



In Vitro Enzyme Inhibitory Properties, Antioxidant Activities, and Phytochemical Profiles of *Moltingia aurea* and *Moltingia coerulea*

Moltingia aurea ve *Moltingia coerulea*'nın *In Vitro* Enzim İnhibitör Özellikleri, Antioksidan Aktiviteleri ve Fitokimyasal Profilleri

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ABSTRACT

Objectives: In Turkey, the genus *Moltingia* Lehm. is represented by two species, namely *Moltingia aurea* Boiss. and *M. coerulea* (Willd.) Lehm., which are used as both food and for medicinal purposes. This study aimed to evaluate the antidiabetic and antioxidant potential and phytochemical profiles of leaf, flower, and root extracts of *Moltingia* species.

Materials and Methods: α -Glucosidase and α -amylase inhibitory activities, antioxidant effects, and total phenol and flavonoid contents of *Moltingia* extracts were evaluated. High-performance liquid chromatography was performed for identifying and quantifying phenolic compounds, which are responsible for various activities of these extracts.

Results: Among the investigated phenolic compounds, caffeic and rosmarinic acids and rutin were determined and quantified in methanol extracts. Rutin was the major compound in the flower extract of *M. aurea*. Rutin and rosmarinic acid were the major compounds in the leaf extract of *M. aurea*. The flowers, leaves and roots of *M. coerulea* were also rich in rosmarinic acid. The antioxidant and antidiabetic potential of these extracts may be attributable to their rutin and rosmarinic acid content.

Conclusion: *Moltingia* species can be used as natural sources of antioxidants. Notably, *M. aurea* extracts can be used for the development of herbal products with antidiabetic potential.

Key words: *Moltingia*, Boraginaceae, antidiabetic, antioxidant, HPLC, phenolic compounds

ÖZ

Amaç: *Moltingia* Lehm. genusu Türkiye'de iki tür ile temsil edilmektedir: *Moltingia aurea* Boiss. ve *M. coerulea* (Willd.) Lehm. Her iki bitki de hem besin olarak hem de tıbbi amaçla kullanılmaktadır. Bu çalışma, *Moltingia* türlerinden elde edilen yaprak, çiçek ve kök ekstraktlarının antidiyabetik ve antioksidan potansiyellerini ve fitokimyasal profillerini incelemeyi amaçlamaktadır.

Gereç ve Yöntemler: *Moltingia* ekstraktlarının α -glukozidaz ve α -amilaz enzim inhibitör aktiviteleri, antioksidan etkileri, total fenol ve flavonoid içerikleri değerlendirilmiştir. Bu ekstraktların farklı aktivitelerinden sorumlu olabileceği düşünülen fenolik bileşiklerin belirlenmesi ve miktar tayini için yüksek performanslı sıvı kromatografisi yöntemi kullanılmıştır.

Bulgular: İncelenen fenolik bileşikler arasında, kafeik asit, rosmarinik asit ve rutin metanol ekstraktlarında tespit edilmiş ve miktarları tayin edilmiştir. Rutin, *M. aurea* çiçek ekstraktının ana bileşeni olarak belirlenmiştir. Rutin ve rosmarinik asit *M. aurea* yaprak ekstraktının ana bileşenleri olarak belirlenmiştir. *M. coerulea* çiçek, yaprak ve köklerinin ise rosmarinik asit açısından da zengin olduğu tespit edilmiştir. Ekstraktların antioksidan ve antidiyabetik etkileri rutin ve rosmarinik asit içeriklerine bağlı olabilir.

Sonuç: *Moltingia* türleri potansiyel doğal antioksidan kaynağı olarak kullanılabilir. Özellikle *M. aurea* ekstraktları antidiyabetik potansiyele sahip bitkisel ürün geliştirilmesinde kullanılabilir.

Anahtar kelimeler: *Moltingia*, Boraginaceae, antidiyabetik, antioksidan, YPSK, fenolik bileşikler

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INTRODUCTION

The Boraginaceae family is distributed in tropical, subtropical, and temperate areas of the world and comprises approximately 2500 species.¹ *Moltkia*, *Heliotropium*, *Cordia*, *Arnebia*, *Echium*, and *Onosma* are some of the important genera of Boraginaceae. Most members of this family are medicinally important plants that contain secondary metabolites, including flavonoids, terpenoids, alkaloids, fatty acids, glycosides, and phytosterols, and various proteins.^{2,3}

Moltkia comprises five species, all of which grow in the Eastern part of the Mediterranean region. *Moltkia coerulea* (Willd.) Lehm. is endemic to Anatolia, Lebanon, and the Crimea; *M. aurea* Boiss. is endemic to Anatolia.⁴ *Moltkia* species of Turkey, known as “emzik cicegi, sancı out, sormuk, sarı kesen”, are traditionally used for various health problems, such as kidney disorders, diarrhea, and abdominal pain.^{5,6} In Sivas, leaves of *M. coerulea* are consumed as food; in Nigde, the sweet flowers of *M. coerulea* are eaten by children.⁷ Studies on *Moltkia* species of Turkey have revealed that these plants possess antioxidant, antibacterial, and cytotoxic activities.^{3,5}

Inhibition of α -glucosidase in the bowel slows oligosaccharide degradation, thereby reducing the glucose level in the circulatory system. α -amylase degrades long-chain carbohydrates. Inhibition of such enzymes has been an important approach to decrease blood glucose levels and diabetes-related complications. Oxidative stress is an important determinant of diabetes-related complications, and the overproduction of free radicals is related to hyperglycemia.⁸ Although studies comparing anatomical and morphological features of *M. aurea* and *M. coerulea* growing in Turkey¹ are available, only a few studies have examined the biological activity and phytochemistry of both species together.^{5,9} Therefore, we investigated the antidiabetic and antioxidant potential of flowers, leaves, and roots of Turkish *Moltkia* species and compared their phytochemical profiles.

