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Composition and content of total phenolics and flavonoids, and antioxidant activity of *Trigonella isthmocarpa*

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ABSTRACT

The aqueous methanolic extracts obtained from aerial parts and seeds of *T. isthmocarpa* Boiss. & Bal. from Turkey were analyzed by reversed-phase HPLC for their phenolic acid and flavonoid composition. Total phenolic content was determined using the Folin-Ciocalteu assay, while total flavonoid content was measured spectrometrically with $AlCl_3$ assay. Primary antioxidant activity in terms of free radical scavenging activities of both extracts was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The highest total flavonoid and total phenolic content was determined in the extract of aerial parts (830.8 mg/100 g and 877.9 mg gallic acid equivalent/100 g, respectively). The extracts of aerial parts and seeds had the high antioxidant activity (87.2 % and 78.2%, respectively). Gallic acid, caffeic acid, luteolin, apigenin and kaempferol were detected as phenolic compounds in both extracts by HPLC methods. In addition, an isoflavone, formononetin was determined in the extract from aerial parts of the plant. The present results suggest that *T. isthmocarpa* have shown potential a source of natural antioxidants.

1. Introduction

Trigonella L. belongs to the family Fabaceae that including about 135 species in the world. Most of the species are distributed in the dry regions around the East Mediterranean, West Asia, South Europe, North and South Africa [1-3]. *Trigonella foenum-graecum* L. commonly called fenugreek is the most widely used species in *Trigonella* genus. It is cultivated as a spice in Mediterranean countries, the Middle East, Russia, the Balkans, West Asia and China [1]. The seeds have been traditionally used to treat disorders such as diabetes, high cholesterol, wounds, inflammation and gastrointestinal ailments [4-6]. The leaves are useful for swelling, burns and baldness [7,8]. Many of the claimed folk medicinal uses of this plant have been scientifically tested and it was reported that fenugreek have antidiabetic, antioxidant, anti-inflammatory, antipyretic, immunomodulatory, anticancer, gastroprotective and chemopreventive effects [5-8]. The main components of the plant are fibers, polyphenolic compounds such as flavonoids, polysaccharides, saponins, fixed and volatile oils, alkaloids, minerals, protein and amino acids [7]. *T. foenum-graecum*is extensively studied but there is little information for other species of the genus in the literature [9,10].

The genus *Trigonella* comprises about 50 taxa in Turkey. These taxa divided 13 sections and 8 groups [11-13]. *Trigonella isthmocarpa* Boiss. & Bal. is an endemic species, which grows wild in Central Anatolia and this species has not been the subject of any study. The aim of the present study was to determine phenolic compounds of using HPLC-DAD system, total phenol, total flavonoid content and free radical scavenging activity of *T. isthmocarpa*.

2. Experimental

2.1. Plant material

T. isthmocarpa Boiss. & Bal. was collected from Central Anatolia (B5 Nigde) in June2011. A voucher specimen was deposited in the Herbarium of the Faculty of Science, Mustafa Kemal University (A. İlçim 1371 MKUH) and identified by Dr. Ahmet İlçim.

2.2. Reagents and chemicals

Methanol, acetic acid, hydrochloric acid, acetonitrile, trifloroacetic acid (TFA), Folin-Ciocalteu's phenol reagent were obtained from Merck (Germany). Caffeic acid, apigenin and formononetin were purchased from Fluka (Italy). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), gallic acid, luteolin and kaempferol were obtained from Sigma Chemicals (USA). The solvents were analytical grade. Distilled and deionized water (ddH_2O) was used for the preparation of all the samples and solutions. Freshly prepared solutions were used for each experiment.

2.3. Instrumentation

The HPLC analyses were performed by an Agilent 1200 HPLC system equipped with an auto-sampler, quaternary pump, DAD-UV detector scanning from 200 to 400 nm, vacuum degasser, a column oven and a data system Agilent Chem. Station. Total flavonoid and total phenol contents were analyzed by Specord 210 plus (Analytic Jena).

