

Total Phenolic and Flavonoid Content, Mineral Composition and Antioxidant Potential of *Trigonella monspeliaca*

Ş. Selma URAS GÜNGÖR¹, Sevda GÜZEL¹, Ahmet İLÇİM², Gamze KÖKDİL^{1*}

¹ Mersin University, Faculty of Pharmacy, Department of Pharmacognosy, 33169 Mersin, TURKEY, ² Mustafa Kemal University, Faculty of Arts and Science, Department of Biology, 31000 Antakya/Hatay, TURKEY

The seeds and aerial parts of *Trigonella monspeliaca* were analyzed by ICP-MS to determine mineral composition. K, P, Ca and Mg were main minerals in the seeds. Ca, K and P were the major elements in the aerial parts. Total phenol and flavonoid content of the aqueous methanol extracts of the plant was measured by Folin-Ciocalteu and the AlCl₃ assay, respectively. Antioxidant potential of the extracts was evaluated by free radical scavenging activity using DPPH assay. The present study showed that the seed extract contained significantly higher amount of total phenolics (150.80±0.33 mg GAE/g) while aerial parts had high amount of total flavonoids. In DPPH radical scavenging assay, inhibition % of the extracts was found to be 51.6-78.0.

Key words: *Trigonella monspeliaca* L., Mineral composition, Phenolics, Antioxidant activity.

Trigonella monspeliaca'nın Mineral Kompozisyonu, Total Fenol ve Flavonoit Miktarı, Antioksidan Etki Potansiyeli

Trigonella monspeliaca tohumları ve topraküstü kısmı mineral bileşimi açısından ICP-MS ile incelendi. Tohumda başlıca mineraller K, P, Ca ve Mg olarak saptandı. Toprak üstü kısımda ana mineraller ise Ca, K ve P olarak belirlendi. Bitkinin sulu metanollü ekstrelerinin total fenol ve flavonoit miktarı sırasıyla Folin-Ciocalteu ve AlCl₃ yöntemleri ile ölçüldü. Ekstrelerin antioksidan etki potansiyeli DPPH yöntemi kullanılarak serbest radikal süpürücü aktivite yönünden değerlendirildi. Çalışma toprak üstü kısmının yüksek miktarda flavonoit içerirken tohum ekstrelerinin total fenol miktarının (150.80±0.33 mg GAE/g) belirgin biçimde yüksek olduğunu gösterdi. DPPH radikal süpürücü teste ekstrelerin % inhibisyonunun 51.6 - 78.0 arasında olduğu belirlendi.

Anahtar kelimeler: *Trigonella monspeliaca* L., Mineral bileşim, Fenolikler, Antioksidan etki.

*Correspondence: E-mail: gkokdil@gmail.com, Tel: +90 324 341 30 21

INTRODUCTION

The genus *Trigonella* L. (Leguminosae) includes about 135 species distributed in Mediterranean regions, Southeastern Europe, Western Asia, North and South Africa (1-3). Among these species *Trigonella foenum-graecum* L., commonly called fenugreek, is one of the oldest medicinal plants and

cultivated in the Mediterranean region, India, North African countries, Yemen and China (4,5). The seeds have been used as a carminative, tonic, aphrodisiac in Ayurvedic, Chinese and Unani systems of medicine (1,6,7). They have been also used for the treatment of kidney-related disorders, bronchial complaints, diabetes, painful menstruation, neurasthenia, gout and arthritis

(1,4,5). *T. foenum-graecum* seeds are known as spice because of its aroma and benefits to human health, and fenugreek leaves are widely used in India as a green leafy vegetable in the diet because it is a rich source of calcium, iron, β -carotene and other vitamins (5,8). The plant contains fixed oils, saponins, flavonoids, alkaloids, fibers, polysaccharides, minerals, protein and amino acids (9,10). *Trigonella foenum-graecum* has been extensively studied and experimental and clinical studies have demonstrated its antidiabetic, antioxidant, anti-inflammatory, antipyretic, antiulcer, hypocholesterolaemic, immunomodulatory, wound-healing, CNS-stimulant, anticancer, gastro protective and chemo preventive effects (3,4,9,11-14).