MATERIALS AND METHODS

Plant material

Plants from *Moltkia* species were collected in the flowering stage from Eryaman-Ankara, Turkey in May, 2015. Plants were collected by N. Orhan and Ç. Orhan and identified by Assoc. Prof. Dr. N. Orhan. Voucher specimens GUEF 3239 and GUEF 3240 (*M. aurea*) and GUEF 3241 and GUEF 3242 (*M. coerulea*) were deposited at Gazi University's Faculty of Pharmacy.

Extract preparation

Water extract: Dried and ground plant parts (leaf, flower, and root) were extracted with 50 mL hot water (4% w/v) on a heating-magnetic stirrer for 6 h and filtered. The residues were treated with 50 mL water using the same procedure. Filtered aqueous extracts were combined and freeze-dried.

Ethyl acetate and methanol (MeOH) extracts: Dried and powdered plant parts (leaf, flower, and root) were treated with 200 mL MeOH and ethyl acetate (2.5% w/v) on a shaker

for 18 h at 25°C and filtered. This procedure was repeated two times; extracts were pooled and solvents were removed using a rotary evaporator. Extract yields are presented in Table 1.

Enzyme inhibitory activity

α -Amylase inhibitory activity

α -Amylase (porcine pancreatic, EC 3.2.1.1, Sigma) was dissolved in distilled water and added to plant extracts. Incubation was performed at 37°C for 3 min. Then, potato starch solution (0.5% w/v) was added to the mix and incubated at 37°C for 5 min. Subsequently, 3,5-dinitrosalicylic acid solution was added to the mix and incubated in an 85°C heater. Finally, distilled water was added, and the tubes were allowed to cool. Absorbance values were recorded at 540 nm. Acarbose was run as the reference.¹⁰ Absorbance (A) due to maltose formation was estimated according to the formula: $A_{\text{Control or sample}} = A_{\text{Test}} - A_{\text{Blank}}$.

The quantity of maltose formation was assessed by using the maltose standard calibration curve (0-0.1% w/v) and the gained net absorbance value. Inhibition ratio was estimated as:

$$\text{Inhibition \%} = \left[\frac{\text{Maltose}_{\text{Control}} - \text{Maltose}_{\text{Sample}}}{\text{Maltose}_{\text{Control}}} \right] \times 100.$$

Table 1. Yield percentages and total phenol and flavonoid contents in *Moltkia* extracts

Plant	Part	Extract	Yield % (w/w)	Total phenol content (mean \pm SD)	Total flavonoid content (mean \pm SD)
<i>Moltkia aurea</i>	Leaf	Water	25.71	232.94 \pm 9.37	38.38 \pm 1.61
		MeOH	14.96	149.72 \pm 12.74	20.60 \pm 1.58
		EA	1.89	122.18 \pm 3.37	34.86 \pm 1.40
	Flower	Water	30.23	219.07 \pm 28.26	49.12 \pm 1.90
		MeOH	24.94	57.52 \pm 11.43	58.80 \pm 2.61
		EA	1.57	20.19 \pm 0.00	51.05 \pm 5.89
	Root	Water	9.47	376.53 \pm 34.19	30.46 \pm 1.33
		MeOH	5.05	369.40 \pm 34.30	32.39 \pm 0.61
		EA	0.53	125.85 \pm 28.81	66.55 \pm 1.90
<i>Moltkia coerulea</i>	Leaf	Water	22.68	201.32 \pm 13.59	36.09 \pm 3.52
		MeOH	9.72	18.97 \pm 0.00	36.62 \pm 1.22
		EA	1.00	77.71 \pm 27.54	127.46 \pm 4.33
	Flower	Water	22.17	224.17 \pm 17.18	29.75 \pm 5.29
		MeOH	12.55	204.79 \pm 29.68	30.63 \pm 1.40
		EA	0.84	25.09 \pm 3.06	62.67 \pm 3.23
	Root	Water	15.39	252.52 \pm 19.05	26.06 \pm 0.81
		MeOH	4.60	136.87 \pm 27.87	29.05 \pm 0.53
		EA	0.49	18.77 \pm 4.09	73.06 \pm 2.13

Total flavonoid content was expressed as mg quercetin equivalent/g extract and total phenol content was expressed as mg gallic acid equivalent/g extract MeOH: Methanol, EA: Ethyl acetate, SD: Standard deviation

α-Glucosidase inhibitory activity

α-Glucosidase inhibitory potential of *Moltkia* samples was evaluated using the assay of Lam et al.¹¹ *α*-Glucosidase (Sigma Co., St. Louis, USA) obtained from *Bacillus stearothermophilus* was dissolved in phosphate buffer (0.5 M) at pH 6.5. Extracts and enzyme solution were suspended in hydroalcohol (80%) and preincubated at 37°C. Then, 20 mM *p*-nitrophenyl-*α*-D-glucopyranoside (NPG, Sigma) was added as the substrate. Samples were incubated for nearly 30 min at 37°C. The difference in absorbance at 405 nm, due to the hydrolysis of NPG by the enzyme, was quantified. The *α*-glucosidase inhibitor acarbose (Bayer Group, Turkey) was chosen as a reference. The inhibition ratio (%) was estimated using the following equation:

$$\text{Inhibition \%} = [(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100$$

Antioxidant activity

Radical [1,1-diphenyl-2-picrylhydrazyl (DPPH)] scavenging activity

A total of 40 μ L DPPH solution was vortexed with 160 μ L of the extract and incubated in the dark. Then, absorbance was measured at 520 nm.¹² All calculations were performed using the Softmax PRO 4.3.2.LS software. Butylated hydroxytoluene was used as a reference at 0.1, 0.3, and 1 mg/mL.

