ORIGINAL ARTICLE

DOI: 10.4274/tjps.galenos.2020.12258

In vitro Enzyme Inhibitory Properties, Antioxidant Activities and Phytochemical Profiles of *Moltkia aurea* and *Moltkia coerulea*

Moltkia aurea ve *Moltkia coerulea*'nın *In vitro* Enzim İnhibitör Özellikleri, Antioksidan Aktiviteleri ve Fitokimyasal Profilleri

Nilufer ORHAN[1], Alper Gökbulut[2], Didem Deliorman Orhan[1]
[1]Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, 06330 Etiler, Ankara, Turkey
[2]Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, 06100 Tandoğan, Ankara, Turkey

Corresponding Author
Alper Gökbulut
gokbulut@pharmacy.ankara.edu.tr
905358282566
orcid.org/0000-0001-8657-6016
12.03.2020
21.04.2020

Abstract
Objectives: The genus *Moltkia* Lehm., is represented by two species; *Moltkia aurea* Boiss., and *M. coerulea* (Willd.) Lehm. in Turkey. They are used both as food and for medicinal purposes. The current study aimed to investigate the antidiabetic and antioxidant potential, and the phytochemical profile of the leaf, flower and root extracts of *Moltkia* species.
Materials and Methods: α-Glucosidase and α-amylase enzyme inhibitory activities of the extracts were studied. Also, antioxidant effects, total phenol and flavonoid contents of the extracts were evaluated. High performance liquid chromatography (HPLC) method was applied for the identification and quantification of the phenolic compounds which could be liable for the observed various activities of the extracts.
Results: Among the investigated phenolic compounds, caffeic and rosmarinic acids and rutin were determined and quantified in the methanol extracts. Rutin was found as the major compound in *M. aurea* flower extract, also it was determined as one of the major compounds of the leaf extract together with rosmarinic acid. *M. coerulea* flowers, leaves and roots were found to be rich in rosmarinic acid. The antioxidant and antidiabetic potentials of the extracts may be thought to be due to their rutin and rosmarinic acid content.
Conclusion: These results propose that tested *Moltkia* species could be used as potential natural antioxidant sources. On the other hand, especially *M. aurea* extracts could be evaluated in the development of herbal products with antidiabetic potential.
Keywords: *Moltkia*, Boraginaceae, antidiabetic, antioxidant, HPLC, phenolic compounds
ÖZET
Amaç: *Moltkia* Lehm. genusu Türkiye'de iki tür ile temsil edilmektedir: *Moltkia aurea* Boiss. ve *M. coerulea* (Willd.) Lehm. Her iki bitki de hem besin olarak hem de tıbbi amaçla kullanılmaktadır. Bu çalışma, *Moltkia* türlerinden elde edilen yaprak, çiçek ve kök

ekstrelerinin antidiyabetik ve antioksidan potansiyellerini ve fitokimyasal profillerini incelemeyi amaçlamaktadır.
Gereç ve Yöntemler: Ekstrelerin *α*-glukozidaz ve *α*-amilaz enzim inhibitor aktiviteleri çalışılmıştır. Ayrıca, antioksidan etkileri, total fenol ve flavonoit içerikleri değerlendirilmiştir. Ekstrelerde saptanan aktivitelerden sorumlu olabileceği düşünülen fenolik bileşiklerin tespiti ve miktar tayini için Yüksek Performanslı Sıvı Kromatografisi (YPSK) yöntemi kullanılmıştır.
Bulgular: Metanol ekstrelerinde, incelenen fenolik bileşikler arasında, kafeik asit, rozmarinik asit ve rutin tespit edilmiş ve miktarları tayin edilmiştir. Rutin, *M. aurea* çiçek ekstresinin ana bileşeni olarak, yaprak ekstresinde de rozmarinik asit ile birlikte ana bileşenlerden biri olarak belirlenmiştir. *M. coerulea* çiçek, yaprak ve köklerinin rozmarinik asit açısından zengin olduğu tespit edilmiştir. Ekstrelerin antioksidan ve antidiyabetik etkilerinin rutin ve rozmarinik asit içeriklerinden kaynaklandığı düşünülmektedir.
Sonuç: Bu sonuçlar *Moltkia* türlerinin potansiyel doğal antioksidan kaynağı olarak kullanılabileceğini göstermektedir. Öte yandan, özellikle *M. aurea* ekstreleri antidiyabetik potansiyele sahip bitkisel ürün geliştirilmesinde değerlendirilebilir.
Anahtar kelimeler: *Moltkia*, Boraginaceae, antidiyabetik, antioksidan, YPSK, fenolik bileşikler

INTRODUCTION

The Boraginaceae family is growing throughout the tropical, subtropical and temperate locations of the world and it comprises about 2500 species.[1] *Moltkia*, *Heliotropium*, *Cordia*, *Arnebia*, *Echium* and *Onosma* are some of the important genera of the Boraginaceae. Most of the members of this family are medicinally important plants that contain secondary metabolites such as flavonoids, terpenoids, alkaloids, fatty acids, glycosides, phytosterols and various proteins.[2,3]
Moltkia genus (Boraginaceae) consists of five species all growing in the Eastern part of the Mediterranean region. Among them, *Moltkia coerulea* (Willd.) Lehm. occurs in Anatolia, Lebanon and the Krimea; and *M. aurea* Boiss. is endemic to Anatolia.[4] *Moltkia* species of Turkey known as "emzik cicegi, sancı out, sormuk, sarı kesen", are used against various health problems such as kidney disorders, diarrhea and abdominal pain traditionally.[5,6] Additionally, leaves of *M. coerulea* are consumed as food in Sivas and its flowers are eaten by kids because of their sweet taste in Nigde.[7] The restricted number of studies on *Moltkia* species of Turkey revealed that plants mainly possess antioxidant, antibacterial, and cytotoxic activities.[3,5]
By the inhibition of *α*-glucosidase enzyme in the bowel, the proportion of oligosaccharide disjunction is slowing down, and so that the glucose level in the circulatory system is reduced. *α*-amylase is responsible for breaking down long-chain carbohydrates. Inhibition of such enzymes has been an important approach to decrease level of blood glucose and to avoid diabetic complications. On the other hand, oxidative stress is considered to be an important determinant of diabetes complications, and the overproduction of free radicals is related to hyperglycemia.[8] Beyond the study comparing anatomical and morphological features of *M. aurea* and *M. coerulea* growing in Turkey[1], there are only a few studies examining the biological activity and phytochemistry of these two species together.[5,9] Therefore, herein, we aimed to investigate antidiabetic and antioxidant potentials of different parts (flowers, leaves and roots) of Turkish *Moltkia* species and to compare their phytochemical profile.

MATERIALS AND METHODS

Plant material

Moltkia species were picked up in its 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 have been placed in the Gazi University Faculty of Pharmacy: GUEF 3239, GUEF 3240 (*M. aurea*) and GUEF 3241, GUEF 3242 (*M. coerulea*).

Preparation of the extracts

Water extract: Dried and grounded plant parts (leaf, flower, 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 with the same procedure again. Filtered aqueous extracts were combined and freeze-dried.

Ethyl acetate and methanol extracts: Dried and powdered plant parts (leaf, flower, root) were treated with 200 mL methanol and ethyl acetate (2.5% w/v) on a shaker for 18 h at 25 °C, and filtered. This procedure was repeated for two more times, extracts were pooled and condensed by a rotary evaporator. Yields of extracts were given in Table 1.