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2.4. Extraction procedure

Aerial parts and seeds (3 g) were powdered mechanically and dispersed with 80% aqueous methanol sonicated for 60 min. at 30 °C. The supernatants were filtered through a Whatman Grade 1 filter paper. The volume of the extracts was adjusted to 50 mL by adding the appropriate volume of 80% aqueous methanol. For the acid hydroliysis, 40 mL of the same aqueous methanol extracts and 10 mL of HCl (37%) were heated at 90 °C for two hours. The mixtures were left to cool at room temperature. The volume of the mixtures were adjusted to 50 mL by adding the appropriate volume of a methanol:water mixture (80:20, *v*:*v*). The extracts were stored at 4 °C for the analysis [14].

2.5. Determination of total phenolic contents

The concentration of total phenolics in extracts was determined using Folin-Ciocalteu procedure as described by Kim *et al*. (2003) [15]. To 1 mL of appropriately diluted extracts or standard solutions of gallic acid (25, 50, 100, 150, 200, 250, 300, 400, 450 and 500 mg/L) was added to a 25 mL volumetric flask containing 9 mL of ddH_2O. A reagent blank using ddH_2O was prepared. One mililiter 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 with mixing. The solution was then immediately diluted to volume (25 mL) with ddH_2O and mixed thoroughly. After incubation for 90 min at 23 °C, the absorbance versus prepared blank was read at 750 nm. The total phenols were determined as gallic acid equivalents (mg/L), and the values are presented as means of triplicate analyses.

2.6. Determination of total flavonoid contents

The amount of total flavonoids in the extracts was measured as the method described by Kim *et al*. (2003) [15] with some modification based on that of the method described by Subhasree *et al*. (2009) [16]. Using spectrophotometrical technique, total flavonoid contents in the extracts were determined by $AlCl_3$ methods. 0.5 mL of the each extract and standard solutions of rutin (25, 50, 100, 150, 200 and 250 mg/L) were added to a 10 mL volumetric flask containing 3 mL ddH_2O. At zero time, 0.3 mL 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 mL 10% $AlCl_3$ was added. At 6 min, 2 mL 1 M NaOH was added to the mixture. Immediately the volume of the mixtures was adjusted to 10 mL by adding the appropriate volume of a ddH_2O. Absorbance of the mixture, pink in colour, was determined at 510 nm versus prepared methanol blank. All determinations were performed in triplicate. Rutin was used as a reference standard and total flavonoids of the extracts were expressed on a dry weight basis as mg/100 g rutin equivalents

2.7. HPLC-DAD analyses

The aqueous methanol extracts were analyzed by reversed-phase HPLC for their flavonoid and phenolic acid contents. A 250 mm x 4.6 mm, 5 µm Hypersil ODS column (Thermo Electron Corporation-Agilent, USA) was used for separation of flavonoids and the mobile phase consisted of water:acetic acid (100:1, *v*:*v*)(A) and methanol (B) [17]. The gradient condition was as follows: 0 min: 40% B; 20 min: 90% B; 30 min: 40% B. All wavelengths were selected as the detection wavelength. The column temperature was set at 20 °C. The flow rate was 0.6 mL/min and the loading volume was 20 µL. A 150 mm x 4.0 mm, 3 µm Inertsil ODS column (Hichrom, UK) was used for separation of phenolic acids. The mobile phase consisted of 40mM H_3PO_4 buffer, pH = 3.0 (adjusted with 1 M KOH), and acetonitrile (32:68, *v*:*v*) and the flow rate was 0.5 mL/min [18].The compounds were identified by comparison with reference flavonoids and phenolic acids with their retention times. Quantification of flavonoids was obtained from peak area using ChemStation 8.02 software and calibration curves of reference compounds.