Superoxide anion scavenging activity

Superoxide radicals were generated in a nonenzymatic system. The reaction mixture, including 25–2000 μ g/mL of the test fraction in 70°C ethanol, 1 mL 468 mmol/L β -NADH, 1 mL 60 mmol/L phenazine methosulphate (PMS), and 1 mL 150 mmol/L nitro blue tetrazolium chloride in phosphate buffer (0.1 mol/L, pH 7.4), was incubated. Absorbance was recorded at 560 nm against blank samples, which were free of PMS. Activity was estimated as scavenging activity (%) = $[(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100$, where A_{Control} is the absorbance of the control and A_{Sample} is the absorbance of the extract.^{13,14} Quercetin was chosen as the reference at 0.1, 0.3, and 1 mg/mL.

Ferric-reducing antioxidant power

The extract or ascorbic acid was incubated with 0.2 mol/L phosphate buffer (pH 6.6) and $K_3Fe(CN)_6$. Trichloroacetic acid was added to the mixture, vortexed, and centrifuged. Water and ferric chloride were added to the supernatant and absorbance was measured at 700 nm.¹⁵ Ascorbic acid was used as reference.

Metal-chelating capacity

Extracts were incubated with $FeCl_2$ (2 mM). The reaction was initiated after the addition of 0.2 mL of ferrozine (5 mM). After a while, absorbance was recorded at 562 nm.¹⁶ Ethylenediaminetetraacetic acid was used as a reference and $FeCl_2$ and ferrozine as controls. Inhibition ratio of ferrozine- Fe^{+2} complex generation was estimated using the following formula: Metal-chelating activity (%) = $[(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100$.

Total antioxidant capacity

Molybdate reagent, water, and extracts were mixed, vortexed, incubated for 90 min at 95°C in test-tubes. Then, tubes were

cooled and absorbance was recorded at 695 nm. Outcomes were shown as ascorbic acid equivalent in the extract (mg/g).¹⁷

Phytochemical content

Estimation of total phenol content

Extracts were mixed and incubated with the Folin-Ciocalteu reagent. Then, sodium carbonate solution was added to the mixture and immediately vortexed. Absorbance was measured at 735 nm after 30 min in the dark. Total phenol content of extracts in mg of gallic acid equivalent (GAE)¹⁸ was calculated using the following equation:

$$\text{Absorbance} = 1.6342 \times (\text{conc.}) + 1.417, r^2 = 0.9986.$$

Determination of total flavonoid content

Extracts (1 mg/mL) were suspended in 80% ethanol. Sodium acetate, aluminum chloride solution, ethyl alcohol, and water were mixed and incubated with extracts for 30 min. Absorbance was measured at 415 nm. Outcomes in mg quercetin equivalent (QE)/g extract¹⁹ were calculated using the following equation: Absorbance = $1.8934 \times (\text{conc.}) - 0.025$, and r^2 value was 0.9996.

Qualitative and quantitative analyses of phenolic compounds using reverse phase-high-performance liquid chromatography (RP-HPLC)-photo diode array (PDA)

HPLC analyses were performed as described^{20,21} using the HP Agilent 1260 series LC system and ACE column (5 μ m, 250 mm \times 4.6 mm) at 30°C. The flow rate of the gradient elution was 0.8 mL/min. The mobile phase was a mixture of water (solution A), MeOH (solution B), and acetonitrile (solution C), each containing 0.1% trifluoroacetic acid. Caffeic and rosmarinic acids were analyzed at 330 nm and rutin at 360 nm by external standardization.

Statistical analysis

Experiments were performed in triplicate and mean values were obtained. All values are given as the mean \pm standard deviation. Computations were performed using GraphPad InStat and Microsoft Excel.

RESULTS

Results of the total phenol content assay revealed that lyophilized water and MeOH extracts of all investigated parts of both *Moltkia* species were rich in total phenolics. Water and MeOH extracts of *M. aurea* roots had the highest total phenolic content (376.53 ± 34.19 and 369.40 ± 34.30 mg GAE/g extract, respectively). The highest amount of total flavonoid was recorded in the ethyl acetate extract of *M. coerulea* leaves (127.46 ± 4.33 mg QE/g extract). In addition, amount of total flavonoid in the MeOH extract of *M. aurea* flowers was high (58.80 ± 2.61 mg QE/g extract), which was consistent with results from HPLC analysis, indicating high rutin content. Extract yields and obtained results for total phenol and flavonoid content are presented in Table 1.

One approach for managing diabetes includes inhibiting *α*-amylase and *α*-glucosidase activity. In this study, *Moltkia* species displayed mild enzyme inhibitory activity compared

to the reference acarbose (Table 2). Ethyl acetate and water extracts of *M. aurea* leaves inhibited α -amylase (24.07% \pm 2.59% and 14.11% \pm 2.59%, respectively) together with ethyl acetate extracts of *M. coerulea* flowers (18.89% \pm 2.43%) at 3 mg/mL. MeOH and ethyl acetate extracts inhibited α -glucosidase activity more strongly than water extracts (Table 2).