Enzyme Inhibitory Activity

α-Amylase Inhibitory Activity

Distilled water was used to dissolve the *α*-amylase (porcine pancreatic, EC 3.2.1.1, Sigma). Enzyme solution was added to the plant extracts, and the reaction was initiated. Then, the incubation of the tubes was performed at 37 °C for 3 min. After the insertion of the solution of potato starch (0.5%, w/v), the incubation of the tubes was done again at 37 °C for 5 min. After the addition of DNS (3,5-dinitrosalicylic acid) solution, mixture was set to a 85 °C heater. Later, distilled water was added, and the tubes were allowed to cool. Absorbances were recorded at 540 nm. Acarbose was run as the reference.[10] The 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 calculated by using the maltose standard calibration curve (0-0.1% w/v) and the gained net absorbance. Inhibition ratio was estimated as:

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

α-Glucosidase Inhibitory Activity

α-Glucosidase enzyme inhibitory potential of the *Moltkia* samples was evaluated by the method of Lam et al.[11] *α*-Glucosidase enzyme (Sigma Co., St. Louis, USA) from *Bacillus stearothermophilus* was dissolved in 0.5 M phosphate buffer (pH 6.5). The extracts and the enzyme solution dissolved in hydroalcohol (80%) were preincubated at 37 °C. Then, [20 mM *p*-nitrophenyl-*α*-d-glucopyranoside (NPG), Sigma] was appended as the substrate. The incubation of the media was performed for nearly half an hour at 37 °C. The differentiation in the absorption at 405 nm, because of the hydrolysis of NPG by the enzyme, was quantified. An *α*-glucosidase inhibitor, acarbose (Bayer Group, Turkey), was chosen as reference. The inhibition ratio (%) was estimated by the following equation: $\text{Inhibition \%} = [(A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}] \times 100$

Antioxidant Activity

Radical (DPPH) scavenging activity

40 µL of DPPH solution was vortexed with 160 µL of extract, and incubation was done in darkness for a while. After that, the absorbance was measured at 520 nm.[12] The calculations were performed by using the software of Softmax PRO 4.3.2.LS. As reference, butylated hydroxytoluene (BHT) was preferred at three different concentrations: 0.1, 0.3 and 1 mg/mL.

Superoxide anion scavenging activity

In a non-enzymatic system, radicals of superoxide were formed. The reaction mixture containing 25–2000 µg/ mL of the test fraction in 70 °C ethanol, 1 mL of 468 mmol/L *β*-NADH, 1 mL of 60 mmol/L PMS, and 1 mL of 150 mmol/L NBT in phosphate buffer (0.1 mol/L, pH 7.4) was incubated for a short time. The absorbance was recorded at 560 nm against blank samples which are free of PMS. The 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 reference at three different concentrations: 0.1, 0.3 and 1 mg/mL.

Ferric-reducing antioxidant power

Extract/ascorbic acid was treated with 0.2 mol/L phosphate buffer (pH 6.6) and $K_3Fe(CN)_6$, then the incubation of the tubes was performed. TCA (trichloroacetic acid) was appended to the media, and the tubes were vortexed. After centrifugation, same amount of water and ferric chlorid was added to the supernatant, and the absorbance was recorded at 700 nm.[15] Reference was Ascorbic acid.

Metal chelating capacity

The incubation of the extracts was performed with $FeCl_2$ (2 mM). Following the 0.2 ml of ferrozine (5 mM) addition, the reaction was initiated. After a while, the absorbance was recorded at 562 nm[16]. Ethylenediaminetetraacetic acid (EDTA) was the reference. $FeCl_2$ and ferrozine were placed in control. Inhibition ratio of the ferrozine-Fe^{+2} complex generation was estimated using the following formula: Metal chelating activity (%)=$[(A_{Control}-A_{Sample})/A_{Control}]\times100$.

Total antioxidant capacity

Molybdate reagent, water and the extracts were mixed in the test tubes. Incubation of the vortexed tubes was performed for 90 min at high temperature. Then, the tubes were allowed to cool, and the absorbance values were recorded at 695 nm wavelength. Results were shown as ascorbic acid equivalent in the extract (mg/g).[17]

Phytochemical Content

Estimation of total phenol content

Folin-Ciocalteu reagent was mixed with the extracts, and the incubation of the samples was done. After the addition of the sodium carbonate solution, samples were immediately vortexed. The absorbances were recorded at 735 nm after half an hour in darkness. Total phenol content of the extracts was presented in mg of gallic acid equivalents (GAE).[18] The equation was; y(Abs.)=1.6342x(Conc.)+1.417, and r^2 value was 0.9986.

Determination of total flavonoid content

80% ethanol was used to dissolve the extracts (1 mg/ml). Sodium acetate, aluminum chloride solution, ethyl alcohol and water were mixed with the extract samples. After incubation for 30 min, the absorbances were recorded at 415 nm. Results were given in mg of quercetin equivalents (QE)/g extracts.[19] The equation was; y(Abs)=1.8934x(Conc.)-0.025, and r^2 value was 0.9996.

Qualitative and quantitative analyses of phenolic compounds using RP-HPLC-PDA

The HPLC analyses were done according to the validated procedure.[20,21] HP Agilent 1260 series LC system was used. ACE column (5µm, 250mm × 4.6 mm) was preferred at 30°C. The flow rate of the gradient elution was set to 0.8 mL/min. The mobile phase was a mixture of trifluoroacetic acid 0.1 % in water (solution A), trifluoroacetic acid 0.1 % in methanol (solution B), and trifluoroacetic acid 0.1 % in acetonitrile (solution C). Caffeic and rosmarinic acids were analyzed at 330 nm, while rutin was at 360 nm by external standardization.

Statistical Analysis

Analyses were done in triplicates and the mean values were obtained. All values are given as the mean ± standard deviation (S.D.). Computations were performed via GraphPad Instat and Microsoft Excel.

RESULTS

According to the total phenolic content assay results, lyophilized water and methanol extracts of all the investigated parts of two *Moltkia* species were found to be rich in total phenolics. Water and methanol extracts of *M. aurea* roots had the highest total phenolic contents (376.53 ± 34.19 and 369.40 ± 34.30 mg GAE/g extract respectively) among all. The highest total flavonoid amount was determined in the ethyl acetate extract of *M. coerulea* leaves (127.46 ± 4.33 mg QE/g extract). Total flavonoid amount of flower methanol extract of *M. aurea* was also quite high as 58.80 ± 2.61 mg QE/g extract, and that was identical with the results of our

HPLC analysis indicating high rutin content. Extract yields and obtained results in terms of total phenol and flavonoid amounts were presented in Table 1.
An approach for the handling of diabetes is inhibition of α-amylase and α-glucosidase enzymes. In this study, *Moltkia* species displayed mild enzyme inhibitory activity compared to the reference acarbose. (Table 2). Ethyl acetate and water leaf extracts of *M. aurea* mostly inhibited α-amylase (24.07 ± 2.59% and 14.11 ± 2.59, respectively), together with ethyl acetate flower extract of *M. coerulea* (18.89 ± 2.43%) at 3 mg /mL concentration. Methanol and ethyl acetate extracts generally inhibited α-glucosidase enzyme stronger than the water extracts (Table 2).
Antioxidant activity is an excellent instance of a functional benefit that plant extracts can provide.[22] To present the antioxidant potential of the aforementioned *Moltkia* species, various *in vitro* tests were performed. Total antioxidant capacity experiment results revealed that ethyl acetate extracts of the species displayed significant antioxidant potential than the other solvent extracts. Also, water and especially methanol extracts showed meaningful ferric reducing power compared to the ethyl acetate extracts. All the water extracts of the investigated parts of the two species exhibited spectacular metal chelating activity (Table 3). While the superoxide scavenging activity of water, methanol and ethyl acetate extracts of the roots of both species were found to be promising, especially methanol extracts of all the samples considerably scavenged DPPH free radical (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, Rt apigenin:40.3 min) only caffeic acid, rutin and rosmarinic acid were detected and quantified in the flower, leaf and root methanol extracts of the species (Figure 1-6). Chromatograms of the standard compounds were placed in Figure 7-8. UV spectra of the quantified compounds which were overlaid with the authentic compounds, were given in Figure 9-11.
Rutin is a flavonoid glycoside, naturally taking place in many fruits and vegetables, which has several biological activities such as antioxidant, antibacterial, antifungal, antiinflammatory effects.[23] As it can be seen from the chromatograms, rutin was determined in significant amount in the flower extract of *M. aurea* as 6.198±0.271% (mean peak area: 3290), distinctly methanol extract of *M. coerulea* flowers contained less amount of rutin as 0.099±0.002% (mean peak area: 81.23). This data was thought to be really spectacular especially revealing a phytochemical difference between these two *Moltkia* taxa growing naturally in Turkey.
Rosmarinic acid is a phenolic compound derived from hydroxycinnamic acid, mostly found in Lamiaceae and Boraginaceae plants, which has many biological activities including antioxidant, antimicrobial, antiinflammatory, antiproliferative and chemopreventive effects.[24] As it was clearly observed from the chromatograms, especially root extracts of the two species were found to be rich in rosmarinic acid with values of 3.459±0.005% and 2.028±0.012%, respectively. Also, rosmarinic acid was detected in remarkable amount in the other investigated parts of the species (Table 5).

