *Bacillus subtilis* was 6 mm. All sections of plant the extract inhibited only *E. coli* with an inhibition zone of 10 mm. Flower extract displayed antimicrobial activity to against *S. aureus* and *P. vulgaris*. Inhibition zone of the flower extract was 9 mm and 13 mm (Table III).

Table III

Screening for antimicrobial activity of the ethanolic extracts of *A. paryadrica*

	Plant extracts (zone), (mm)				Plant Extracts (MIC), (mg/mL)				+ Control/ AMP	- Control/ DMSO
Bacteria	**Flower**	**Leaf**	**Root**	**All of section**	**Flower**	**Leaf**	**Root**	**All of section**		
PA	-	-	-	-	-	-	-	-	30	-
EC	-	-	-	10	-	-	-	26.5	-	-
BS	-	6	-	6	-	10.5	26.5	-	30	-
YP	14	-	-	-	7	-	-	-	30	-
SA	9	-	-	-	14	-	-	-	30	-
PV	13	-	-	-	3.5	-	-	26.5	30	-
SP	6	-	-	-	-	-	-	26.5	30	-

PA: *Pseudomonas aeruginosa* ATCC43288, EC: *Escherichia coli* ATCC 25922, BS: *Bacillus subtilis* ATCC 6633, YP: *Yersinia pseudotuberculosis* ATCC 911, SA: *Staphylococcus aureus* ATCC 25923, PV: *Proteus vulgaris* ATCC 13315, SP: *Streptococcus pyogenes* ATCC 19615

MICs of plant extracts were found to be between 3.5 - 26.5 mg/mL. The flower extract display a very potent activity against *P. vulgaris* with 3.5 mg/mL MIC. MIC values of the plant flower extracts for *S. aureus* and *Y. pseudotuberculosis* were 14 mg/mL and 7 mg/mL respectively. The extract of all section of plant had a lower MIC value (26 mg/mL) against *E. coli*. The MIC value for the leaf extract was determined as of 10.5 mg/mL against *B. subtilis* (Table III). The methanolic extract of *A. wolgensis* was effective against *S. enteritidis* (48 mg/mL), *S. aureus* (50 mg/mL) and *E. coli* (50 mg/mL), but no activity was observed against *B. subtilis* [17]. It was determined during a study conducted in Iran that essential oils of *A. wolgensis* did not display any antimicrobial activity [24]. It was determined as a result of in a study on the Ranunculus species in Turkey that plant extracts are more effective on Gram positive bacteria [26]. Ethanolic extracts of *A. paryadrica* display antimicrobial activity against Gram negative and Gram positive bacteria according to the obtained results of the agar well diffusion test and MIC experiments. Antimicrobial activity of the plant flower extract was found to be higher in the present study.

Conclusions

This report on the secondary metabolite profiles and biological activity analyses of the ethanolic extracts from flower, leaf and root of *A. paryadrica* is the first known study until today. A total of 8, 7 and 3 secondary metabolites were identified *via* LC-MS/MS in ethanol extracts from flower, leaf and root of *A. paryadrica*, respectively. With its astringent and different pharmacological effects, quinic acid is the main component among the detected 37 phytochemicals in all parts of the plant. The ethanol extracts of *A. paryadrica* have displayed various antioxidant and antimicrobial effects.

Nowadays, many bacteria species have begun to display resistance to all known antibiotics. For this reason, researchers are tending towards new natural resources as an alternative to drugs.

According to literature, extracts of genus *Adonis* displayed various pharmacologic effects. The plants of genus have the potential for effective treatment of various illnesses (cardiovascular, inflammation, Alzheimer's and microbial diseases). We are of the opinion that the present study can yield to different

pharmacological studies for similar plants, especially *Adonis* species.

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Conflict of interest

The authors declare no conflict of interest.

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