Plant extracts have excellent antioxidant activity.²² To evaluate the antioxidant potential of *Moltkia* species, various *in vitro* tests were performed. Analysis of total antioxidant capacity revealed that ethyl acetate extracts displayed significant antioxidant potential compared with extracts prepared using other solvents. Water, and especially MeOH, extracts exhibited significant ferric-reducing power compared to ethyl acetate extracts. All water extracts exhibited significant metal-chelating activity (Table 3). Although the superoxide-scavenging activity of water, MeOH, and ethyl acetate extracts of roots of both species were found to be promising, MeOH extracts of all samples scavenged the DPPH free radical significantly (Table 4).

HPLC analyses revealed that among the investigated phenolics (Rt chlorogenic acid: 8.9 min, Rt caffeic acid: 12.3 min, Rt ferulic acid: 20.25 min, Rt rutin: 23.1 min, Rt rosmarinic acid: 30.6 min, Rt quercetin: 36.5 min, Rt luteolin: 37.3 min, and Rt

apigenin: 40.3 min) only caffeic acid, rutin, and rosmarinic acid were detected and quantified in methanolic extracts of flowers, leaves, and roots (Figures 1-6). Chromatograms of standard compounds are shown in Figures 7-8. Ultraviolet spectra of quantified compounds, which were overlaid with those of standard compounds, are shown in Figures 9-11.

Rutin a flavonoid glycoside, occurs naturally in many fruits and vegetables and has several biological activities, such as antioxidant, antibacterial, antifungal, and antiinflammatory effects.²³ As seen in the chromatograms, a significant amount of rutin was present in flower extracts of *M. aurea* (6.198% \pm 0.271%; mean peak area: 3290), and MeOH extract of *M. coerulea* flowers contained less amount rutin (0.099% \pm 0.002%; mean peak area: 81.23). We believe our findings reveal significant phytochemical differences between these two *Moltkia* taxa endemic to Turkey. Rosmarinic acid, a phenolic compound derived from hydroxycinnamic acid, and mostly found in Lamiaceae and Boraginaceae, has many biological activities, including antioxidant, antimicrobial, antiinflammatory, antiproliferative, and chemopreventive effects.²⁴ As seen from the chromatograms, root extracts of both species were rich in rosmarinic acid (3.459% \pm 0.005% and 2.028% \pm 0.012%, respectively). Moreover, remarkable

Table 2. Enzyme inhibitory effects of *Moltkia* extracts

Plant	Part	Extract	α -Glucosidase inhibition % \pm SD			α -Amylase inhibition % \pm SD	
			3 mg/mL	1 mg/mL	0.3 mg/mL	3 mg/mL	
<i>Moltkia aurea</i>	Leaf	Water	52.77 \pm 7.26	29.29 \pm 1.71	3.31 \pm 1.74	14.11 \pm 2.59	
		MeOH	51.87 \pm 2.41	28.31 \pm 3.50	13.47 \pm 2.06	-	
		EA	86.02 \pm 1.23	76.17 \pm 0.22	59.41 \pm 1.23	24.07 \pm 2.59	
	Flower	Water	48.10 \pm 7.15	30.49 \pm 3.85	19.42 \pm 3.00	12.86 \pm 3.80	
		MeOH	61.01 \pm 4.07	47.86 \pm 2.98	25.26 \pm 2.54	-	
		EA	78.74 \pm 1.18	63.92 \pm 1.46	46.55 \pm 3.98	8.43 \pm 5.84	
	Root	Water	85.67 \pm 2.76	67.66 \pm 0.66	25.42 \pm 0.59	-	
		MeOH	77.68 \pm 3.85	68.35 \pm 0.72	31.04 \pm 4.81	8.28 \pm 1.10	
		EA	84.93 \pm 2.09	69.12 \pm 1.39	33.43 \pm 3.48	9.47 \pm 1.58	
<i>Moltkia coerulea</i>	Leaf	Water	49.84 \pm 0.99	33.64 \pm 3.31	36.78 \pm 6.07	-	
		MeOH	66.84 \pm 2.54	48.25 \pm 4.24	37.44 \pm 3.92	3.68 \pm 9.65	
		EA	66.26 \pm 2.58	64.69 \pm 2.12	41.08 \pm 5.87	-	
	Flower	Water	44.12 \pm 5.43	36.89 \pm 4.06	13.70 \pm 0.91	-	
		MeOH	60.04 \pm 0.65	41.28 \pm 0.97	16.64 \pm 2.20	-	
		EA	84.11 \pm 1.79	73.24 \pm 1.05	53.95 \pm 3.91	18.89 \pm 2.43	
	Root	Water	46.44 \pm 3.10	35.03 \pm 1.94	5.68 \pm 1.43	-	
		MeOH	74.65 \pm 1.54	48.66 \pm 1.83	19.00 \pm 4.25	-	
		EA	71.56 \pm 1.00	62.12 \pm 0.30	42.75 \pm 3.10	-	
Reference	Acarbose	1 mg/mL	0.3 mg/mL	0.1 mg/mL	1 mg/mL	0.3 mg/mL	0.1 mg/mL
	-	98.88 \pm 0.07	97.98 \pm 0.03	96.37 \pm 0.56	44.72 \pm 2.76	31.51 \pm 3.18	26.26 \pm 5.74

--: No activity, SD: Standard deviation, MeOH: Methanol, EA: Ethyl acetate

amount of rosmarinic acid was detected in the other investigated parts (Table 5).