DISCUSSION

To our comprehensive literature survey, just a few studies on pharmacological activities and chemical profile of related *Moltkia* species were scared up. Harput et al.[5] investigated cytotoxic activity of the aerial parts of *M. aurea* on three different cell lines and determined antioxidant activity. Cytotoxic activity has been shown against cancer cell lines dose dependantly as well as promising antioxidant activity. Additionally, some phenolic compounds were isolated from the water sub-extract. Erdemoglu et al.[25] studied on the chemical constituents of seed oil of *M. aurea*.
In a recent study, the effect of ointment prepared from the aerial parts of *M. coerulea* collected from Iran on wound healing were investigated by Farahpour et al.[26] Additionally,

chemical constituents of flowers of *M. coerulea* collected from India were determined as fatty acids (capric, myristic, palmitic, behenic, undecylic acid), flavonoids (kaempferol, quercetin, nortangeretin, rebinetin) and amino acids (ornithine, lysine, Dopa, serine, glutamic acid, proline, amino-n-butyric acid, phenylalanine, leucine).[27]

Zengin et al.[9] investigated the antioxidant, antimicrobial, antityrosinase, antiacetylcholinesterase, antibutyrylcholinesterase, and antidiabetic capacities of the methanolic extracts of the aerial parts of *M. aurea* and *M. coerulea in vitro*. Also, the phenolic profile of the species was studied and an *in vivo* assay was performed to evaluate genotoxicity. According to the results, it was indicated that rutin, hesperidin, and protocatechuic acid were mainly detected in the aerial parts of *M. aurea*, while *M. coerulea* showed a close profile with rutin, protocatechuic acid, malic acid, and hesperidin. In our study, we checked three different parts of the species in terms of antioxidant and antidiabetic activity and the phenolic compounds profile. Similarly, with the above given study, rutin was determined in significant amount in *M. aurea* flower methanol extract, but also, we determined rosmarinic acid as one of the most abundant compound in both species, especially in the leaves and roots of *M. aurea* and in all the investigated parts of *M. coerulea*. The rosmarinic acid content of the species could be placed as the main difference among these two studies. Rutin amount was determined too high in the flower extract of *M. aurea* compared to *M. coerulea*. Also, caffeic acid should contribute to the antioxidant feature of the species. According to the Zengin et al.[9] study, the antioxidant effect of *M. aurea* and *M. coerulea* was assessed. The results depicted that although both of the species showed antioxidant properties, *M. aurea* was better. We can't exactly indicate or categorize the species according to their antioxidant potential, although different results were obtained from different extracts so that building a correlation was difficult. On the other hand, the results depicted that *M. aurea* and *M. coerulea* possessed antidiabetic activity as inhibitors of carbohydrate digestive enzymes.

CONCLUSIONS

This study revealed that *M. aurae* and *M. coerulea* possessed potent antioxidant and mild carbohydrate digestive enzyme inhibitory activity in *in vitro* assays. It was demonstrated that both plants appeared as a significant source of rosmarinic acid together with rutin and caffeic acid which are possibly responsible for the aforementioned activities. These results propose that tested *Moltkia* species could be used as potential natural antioxidant sources. On the other hand, especially *M. aurea* extracts could be evaluated in the development of herbal products with antidiabetic potential.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

1.Dogu S, Dinc M, Pınar NM. Anatomical and micromorphological differentiation in the genus *Moltkia* Lehm in Turkey. Pakistan J Bot. 2012;44:1083-1090.

2.Sharma RA, Singh B, Singh D, Chandrawat P. Ethnomedicinal, pharmacological properties and chemistry of some medicinal plants of Boraginaceae in India. J Medic Plants Res. 2009;3:1153-1175.

3.Balpınar N, Ökmen G. The biological activities of *Moltkia aurea* Boiss., an endemic species to Turkey. Afr J Tradit Complement Altern Med. 2017;14:60-64.

4 Georgiou O, Dimitrellos G, Georgiadis T. *Moltkia petraea* (Boraginaceae) in Greece. Phyton. 2000;40:57-69.

5.Harput US, Nagatsu A, Saracoglu, I. Antioxidant and cytotoxic effects of *Moltkia aurea* Boiss. Rec Nat Prod. 2012;6:62-66.

6.Ozdemir E, Alpınar K. The wild edible plants of western Nigde Aladaglar Mountains (Central Turkey). J Fac Pharm Ist Univ. 2010-2011;41:66-74.

7.Ozdemir E, Alpınar K. An ethnobotanical survey of medicinal plants in western part of central Taurus Mountains: Aladaglar (Nigde-Turkey). J Ethnopharmacol. 2015;166:53-65.
8.Van Quan N, Xuan TD, Tran H-D, Thuy NTD, Trang LT, Huong CT, Andriana Y, Tuyen PT. Antioxidant, α-amylase and α-glucosidase inhibitory activities and potential constituents of *Canarium tramdenum* Bark, Molecules. 2019;24:605.
9.Zengin G, Ceylan R, Katanic J, Mollica A, Aktumsek A, Boroja T, Matic S, Mihailovic V, Stanic S, Aumeerudy-Elalfi Z, Yılmaz MA, Mahomoodally MF. Combining *in vitro*, *in vivo* and *in silico* approaches to evaluate nutraceutical potentials and chemical fingerprints of *Moltkia aurea* and *Moltkia coerulea*, Food Chem Toxicol. 2017;107:540-553.
10.Ali H, Houghton PJ, Soumyanath A. *α*-Amylase inhibitory activity of some Malaysian plants used to treat diabetes, with particular reference to *Phyllanthus amarus*. J Ethnopharmacol. 2006;107:449-455.
11.Lam SH, Chen JM, Kang CJ, Chen CH, Lee SS. α-Glucosidase inhibitors from the seeds of *Syagrus romanzoffiana*. Phytochemistry. 2008;69:1173-1178.
12.Jung HA, Jin SE, Choi RJ, Manh HT, Kim YS, Min BS, Son YK, Ahn BR, Kim BW, Sohn HS, Choi JS. Anti tumorigenic activity of sophoraflavescenol against lewis lung carcinoma *in vitro* and *in vivo*. Arch Pharm Res. 2011;34:2087-2099.
13.Liu F, Ooi VFC, Chang ST. Free radical scavenging activity of mushroom polysaccharide extracts. Life Sci. 1997;60:763-771.
14.Adaramoye OA, Farombi EO, Adeyemi EO, Emerole GO. Comparative study on the antioxidant properties of flavonoids of *Garcinia kola* seeds. Pak J Med Sci. 2005;21:331-339.
15.Oyaizu M. Studies on products of browning reactions-antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr. 1986;44:307-315.
16.Dinish TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys. 1994;315:161-169.
17.Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex, specific application to the determination of vitamin E. Anal Biochem. 1999;269:337-341.
18.Zongo C, Savadogo A, Ouattara L, Bassole IHN, Ouattara CAT, Ouattara AS, Barro N, Koudou J, Traore AS. Polyphenols content, antioxidant and antimicrobial activities of *Ampelocissus grantii* (Baker) Planch. (Vitaceae): A medicinal plant from Burkina Faso. Int J Pharmacol. 2010;6:880-887.
19.Kosalec I, Bakmaz M, Pepeljnjak S, Vladimir-Knezevic S. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. Acta Pharmaceut. 2004;54:65-72.
20.Gökbulut A. Validated RP-HPLC method for quantification of phenolic compounds in methanol extracts of aerial parts and roots of *Thymus sipyleus* and evaluation of antioxidant potential. Trop J Pharm Res. 2015;14:1871-1877.
21.Orhan Deliorman D, Hartevioğlu A, Orhan N, Berkkan A, Gökbulut A, Günhan Ö, Pekcan M. Subacute effects of standardized *Fumaria vaillantii* Lois. ethanol extract on trace element levels, biochemical and histopathological parameters in experimental liver toxicity. J Food Biochem. 2016;40:180-189.
22.Meot-Duros L, Le Floch G, Magne C. Radical scavenging, antioxidant and antimicrobial activities of halophytic species. J Ethnopharmacol. 2008;116:258-262.
23.Gullón B, Lú-Chau TA, Moreira MT, Lema JM, Eibes G. Rutin: A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. Trends Food Sci Tech. 2017;67:220-235.
24.Al-Dhabi NA, Arasu MV, Park CH, Park SU. Recent studies on rosmarinic acid and its biological and pharmacological activities. EXCLI J. 2014;13:1192-1195.