According to the literature, *T. foenum-graecum* is extensively studied but there isn't much information for other species of the genus *Trigonella* (15,16). The genus *Trigonella* represented by 54 taxa which are divided 13 sections and 8 groups in Turkey (17-21). *T. monspeliaca* L. is one of these taxa, which grows Mediterranean area, the Syrian Desert, North Iraq, Iran, South Russia and Caucasia (17). This taxon has not been studied phytochemically and pharmacologically. The aim of the present study was to analyze the plant parts for mineral composition and to determine total phenol and flavonoid contents for their possible nutritional value and antioxidant activity.

MATERIALS AND METHODS

Plant material

Trigonella monspeliaca L. (Leguminosae) was collected from Çağlayanerit, Maraş during the seedling period in June 2011. The plant was identified by Assoc. Prof. Dr. Ahmet İlçim (Department of Biology, Faculty of Arts and Sciences, Mustafa Kemal University, Antakya, Hatay, Turkey). A herbarium voucher (MKU 1786) were deposited in the Herbarium of the Faculty of Arts and Sciences, Mustafa Kemal University

Chemicals

All chemicals were analytical-reagent grade and obtained from the following sources: Methanol, Folin-Ciocalteu reagent, nitric acid, hydrochloric acid, HClO_4 and trifluoroacetic acid (Merck, Darmstadt, Germany); 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), butylatedhydroxyanisole (BHA), gallic acid (Sigma Chemical Company, MO, USA). Rutin was obtained from Fluka Chemie (Buchs, Switzerland). Standard sample solutions of Na, Mg, K, Ca, Fe (1-9 mg/L) and P, Cr, Mn, Co, Ni, Cu, Zn, Se, Mo, Cd, Pb (10-90 $\mu\text{g/L}$) were prepared. Those standards were obtained from Environmental Calibration Standard (Agilent Technologies).

Extraction procedure

Powdered aerial part (3 g) and seed (3 g) samples were sonicated with 80% aqueous methanol for 60 min at 30°C. After filtration, the volumes were adjusted to 100 mL by adding 80% aqueous methanol and were stored at 4°C until the analysis (22).

Determination of total phenolic content

The amount of total phenolic content was performed by the Folin-Ciocalteu method as described by Kim *et al.* (23). The 1 mL of appropriately diluted extracts was mixed 9 mL of distilled water. One milliliter of Folin-Ciocalteu's phenol reagent was added to the mixtures and shaken. After 5 min, 10 mL of 7 % Na_2CO_3 solution was added. The solution was diluted to 25 mL with distilled water and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance versus blank was measured at 750 nm. Total phenolics were quantified by calibration curve obtained from measuring the standard solutions of gallic acid (400-1000 mg/L) and were expressed as mg gallic acid equivalents (GAE)/g of dried sample parts. The values are presented as means of triplicate analyses.

Determination of total flavonoid content

The concentration of total flavonoid content was measured as the method described by Kim *et al.* (23) with some modification based on that of the method described by Subhasree

et al. (24). The measurement was based on reaction with AlCl_3 and spectrophotometrical technique. Briefly, 0.5 mL of the each extract were added to a 10 mL volumetric flask containing 3 mL distilled water and then 0.3 mL 5% NaNO_2 was added. After 5 min, 0.3 mL 10% AlCl_3 was added. At 6 min, 2 mL 1 M NaOH was added to the mixture. The volume of the mixtures was adjusted to 10 mL by adding the appropriate volume of distilled water. Absorbance of the mixture was determined at 510 nm versus prepared methanol blank. All determinations were performed in triplicate. Rutin was used for the standard calibration curve. The data were expressed as mg rutin (25-250 mg/L) equivalents (RE)/g of dried sample parts. The values are presented as means of triplicate analyses.