DISCUSSION

Based on our comprehensive literature survey, only a few studies on pharmacological activities and chemical profiles

of related *Moltkia* species have been preformed. Harput et al.⁵ investigated the cytotoxic effect of the aerial parts of *M. aurea* on three cell lines and determined antioxidant activity. Moreover, dose-dependent cytotoxic activity against cancer cell lines as well as promising antioxidant activity have been reported. Additionally, some phenolic compounds were obtained from

Table 3. Metal-chelating activity, ferric-reducing power, and total antioxidant capacity of *Moltkia* extracts

Plant	Part	Extract	Metal-chelating activity % \pm SD			Ferric-reducing power absorbance \pm SD			Total antioxidant capacity*
			3 mg/mL	1 mg/mL	0.3 mg/mL	3 mg/mL	1 mg/mL	0.3 mg/mL	
<i>Moltkia aurea</i>	Leaf	Water	>100	90.33 \pm 4.19	48.96 \pm 5.72	1.5307 \pm 0.0370	0.7410 \pm 0.0605	0.2343 \pm 0.0090	-
		MeOH	22.42 \pm 3.58	-	-	2.3673 \pm 0.0393	1.9194 \pm 0.0615	0.1960 \pm 0.0149	-
		EA	-	-	-	0.7040 \pm 0.0867	0.2143 \pm 0.0084	0.0477 \pm 0.0042	66.30 \pm 9.08
	Flower	Water	>100	98.11 \pm 5.66	33.25 \pm 6.17	2.0137 \pm 0.1373	1.3583 \pm 0.0741	0.4033 \pm 0.0086	-
		MeOH	70.17 \pm 3.86	-	-	2.5244 \pm 0.0674	2.4727 \pm 0.0665	0.3080 \pm 0.0218	-
		EA	12.07 \pm 6.29	-	-	0.6154 \pm 0.0285	0.0847 \pm 0.0110	0.0190 \pm 0.0036	299.53 \pm 27.23
	Root	Water	>100	35.99 \pm 2.92	-	3.1090 \pm 0.0210	2.7980 \pm 0.0936	1.1257 \pm 0.0528	-
		MeOH	-	-	-	1.7140 \pm 0.0875	1.4720 \pm 0.0339	1.0083 \pm 0.1198	8.65 \pm 0.00
		EA	43.03 \pm 7.53	-	-	1.2424 \pm 0.0101	0.9977 \pm 0.1124	0.3130 \pm 0.0300	687.37 \pm 9.08
<i>Moltkia coerulea</i>	Leaf	Water	>100	80.00 \pm 3.47	15.90 \pm 3.05	1.9434 \pm 0.0248	0.7843 \pm 0.5653	0.4973 \pm 0.0138	-
		MeOH	38.79 \pm 9.22	-	-	2.9394 \pm 0.0418	2.4310 \pm 0.0752	1.2323 \pm 0.0594	-
		EA	-	-	-	0.3470 \pm 0.0172	0.1353 \pm 0.0195	0.0333 \pm 0.0087	540.62 \pm 29.76
	Flower	Water	>100	99.87 \pm 2.63	29.77 \pm 0.61	1.8684 \pm 0.0300	1.4033 \pm 0.1028	0.2910 \pm 0.0115	-
		MeOH	80.31 \pm 8.32	-	-	2.5758 \pm 0.0979	2.1333 \pm 0.1092	0.9640 \pm 0.0624	-
		EA	-	-	-	1.2720 \pm 0.0966	0.1057 \pm 0.0176	0.0157 \pm 0.0049	485.59 \pm 12.01
	Root	Water	>100	80.36 \pm 8.16	40.00 \pm 7.18	1.9684 \pm 0.0835	0.9477 \pm 0.0761	0.1500 \pm 0.0394	11.27 \pm 4.54
		MeOH	72.42 \pm 6.87	-	-	1.8060 \pm 0.0095	0.9323 \pm 0.0595	0.6330 \pm 0.0442	21.75 \pm 4.54
		EA	-	-	-	1.8114 \pm 0.0166	0.3910 \pm 0.0231	0.1100 \pm 0.0092	178.98 \pm 12.01
Reference		1 mg/mL	0.3 mg/mL	0.1 mg/mL	3 mg/mL	1 mg/mL	0.3 mg/mL	-	
EDTA		98.87 \pm 0.14	96.60 \pm 3.39	95.75 \pm 0.08	NT	NT	NT	NT	
Ascorbic acid		NT	NT	NT	3.5870 \pm 0.0874	3.4547 \pm 0.0852	3.0787 \pm 0.0587	NT	

*Total antioxidant capacity is expressed as ascorbic acid equivalent/g extract \pm SD. NT: Not tested, -: No activity, SD: Standard deviation, EDTA: Ethylenediaminetetraacetic, MeOH: Methanol, EA: Ethyl acetate

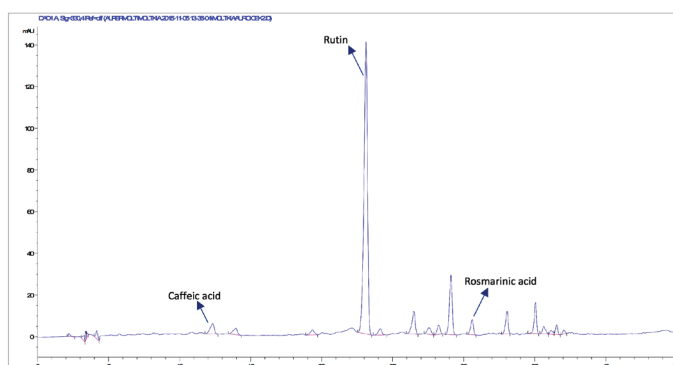


Figure 1. High-performance liquid chromatography analysis of the methanolic extract of *Moltkia aurea* flowers