25.Erdemoglu N, Kusmenoglu S, Vural M. γ-Linolenic acid content and fatty acid composition of Boraginaceae seed oils. Eur J Lipid Sci Tech. 2004;106:160-164.
26.Farahpour MR, Dilmaghanian A, Faridy M, Karashi E. Topical *Moltkia coerulea* hydroethanolic extract accelerates the repair of excision wound in a rat model. Chin J. Traumatol. 2016;19:97-103.
27.Meshkatalsadat MH, Parekh HH. Chemical investigations of the flowers of *Moltkia coerulea*. Planta Med. 1990;56:556-557.

Table 1. Yield percentages, total phenol and flavonoid contents of the *Moltkia* extracts

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

Total flavonoid content was expressed as mg QE/g extract and total phenol content was expressed as mg GAE/g extract.

Table 2. Enzyme inhibitory effects of the *Moltkia* extracts

Plant	Part	Extract	*α*-Glucosidase Inh. Inh. % ± S.D.			*α* I
			3 mg/mL	1 mg/mL	0.3 mg/mL	
M. aurea	Leaf	Water	52.77 ± 7.26	29.29 ± 1.71	3.31 ± 1.74	
		MeOH	51.87 ± 2.41	28.31 ± 3.50	13.47 ± 2.06	
		EA	86.02 ± 1.23	76.17 ± 0.22	59.41 ± 1.23	
	Flower	Water	48.10 ± 7.15	30.49 ± 3.85	19.42 ± 3.00	
		MeOH	61.01 ± 4.07	47.86 ± 2.98	25.26 ± 2.54	
		EA	78.74 ± 1.18	63.92 ± 1.46	46.55 ± 3.98	
	Root	Water	85.67 ± 2.76	67.66 ± 0.66	25.42 ± 0.59	
		MeOH	77.68 ± 3.85	68.35 ± 0.72	31.04 ± 4.81	
		EA	84.93 ± 2.09	69.12 ± 1.39	33.43 ± 3.48	

M. coerulea	Leaf	Water	49.84 ± 0.99	33.64 ± 3.31	36.78 ± 6.07	
		MeOH	66.84 ± 2.54	48.25 ± 4.24	37.44 ± 3.92	
		EA	66.26 ± 2.58	64.69 ± 2.12	41.08 ± 5.87	
	Flower	Water	44.12 ± 5.43	36.89 ± 4.06	13.70 ± 0.91	
		MeOH	60.04 ± 0.65	41.28 ± 0.97	16.64 ± 2.20	
		EA	84.11 ± 1.79	73.24 ± 1.05	53.95 ± 3.91	
	Root	Water	46.44 ± 3.10	35.03 ± 1.94	5.68 ± 1.43	
		MeOH	74.65 ± 1.54	48.66 ± 1.83	19.00 ± 4.25	
		EA	71.56 ± 1.00	62.12 ± 0.30	42.75 ± 3.10	
Reference	**Acarbose**		**1 mg/mL**	**0.3 mg/mL**	**0.1 mg/mL**	**1 mg/mL**
			98.88 ± 0.07	97.98 ± 0.03	96.37 ± 0.56	44.72 ± 2.76

-: No activity, S.D.: Standard Deviation

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

Plant	**Part**	**Extract**	**Metal Chelating Activity % ± S.D.**			**Ferric Reducing Power Absorbance ± S.D.**	
			3 mg/mL	**1 mg/mL**	**0.3 mg/mL**	**3 mg/mL**	**1 mg/mL**
M. aurea	Leaf	Water	>100	90.33 ± 4.19	48.96 ± 5.72	1.5307 ± 0.0370	0.7410 ± 0.0605
		MeOH	22.42 ± 3.58	-	-	2.3673 ± 0.0393	1.9194 ± 0.0615
		EA	-	-	-	0.7040 ± 0.0867	0.2143 ± 0.0084
	Flower	Water	>100	98.11 ± 5.66	33.25 ± 6.17	2.0137 ± 0.1373	1.3583 ± 0.0741
		MeOH	70.17 ± 3.86	-	-	2.5244 ± 0.0674	2.4727 ± 0.0665
		EA	12.07 ± 6.29	-	-	0.6154 ± 0.0285	0.0847 ± 0.0110
	Root	Water	>100	35.99 ± 2.92	-	3.1090 ± 0.0210	2.7980 ± 0.0936
		MeOH	-	-	-	1.7140 ± 0.0875	1.4720 ± 0.0339
		EA	43.03 ± 7.53	-	-	1.2424 ± 0.0101	0.9977 ± 0.1124
M. coerulea	Leaf	Water	>100	80.00 ± 3.47	15.90 ± 3.05	1.9434 ± 0.0248	0.7843 ± 0.5653
		MeOH	38.79 ± 9.22	-	-	2.9394 ± 0.0418	2.4310 ± 0.0752
		EA	-	-	-	0.3470 ± 0.0172	0.1353 ± 0.0195
	Flower	Water	>100	99.87 ± 2.63	29.77 ± 0.61	1.8684 ± 0.0300	1.4033 ± 0.1028
		MeOH	80.31 ± 8.32	-	-	2.5758 ± 0.0979	2.1333 ± 0.1092
		EA	-	-	-	1.2720 ± 0.0966	0.1057 ± 0.0176
	Root	Water	>100	80.36 ± 8.16	40.00 ± 7.18	1.9684 ± 0.0835	0.9477 ± 0.0761
		MeOH	72.42 ± 6.87	-	-	1.8060 ± 0.0095	0.9323 ± 0.0595
		EA	-	-	-	1.8114 ± 0.0166	0.3910 ± 0.0231
Reference			**1 mg/mL**	**0.3 mg/mL**	**0.1 mg/mL**	**3 mg/mL**	**1 mg/mL**
EDTA			98.87 ± 0.14	96.60 ± 3.39	95.75 ± 0.08	NT	NT
Ascorbic acid			NT	NT	NT	3.5870 ± 0.0874	3.4547 ± 0.0852

NT: Not tested, -: No activity, S.D.: Standard Deviation

* Total antioxidant capacity is expressed as ascorbic acid equivalent/g extract ± S.D.