DPPH free radical scavenging assay

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was conducted as described by Yen and Duht, 1994 (25). The each extract (0.1 mL) or standard antioxidant (BHA, 25-250 mg/L) was added to 2.9 mL methanolic solution of DPPH. The mixture was shaken vigorously and incubated in the dark for 45 min at room temperature and the decreases in the absorbance values were measured at 517 nm. The values are presented as means of triplicate analyses. The percentage of DPPH scavenging activity was calculated using the following equation: % DPPH scavenging activity: $100 \times [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}]$, where A_{control} is the absorbance of the control reaction mixture without the test material, and A_{sample} is the absorbance of the test materials.

Mineral analysis

A commercial domestic microwave oven CEM MARS 240/50 model with a timer and variable temperature settings were used for microwave-assisted digestion of plant materials. Each of dried samples of seeds and aerial parts (0.25 g) was prepared for digestion according to the method of Başgel and Erdemoğlu (26). The mineral constituents present in the examined seed and aerial parts of samples were analyzed separately, using an

Agilent 7500ce ICP-MS (Tokyo, Japan) equipped with a collision/reaction cell in the form of octopole reaction system (ORS). Instrument configuration and general experimental conditions for ICP-MS were as follows: Rf power (W): 1600; gas flow rate (L/min): Plasma gas: 15; carrier gas:1; makeup gas: 1; aux gas: 1; spray chamber temperature: 2°C; torch: quartz; auto sampler: CETAC ASX-520; read time (s): 30; delay time (s); 60; wash time (s): 20.

RESULTS AND DISCUSSION

The concentrations of seventeen elements determined in seeds and aerial parts of the plant. The results for element compositions of examined plant materials were presented in Table 1. Three macro minerals, namely potassium, calcium and phosphorus were relatively high in seeds and aerial parts. Calcium (13757.33 ± 25.48) is the main element in the aerial parts while potassium (10960.67 ± 5.51) was the most abundant mineral in the seeds. P, Mg, Ca, Na and Fe were present in moderate quantity in the seeds. The other main elements, in descending order by quantity, were Zn, Na, Cr, Fe, and Mn in aerial parts. Besides these findings, the plant had high content of selenium (38.19 ± 0.12) as compared to seeds.

In the literature, there is no information about mineral content of *T. monspeliaca* but only mineral composition of fenugreek (*T. foenum-graecum*) was studied extensively because of its using as a spice. Gupta et al. indicated that fenugreek seeds contained Ca, P and Mg in high concentrations and the level of Fe were 0.36 g/kg in the seeds. They were not determined K and Na in the seeds (27). It had been demonstrated that fenugreek seeds had high content of K (530 mg/100 g), P (370 mg/100 g), Ca (160 mg/100 g) and Mg (160 mg/100 g) by Srinivasan et al. (9). Cu, Na and Fe were determined in moderate concentrations in that study. Kan et al. reported the amounts of 12 minerals for fenugreek seeds cultivated in Turkey and Ca and Mg were found to be as major minerals in the seeds (28). Shakuntala et al. were

determined mineral content particularly Ca, Cu, Fe, K, Mn and Mg of fenugreek seed fractions such as endosperm, seed coat etc. They showed that Ca, K and Mg levels were present in high levels in all fractions of the

seeds (3). These mineral compositions of fenugreek seeds are in agreement with the present results but quantitative differences were found in *T. monspeliaca* seeds.