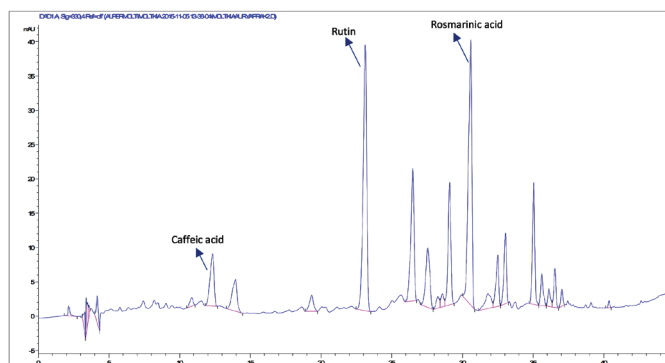


Figure 2. High-performance liquid chromatography analysis of the methanolic extract of *Moltkia aurea* leaves

the water subextract. Erdemoglu et al.²⁵ studied the chemical constituents of the seed oil of *M. aurea*.

The effect of an ointment prepared from the aerial parts of *M. coerulea* (collected from Iran) on wound healing was investigated by Farahpour et al.²⁶ Chemical constituents of

M. coerulea flowers collected from India included fatty acids (capric, myristic, palmitic, behenic, and undecylic acids), flavonoids (kaempferol, quercetin, nortangeretin, and rebisetin), and amino acids (ornithine, lysine, dopa, serine, glutamic acid, proline, amino-n-butyric acid, phenylalanine, and leucine).²⁷

Table 4. Superoxide anion and DPPH radical scavenging activities of *Moltkia* extracts

Plant	Part	Extract	Superoxide anion scavenging activity inhibition % ± SD			DPPH radical scavenging activity inhibition % ± SD		
			3 mg/mL	1 mg/mL	0.3 mg/mL	3 mg/mL	1 mg/mL	0.3 mg/mL
<i>Moltkia aurea</i>	Leaf	Water	65.31±8.13	26.32±3.98	-	-	-	20.33±5.97
		MeOH	7.85±2.04	-	-	62.21±3.66	53.50±2.29	45.50±0.00
		EA	44.14±6.49	-	-	-	26.73±4.40	14.17±3.21
	Flower	Water	75.80±7.45	58.30±1.43	4.63±1.28	-	7.37±3.94	37.33±5.58
		MeOH	20.46±4.09	-	-	43.32±2.80	42.09±6.92	45.00±0.50
		EA	40.37±4.29	-	-	-	6.30±1.40	11.33±4.65
	Root	Water	96.30±6.47	81.43±9.18	45.73±5.82	-	-	-
		MeOH	91.36±0.37	58.92±1.49	30.96±5.07	10.91±7.04	24.17±3.25	35.00±9.99
		EA	80.18±1.56	34.19±6.43	-	-	-	23.50±4.33
<i>Moltkia coerulea</i>	Leaf	Water	61.65±3.12	24.60±4.28	-	-	-	9.00±0.00
		MeOH	34.69±5.74	5.31±1.78	-	24.88±3.94	40.17±4.04	47.00±2.50
		EA	51.09±4.82	11.56±0.74	-	-	15.21±0.00	30.67±6.53
	Flower	Water	59.63±6.16	45.03±3.15	16.97±4.47	-	-	23.17±5.80
		MeOH	14.94±4.63	-	-	44.09±2.70	41.50±0.50	44.67±0.76
		EA	51.74±1.82	14.51±1.64	-	-	4.15±0.46	-
	Root	Water	98.07±4.44	27.78±3.32	-	-	-	8.33±4.25
		MeOH	87.78±0.64	41.58±1.47	24.37±1.44	34.56±0.46	27.00±3.77	38.67±4.01
		EA	76.58±1.46	31.61±1.40	-	-	36.87±4.44	29.5±0.00
Reference		1 mg/mL	0.3 mg/mL	0.1 mg/mL	1 mg/mL	0.3 mg/mL	0.1 mg/mL	
Quercetin		86.52±3.26	75.19±3.22	68.45±0.27	ND	ND	ND	
BHT		>100	62.74±2.08	18.07±2.40	50.67±3.40	47.17±4.31	40.83±7.69	

DPPH: 1,1-diphenyl-2-picrylhydrazyl, BHT: Butylated hydroxytoluene, -: No activity, ND: Not detected, SD: Standard deviation, MeOH: Methanol, EA: Ethyl acetate

Table 5. Content of phenolic compounds in methanol extracts of *Moltkia* species

Species	Content (g/100g extract) ^a		
	Caffeic acid	Rosmarinic acid	Rutin
<i>Moltkia aurea</i> leaf	0.026±0.001	0.299±0.005	0.836±0.004
<i>Moltkia aurea</i> flower	0.032±0.003	0.111±0.005	6.198±0.271
<i>Moltkia aurea</i> root	0.024±0.001	3.459±0.005	0.043±0.002
<i>Moltkia coerulea</i> leaf	0.067±0.003	0.261±0.009	0.585±0.024
<i>Moltkia coerulea</i> flower	0.026±0.001	0.342±0.002	0.099±0.002
<i>Moltkia coerulea</i> root	0.011±0.001	2.028±0.012	ND ^b