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

Plant	**Part**	**Extract**	**Superoxide anion scavenging activity Inhibition % ± S.D.**			**DPPH radical scavenging activity Inhibition % ± S.D.**		
			3 mg/mL	**1 mg/mL**	**0.3 mg/mL**	**3 mg/mL**	**1 mg/mL**	**0.3 mg/mL**
M. aurea	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
M. coerulea	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

-: No activity, ND: Not detected, S.D.: Standard Deviation

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

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

[a] *mean ± SD (n=3);* [b] *ND=not detected*

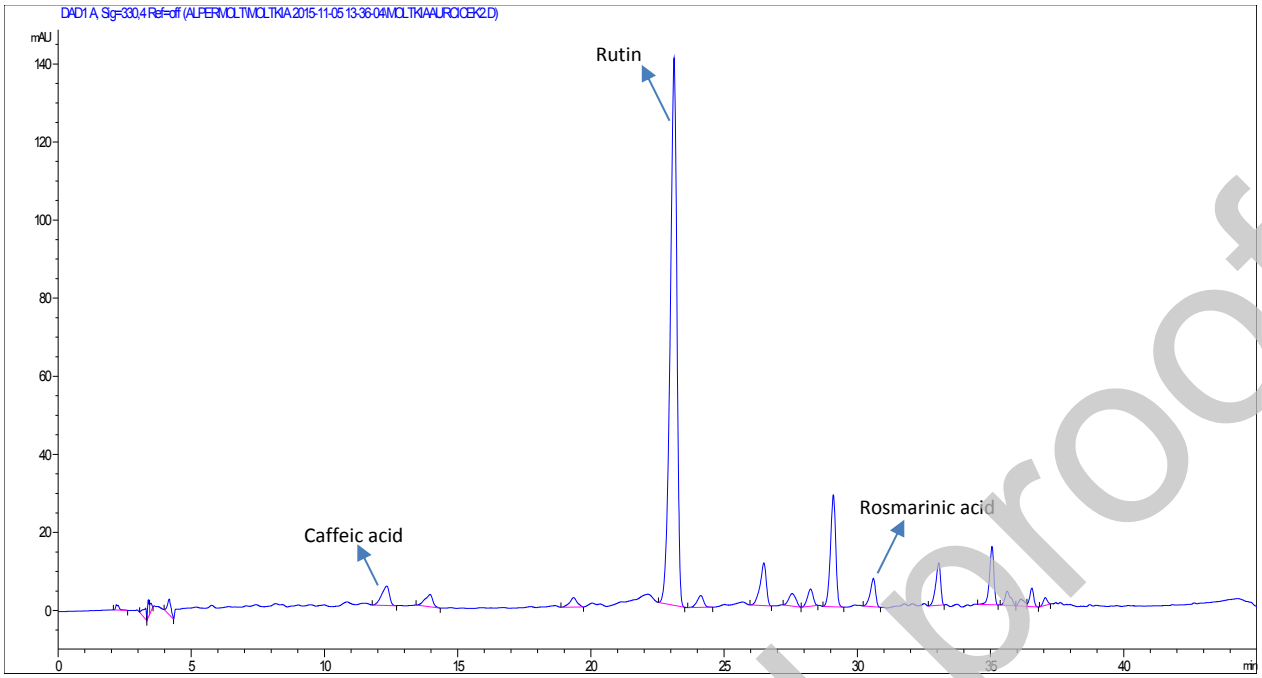

Figure 1. HPLC chromatogram of *M. aurea* flower extract (MeOH)

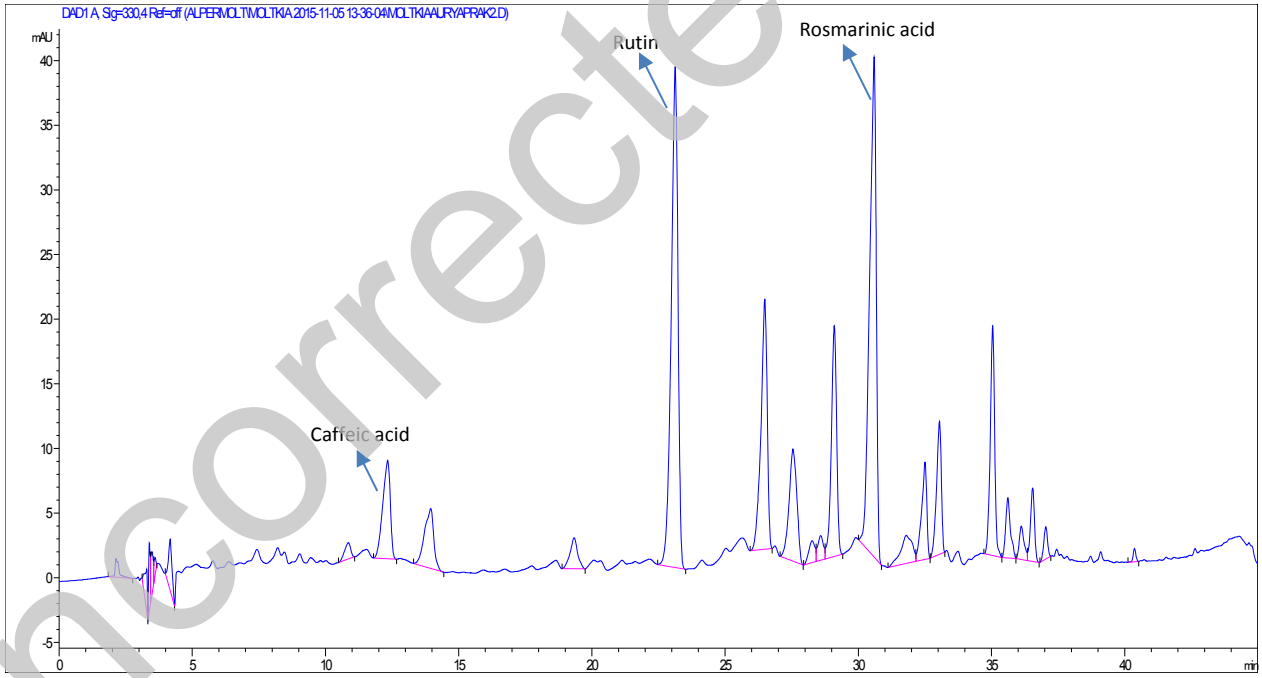

Figure 2. HPLC chromatogram of *M. aurea* leaf extract (MeOH)

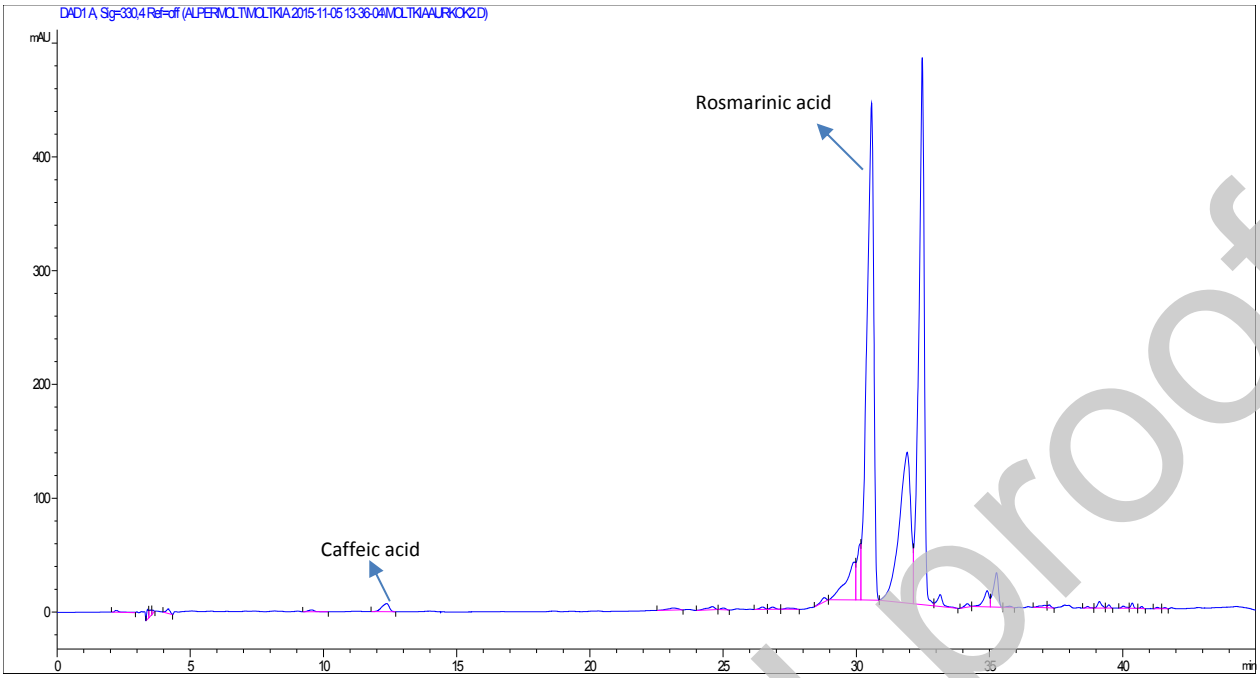

Figure 3. HPLC chromatogram of *M. aurea* root extract (MeOH)