Table 1. Mineral contents of the seed and aerial parts of *T. monspeliaca* (µg/g of dry samples)^a

Minerals	Seed	Aerial parts
Macro minerals		
	369.1±0.17	316.5±0.44
Magnesium (Mg)	3555±7.94	1435±7.2
Phosphorus (P)	4730.33±7.09	3707.5±2.18
Potassium (K)	10960.67±5.51	7802.17±0.15
Calcium (Ca)	3352.67±5.03	13757.33±25.48
Essential trace minerals		
Chromium (Cr)	32.56±0.08	215.47±0.42
Manganese (Mn)	23.08±1.16	134.25±0.21
Iron (Fe)	158.94±0.13	162.79±0.11
Nickel (Ni)	3.34±0.09	16.4±0.16
Zinc (Zn)	47.7±0.25	415.47±0.5
Copper (Cu)	11.13±0.06	25.57±0.1
Selenium (Se)	7.78±0.18	38.19±0.12
Other elements		
Aluminum (Al)	45.14±0.25	1962.37±0.35
Cobalt (Co)	0.66±0.06	6.66±0.12
Molybdenum (Mo)	7.66±0.24	24.86±0.09
Cadmium (Cod)	1.44±0.15	10.1±0.07
Lead (Pb)	4.22±0.08	45.41±0.26

^aValues are means±standard deviations. (n=3).

The mineral content of fenugreek leaves has been investigated by other authors. Ca and Zn concentrations in the leaves of fenugreek were reported by Yadav and Sehgal (29). These authors indicated that the Ca content ranged from 940 to 970 mg/100g and the concentration of Zn was 11.7-12.3 mg/100g in the leaves. Similar and higher Ca content of fenugreek leaves has been reported by Gupta et al. (30). Srinivasan reported 395 mg Ca, 76 mg Na and 67 mg Mg per 100g in fresh fenugreek leaves (9). In comparison with the literature data, current results showed higher

content of Ca and K than reported that of fenugreek leaves.

In this study, Se content of the plant materials was also detected. Se is a nutritionally essential element to the life of human because of its acting as antioxidant, anticarcinogenic agent and regulator of thyroid function. The aerial parts of *T.monspeliaca* have high content of selenium (38.19±0.12).

In the present study, the total phenol and flavonoid content of the extracts prepared from *T. monspeliaca* determined using Folin-Ciocalteu and AlCl₃ spectrophotometrically

method. The seed extract exhibited the higher phenol content (150.8 ± 0.33 mg GAE/g) than the extract of aerial parts of the plant (Table 2).

The highest total flavonoid content was determined in the aerial parts. The antioxidant activity of the plant materials carried out by DPPH radical scavenging activities. The extract obtained from aerial parts of the plant showed the high antioxidant activity (78.0% inhibition).

There are several studies on the total phenol and antioxidant activities of fenugreek extracts prepared with different solvents in the literature. Naidu et al. found that fenugreek seed parts had 65.81-85.88 mg GAE/g total polyphenols and exhibited good free-radical scavenging activities from 50 to 70 % inhibition (31). Aqil et al. reported that the

extract of fenugreek leaves had 74.33 ± 5.13 mg GAE/g total phenolics and showed 57.45 ± 2.44 % inhibition by DPPH method (32). Gupta and Prakash found that fenugreek leaves contained 158.33 ± 20.41 mg tannic acid equivalent/100 g total phenol and they were also reported different concentrations of the extracts (4-20 mg/mL) had showed free radical scavenging activity ranging from 15.23 to 41.46 % (33). In this study, it was observed that *T. monspeliaca* had higher content of phenolics than the phenol content of fenugreek. Additionally the present study showed that a correlation exists between total flavonoid content and free radical scavenging activity. Furthermore, the extract obtained from aerial parts of the plant showed higher radical scavenging activity than that of the seed extract (Table 2).

Table 2. Total phenol and flavonoid content, and free radical scavenging activity of test samples from *T. monspeliaca*.