^aMean ± SD (n=3); ^bND: Not detected, SD: Standard deviation

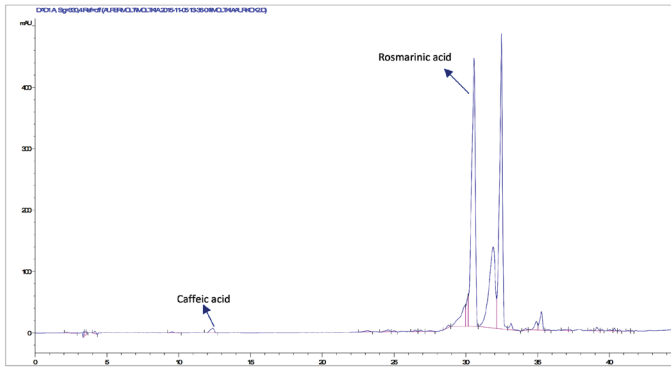


Figure 3. High-performance liquid chromatography analysis of the methanolic extract of *Moltkia aurea* roots

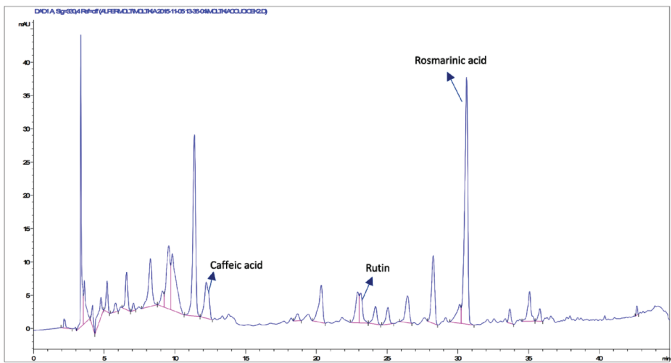


Figure 4. High-performance liquid chromatography analysis of the methanolic extract of *Moltkia coerulea* flowers

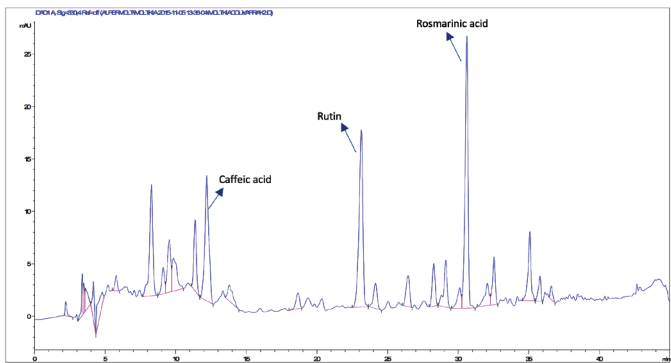


Figure 5. High-performance liquid chromatography analysis of the methanolic extract of *Moltkia coerulea* leaves

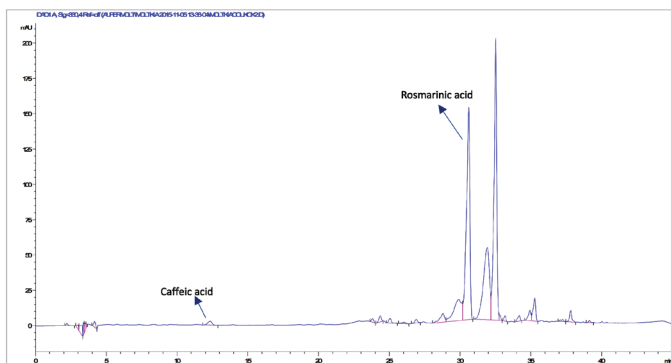


Figure 6. High-performance liquid chromatography analysis of the methanolic extract of *Moltkia coerulea* roots

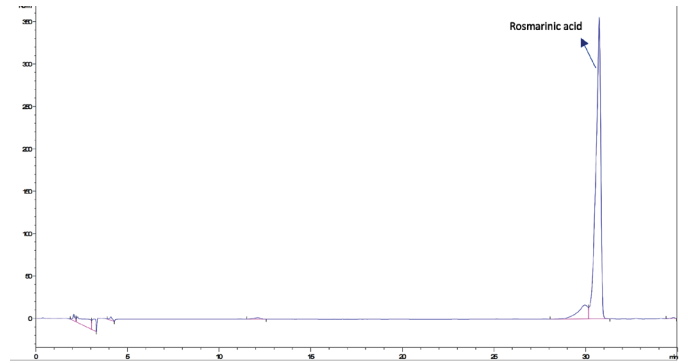


Figure 7. High-performance liquid chromatography analysis of the rosmarinic acid standard

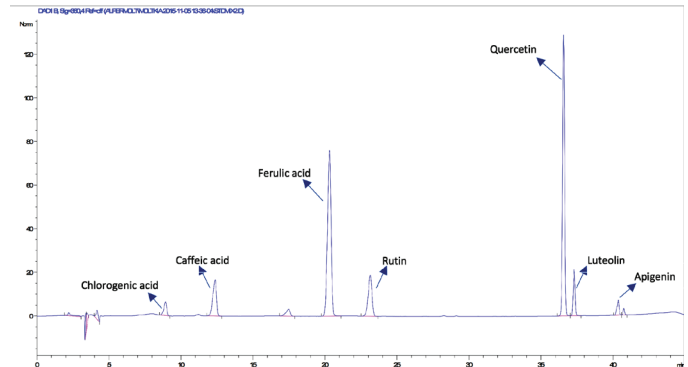


Figure 8. High-performance liquid chromatography analysis of a standard mixture containing chlorogenic acid (Rt: 8.9 min), caffeic acid (Rt: 12.3 min), ferulic acid (Rt: 20.25 min), rutin (Rt: 23.1 min), quercetin (Rt: 36.5 min), luteolin (Rt: 37.3 min), apigenin (Rt: 40.3 min)