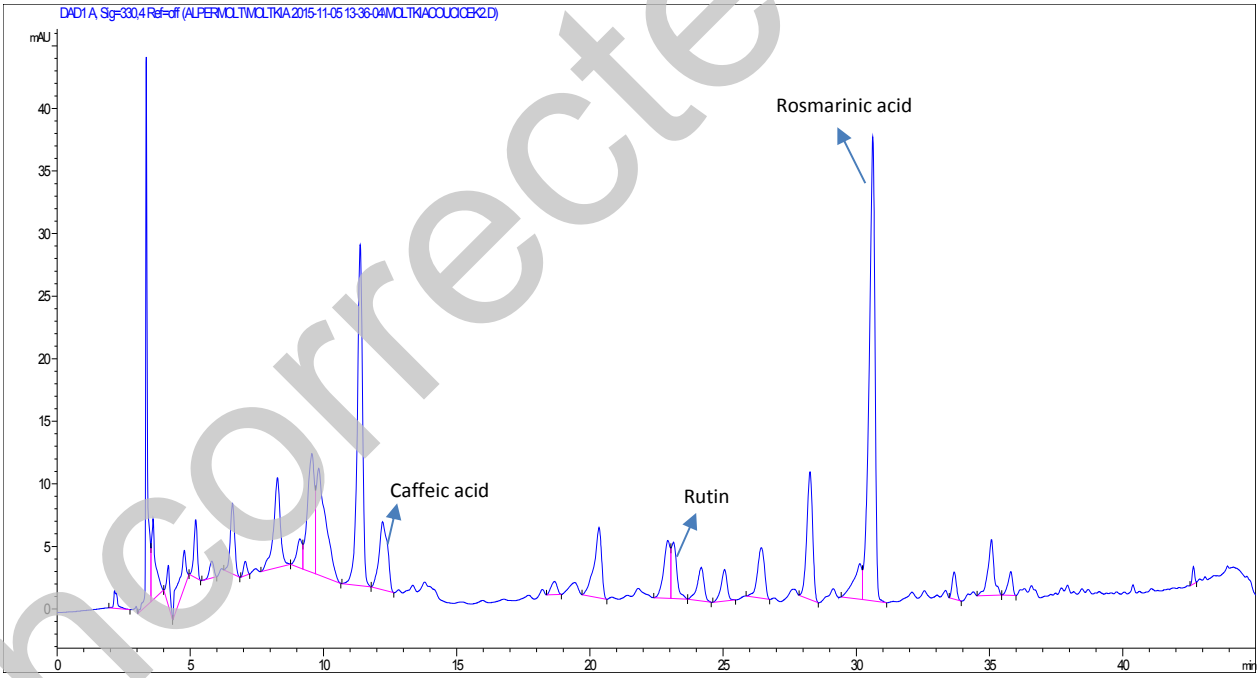

Figure 4. HPLC chromatogram of *M. coerulea* flower extract (MeOH)

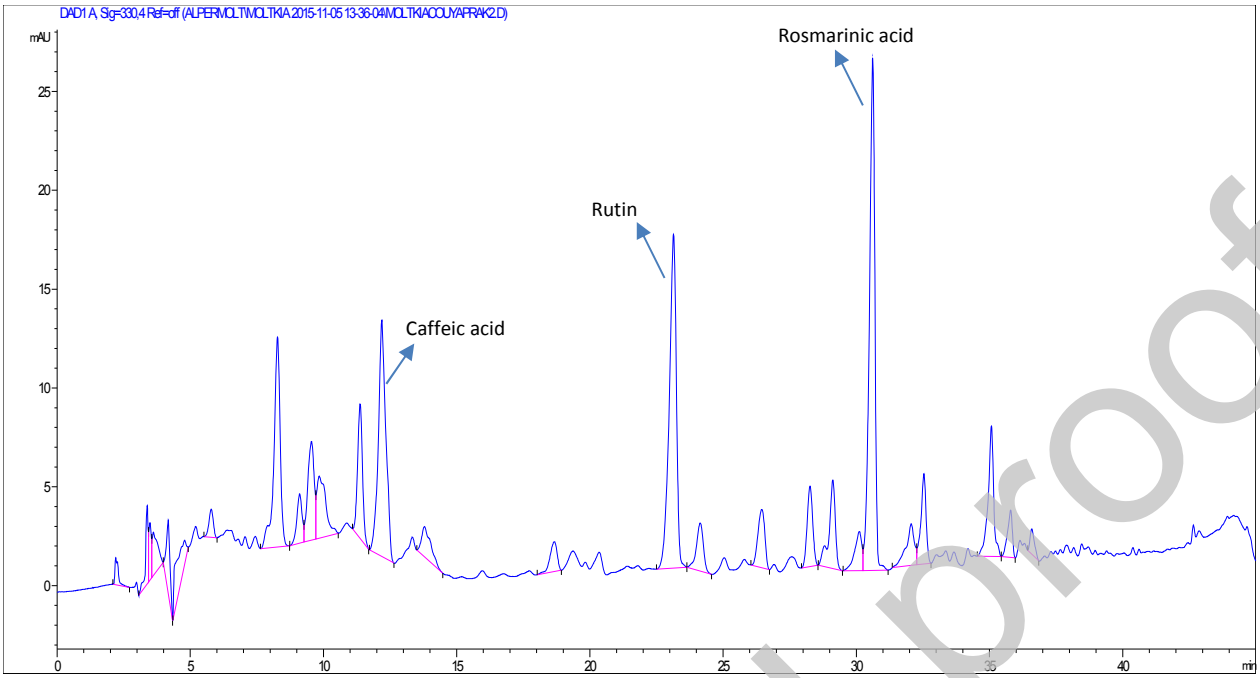

Figure 5. HPLC chromatogram of *M. coerulea* leaf extract (MeOH)

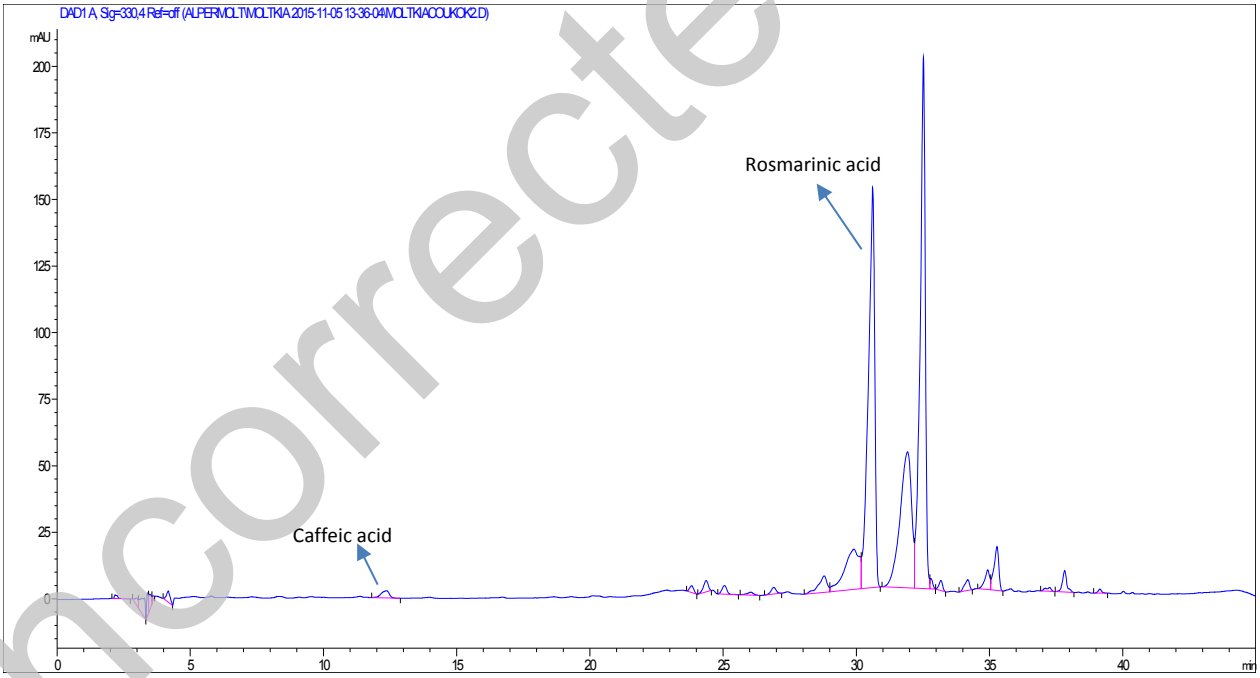

Figure 6. HPLC chromatogram of *M. coerulea* root extract (MeOH)