Sample	Total Phenolic content (mg of GAE/g)	Total Flavonoid content (mg of RE/g)	Inhibition (%)
Seed extract	150.80 ± 0.33	72.70 ± 0.04	51.6 ± 0.07
Aerial parts extract	100.35 ± 0.50	91.08 ± 0.01	78.0 ± 0.02
BHA (200 mg/L)	-	-	80.96 ± 0.09

All results are reported for the first time for *T. monspeliaca*. The results obtained in this study demonstrated that *T. monspeliaca* is important source of minerals and phenolics. The aerial parts of the plant have strong free radical scavenging activity. The concentration of total flavonoid of the extract exhibited positive correlation with antioxidant activity. Further studies are needed to evaluation of the plant in health and food industry.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge to Cihan GEÇGEL, MSc. and Mehmet Ali KURT, PhD. (Advanced Technology of Education, Research and Application Center,

Mersin University, Mersin) for excellent technical support using microwave oven and ICP-MS analyses.

REFERENCES

1. Bown D, Encyclopedia of Herbs & Their Uses, First Edition, pp 393, London, UK, Darling Kindersley Limited, 2002.
2. Evans WC, Trease and Evans Pharmacognosy, 15th Edition, pp 26, UK, University of Nottingham, 2002.
3. Shakuntala S, Naik JP, Jeyarani T, Naidu MM., Srinivas P, Characterisation of germinated fenugreek (*Trigonella foenum-graecum* L.) seed fractions, Int J Food Sci Tech 46, 2337-2343, 2011.