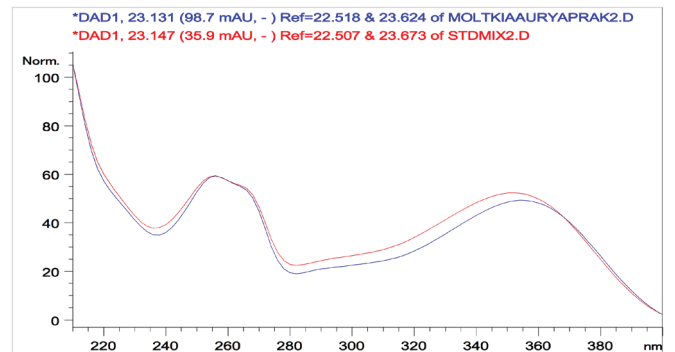


Figure 9. Overlaid ultraviolet spectra of standard rutin and rutin detected in the *Moltkia aurea* leaf extract

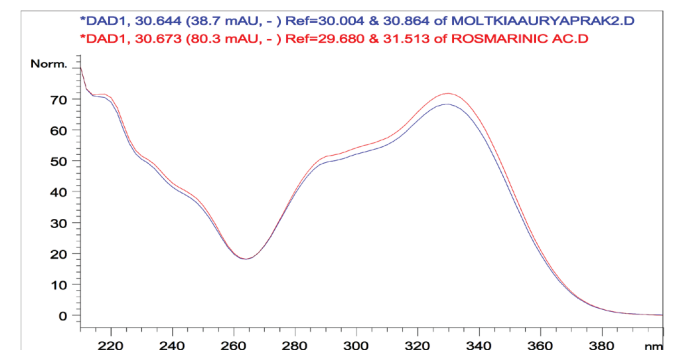


Figure 10. Overlaid ultraviolet spectra of standard rosmarinic acid and rosmarinic acid detected in the *Moltkia aurea* leaf extract

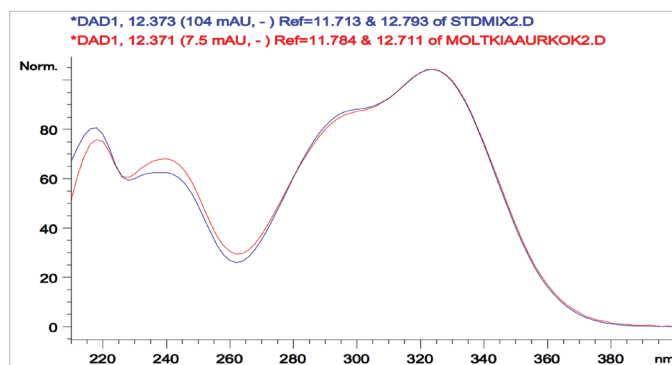


Figure 11. Overlaid ultraviolet spectra of standard caffeic and caffeic acid detected *Moltkia aurea* in the root extract

Zengin et al.⁹ investigated antioxidant, antimicrobial, antityrosinase, antiacetylcholinesterase, antibutyrylcholinesterase, and antidiabetic activities of methanolic extracts of aerial parts of *M. aurea* and *M. coerulea* *in vitro*. Moreover, they studied the phenolic profile of the species and performed an *in vivo* assay to evaluate genotoxicity. Rutin, hesperidin, and protocatechuic acid were detected as the main components of the aerial parts of *M. aurea*, whereas rutin, protocatechuic acid, malic acid, and hesperidin were detected as the main components of the aerial parts of *M. coerulea*. In this study, we evaluated antioxidant and antidiabetic activities and phenolic profiles of three parts of the species. Consistent with the results of Zengin et al.⁹, we found that a significant amount of rutin was present in the MeOH extract of *M. aurea* flowers. Moreover, rosmarinic acid was one of the most abundant compound in both species, especially in the leaf and root of *M. aurea* and all investigated parts of *M. coerulea*. The content of rosmarinic acid is the main difference between the two studies. Rutin amount was determined too high in the flower extract of *M. aurea* compared to that of *M. coerulea*. Moreover, caffeic acid should contribute to the antioxidant feature of the species.

Zengin et al.⁹ reported that although both species possessed antioxidant properties, *M. aurea* was a better antioxidant. We could not categorize the species according to their antioxidant potential. Different results were obtained with different extracts, and making correlation was difficult. Both *M. aurea* and *M. coerulea* possessed antidiabetic activity as inhibitors of carbohydrate-digesting enzymes.

CONCLUSION

M. aurea and *M. coerulea* exhibited potent antioxidant and mild carbohydrate-digesting enzyme inhibitory activity in *in vitro* assays. Both species are a significant source of rosmarinic acid, rutin, and caffeic acid, which are possibly responsible for these activities. Thus, *M. aurea* and *M. coerulea* can be used as potential natural antioxidant sources. Notably, *M. aurea* extracts should be studied for the development of herbal products with antidiabetic potential.

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