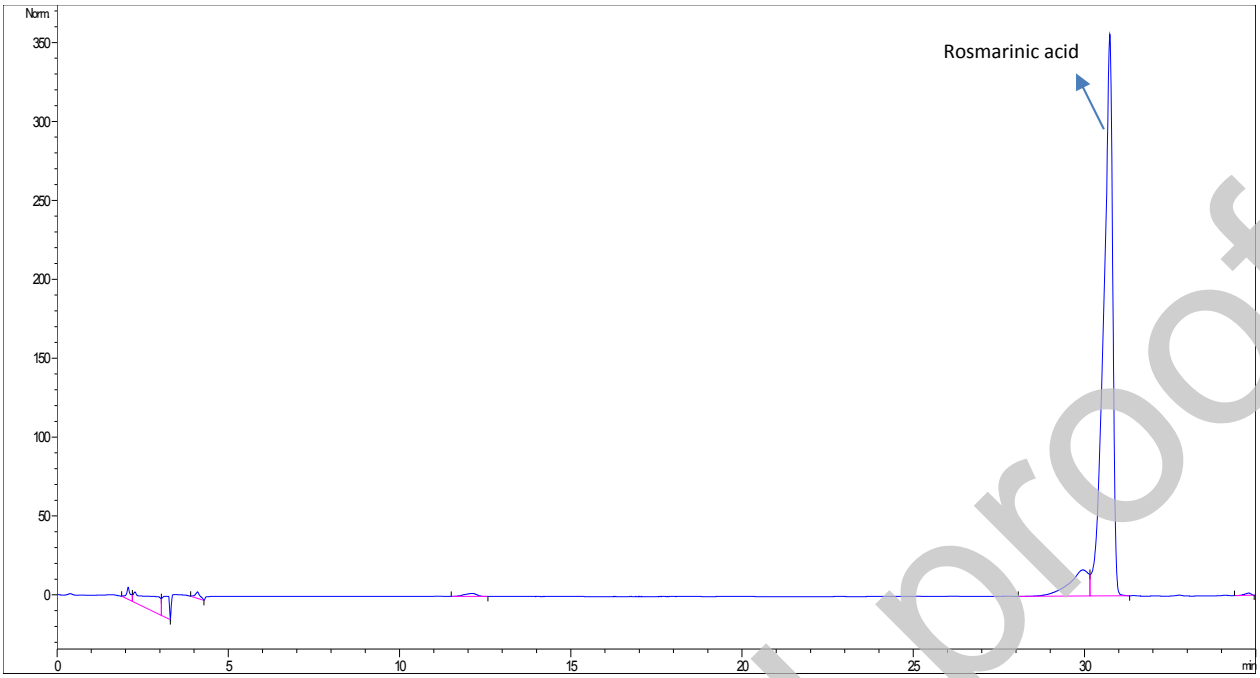

Figure 7. HPLC chromatogram of rosmarinic acid standard

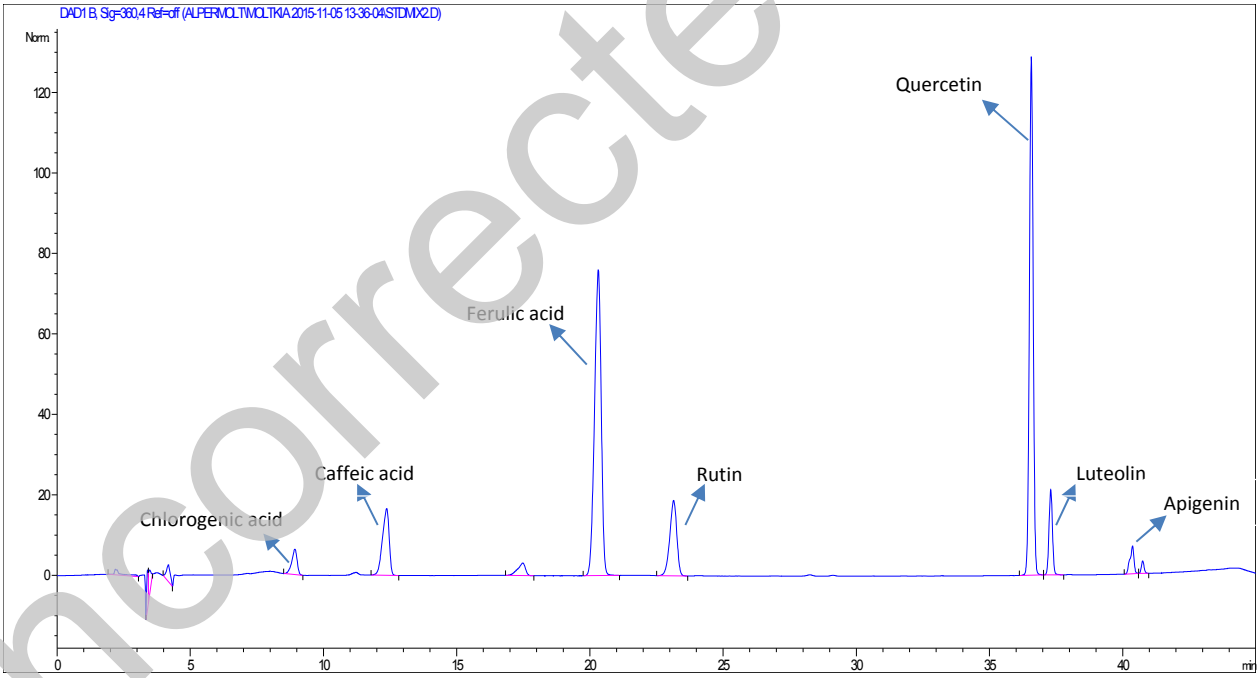

Figure 8. HPLC chromatogram of standard mixture: Chlorogenic acid (Rt: 8.9 min), caffeic acid (Rt: 12.3 min), ferulic acid (Rt: 20.25 min), rutin (Rt:23.1 min), quercetin (Rt: 36.5 min), luteolin (Rt: 37.3 min), apigenin (Rt:40.3 min)