4. Zia T, Nazrul Hasnain S, Hasan SK, Evaluation of the oral hypoglycaemic effect of *Trigonella foenum-graecum* L. in normal mice, J Ethnopharmacol 75, 191-195, 2001.
5. Devi BA, Kamalakkannan N, Prince PSM, Supplementation of fenugreek leaves to diabetic rats. Effect on carbohydrate metabolic enzymes in diabetic liver and kidney, Phytother Res 17, 1231-1233, 2003.
6. Gopu CL, Gilda SS, Paradkar AR, Mahadik KR, Development and validation of a densitometric TLC method for analysis of trigonelline and 4-hydroxyisoleucine in fenugreek seeds, Acta Chromatogr 20, 709-719, 2008.
7. Morani AS, Bodhankar SL, Mohan V, Thakurdesai PA, Ameliorative effects of standardized extract from *Trigonella foenum-graecum* L. seeds on painful peripheral neuropathy in rats, Asian Pacific Journal of Tropical Medicine 5(5), 385-390, 2012.
8. Naidu MM, Khanum H, Sulochanamma G, Sowbhagya HB, Hebbar, UH, Prakash M, Srinivas P, Effect of drying methods on the quality characteristics of fenugreek (*Trigonella foenum-graecum*) greens, Dry Technol 30, 808-816, 2012.
9. Srinivasan K. Fenugreek (*Trigonella foenum-graecum*): A review of health beneficial physiological effects, Food Rev Int 22, 203-224, 2006.
10. Shailajan S, Menon S, Singh A, Mhatre M, Sayed N. A validated RP-HPLC method for quantitation of trigonelline from herbal formulations containing *Trigonella foenum-graecum* (L.) seeds, Pharmaceutical Methods 2, 157-158, 2011.
11. Pandian RS, Anuradha CV, Viswanathan P, Gastroprotective effect of fenugreek seeds (*Trigonella foenum-graecum*) on experimental gastric ulcer in rats, J Ethnopharmacol 81, 393-397, 2002.
12. Toppo FA, Akhand R, Pathak AK, Pharmacological actions and potential uses of *Trigonella foenum-graecum*: A Review, Asian J Pharm Clin Res 2(4), 28-38, 2009.
13. SatheeshKumar N, Mukherjee PK, Bhadra S, Saha BP, Acetylcholinesterase enzyme inhibitory potential of standardized extract of *Trigonella foenum graecum* L and its constituents, Phytomedicine 17, 292-295, 2010.
14. Yadav R, Kaushik, R, Gupta D, The health benefits of *Trigonella foenum-graecum*: A Review, Int J Eng Res Appl 1, 32-35, 2011.
15. Kawashyt SA, Abdalla MF, El Din EMG, Saleh, NAM, The chemosystematics of Egyptian *Trigonella* species, Biochem Syst Ecol 26, 851-856, 1998.
16. Yuldashev MP, Flavonoids of the Aerial Part of *Trigonella grandiflora*, Chem Nat Comp 38, 291-292, 2002.
17. Huber-Morath A, *Trigonella* L. In: Flora of Turkey and the East Aegean Islands (edited by PH Davis), Vol 3, pp 452-482, Edinburgh: Edinburgh University Press, 1970.
18. Davis PH, Mill RR, Tan K, Flora of Turkey and The East Aegean Island, Vol 10, pp 324-326, Edinburgh, UK: Edinburgh University Press, 1988.
19. Güner A, Özhatay N, Ekim T, Başer KHC, Leguminosae, Flora of Turkey and the East Aegean Islands (Suppl 2), Vol 11, pp 193, Edinburgh, UK, Edinburgh University Press, 2000.
20. Gokturk RS, A new subspecies *Trigonella coeruleascens* (Fabaceae), from Turkey, Ann Bot Fenn 46, 62-64, 2009.
21. Martin E, Akan H, Ekici M, Aytaç Z, Karyology of ten Turkish *Trigonella* L. (Leguminosae) species from section *Cylindricae* Boiss, Turk J Bot 34, 485-494, 2010.
22. Gikas E, Bazoti FN, Papadopoulos N, Alesta A, Economou, G, Tsarbopoulos A, Quantitation of the flavanols quercetin and kaempferol in the leaves of *Trigonella foenum-graecum* by high-performance liquid chromatography-diode array detection, Anal Lett 44, 1463-1472, 2011.
23. Kim DO, Jeong SW, Lee CY, Antioxidant capacity of phenolic phytochemicals from various cultivars of plums, Food Chem 81, 321-326, 2003.
24. Subhasree B, Baskar R, Keerthana RL, Susan RL, Rajasekaran P, Evaluation of antioxidant potential in selected green leafy vegetables, Food Chem 115, 1213-1220, 2009.
25. Yen GC, Duht PDJ, Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species, Agr Food Chem 42, 629-632, 1994.
26. Başgel S, Erdemoğlu SB, Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey, Sci Total Environ 359, 82-89, 2006.
27. Gupta KK, Bhattacharjee S, Kar S, Chakrabarty S, Thakur P, Bhattacharyya G, Srivastava SC, Mineral compositions of eight common spices, Commun Soil Sci Plan 34, 681-693, 2003.
28. Kan Y, Kan A, Ceyhan T, Sayar E, Kartal, M, Altun L, Aslan S, Cevheroğlu Ş, Atomic

- absorption spectrometric analysis of *Trigonella foenum-graecum* L. seeds cultivated in Turkey, Turk J Pharm Sci 2 (3), 187-191,2005.
29. Yadav SK, Sehgal S, Effect of domestic processing on total and extractable calcium and zinc content of Bathua (*Chenopodium album*) and Fenugreek (*Trigonella foenum-graecum*) leaves, Plant Food Hum Nutr 53, 255-263,1999.
 30. Gupta K, Barat GK, Wagle DS, Chawla HKL, Nutrient contents and antinutritional factors in conventional and non-conventional leafy vegetables, Food Chem 31, 105-116, 1989.
 31. Naidu MM, Shyamala BN, Naik JP, Sulochanamma G, Srinivas P, Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds, Food Sci Technol 44, 451-456, 2011.
 32. Gupta S, Prakash J, Studies on Indian green leafy vegetables for their antioxidant activity, Plant Foods Hum Nutr 64, 39-45, 2009.
 33. Aqil F, Ahmad I, Mehmood Z, Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants, Turk J Biol 30, 177-183, 2006.

Received: 19.09.2013
Accepted: 06.03.2014