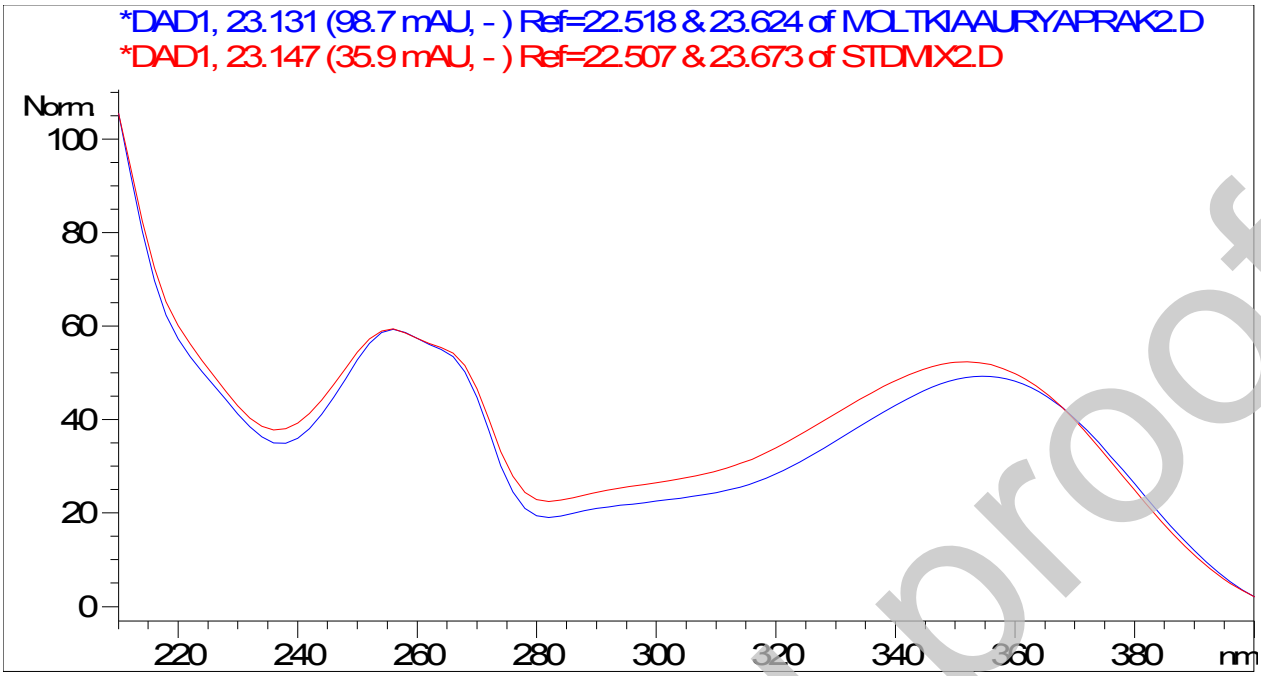

Figure 9. Overlaid UV spectra of standard rutin and rutin detected in the *M. aurea* leaf extract

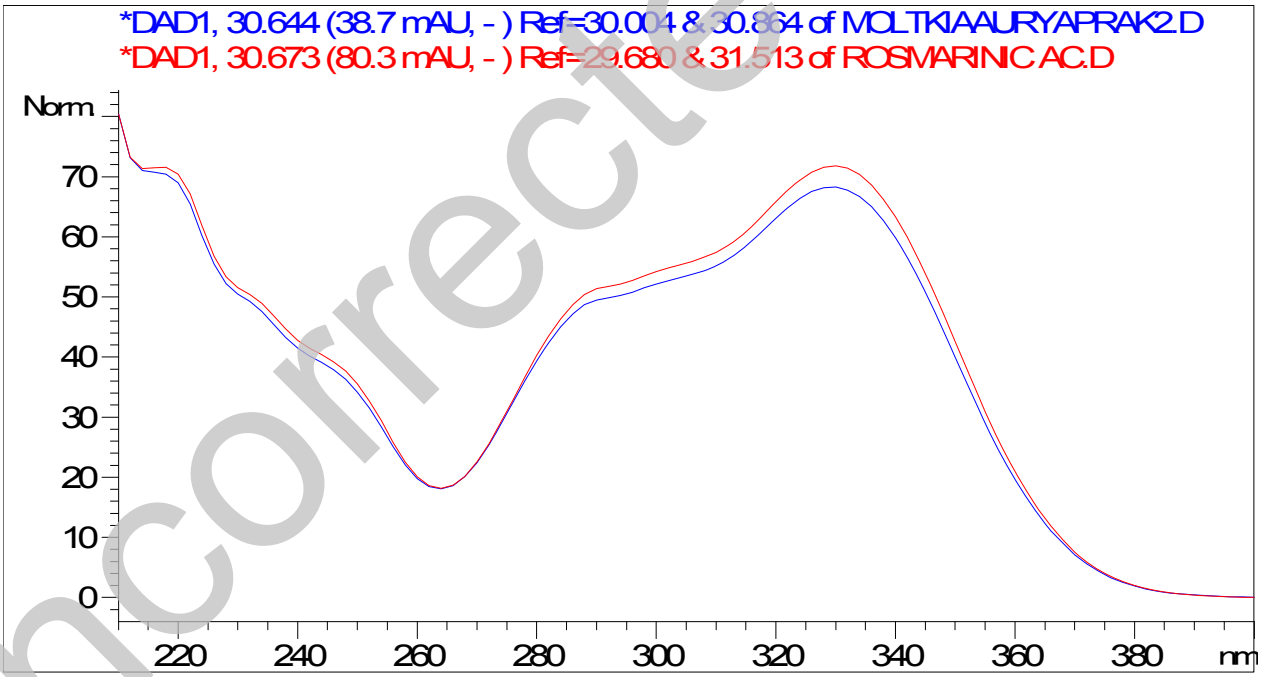

Figure 10. Overlaid UV spectra of standard rosmarinic acid and rosmarinic acid detected in the *M. aurea* leaf extract

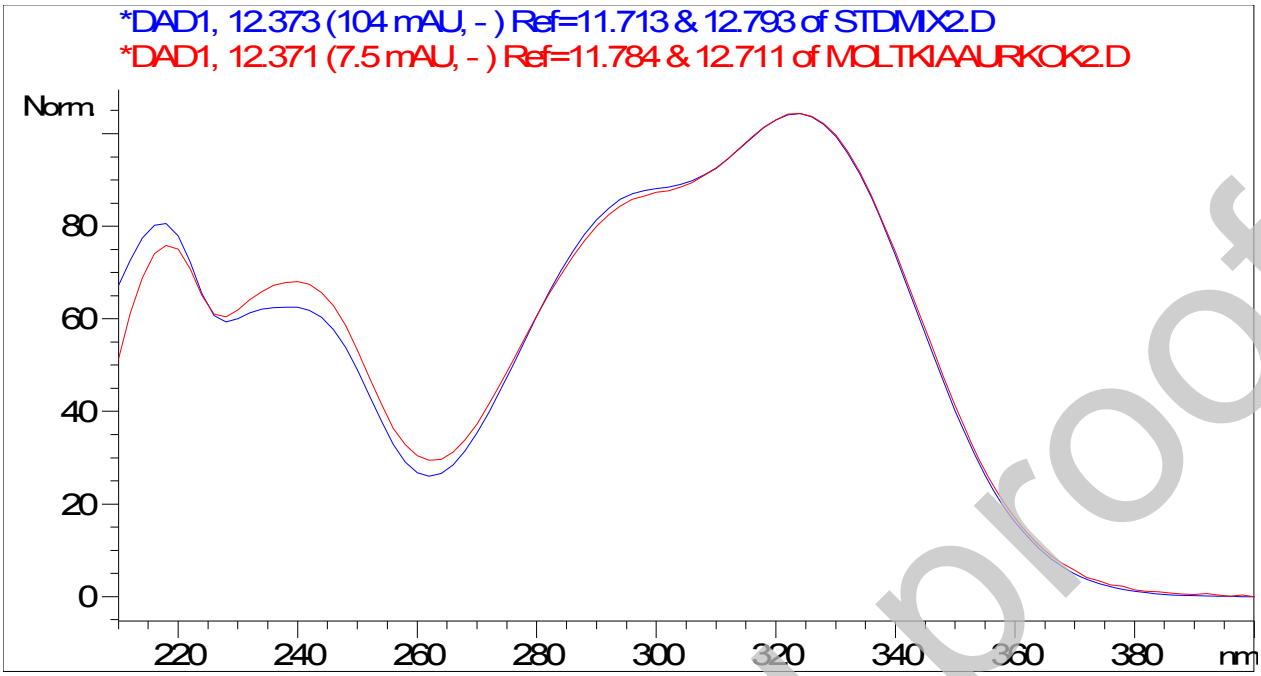

Figure 11. Overlaid UV spectra of standard caffeic acid and caffeic acid detected in the *M. aurea* root extract