2.8. DPPH radical-scavenging activity

Radical scavenging activity of the extracts was determined as the method described by Yen and Duht (1994) [19]. The extracts were tested for their possible *in vitro* antioxidant activities by qualitative 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. 0.1 mL of the each sample and standard solutions of butylated hydroxyanisole (BHA) (25, 50, 100, 150 and 200 mg/L) were added 2.9 mL methanolic solution of DPPH. The mixtures were shaken vigorously and incubated in the dark for 45 min at room temperature. The decrease in absorbance of samples was measured at 517 nm with a spectrophotometer. 80% Methanol was substituted for samples. All the analysis were carried out in triplicate. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the equation 1.

% Free radical scavenging activity: [(Control absorbance-Sample absorbance)/ (Control absorbance)] x100 (1)

3. Results and discussion

The content of the total phenolic compound of the extracts from *T. isthmocarpa* determined using Folin-Ciocalteu method expressed as gallic acid equivalents is shown in Figure 1. The extract obtained from aerial parts of the plant exibited higher phenol content of 877.9 mg/100 g gallic acid equivalent (GAE). Aluminium chloride colorimetric assay was used to determine total flavonoid content of the extracts. The greatest total flavonoid content was revealed in the aerial parts of the plant (830.8 mg rutin equivalents/100).

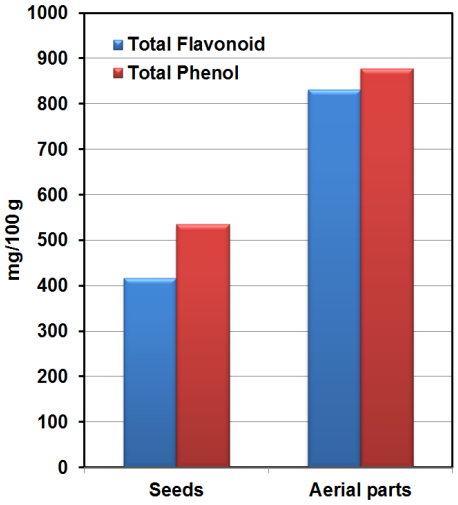

Figure 1. Total phenolic and flavonoid content in *T. isthmocarpa* seeds and aerial parts.

The qualitative and quantitative determination of the phenolic acids and flavonoids of the extracts HPLC-DAD method was used. Gallic acid and caffeic acid were identified as phenolic acids in both hydrolised extracts. Luteolin, apigenin and kaempferol was determined as flavonoids in seed and aerial parts of the extracts (Table 1). The extract of aerial parts of *T. isthmocarpa* contains formononetin besides these flavonoids. Formononetin was not determined quantitatively, since peaks were very small and amounts were below the limit of quantification.

Table 1. Phenolic acid and flavonoid content (%) of the seeds and aerial parts of *T.isthmocarpa.*

Compound	Seeds (mg/g)	Aerial parts (mg/g)
Gallic acid	0.66	0.83
Caffeic acid	0.16	0.02
Luteolin	0.16	0.50
Apigenin	0.01	0.02
Kaempferol	+	0.05
Formononetin	-	+

+ = Not detected quantitatively.
- = Not detected.

The DPPH (1,1-Diphenyl-2-picrylhydrazyl) method was used to evaluate the antioxidant activity of the extracts. The DPPH radical has been widely used to test the free radical scavenging ability of different plants [20]. The DPPH scavenging activities of the extracts are shown in Table 2. The extracts had appreciable free radical scavenging activity. The extract of aerial parts of *T. isthmocarpa* showed the high antioxidant activity (87.2% inhibition) when compared with BHA (Butylated hydroxyanisole) which showed 73.1% inhibition.

Table 2. DPPH. radical scavenging activity (% inhibition) of extracts obtained from *T. isthmocarpa.*

Extract	Inhibition (%)
Seed	78.2
Aerial part	87.2
Butylated hydroxyanisole (BHA)	73.1

This investigation indicated the presence of compounds possessing antioxidant activity in the extracts of *T. isthmocarpa*. The extract of aerial parts of the plant has a higher phenolic and flavonoid content 877.9 and 830.8 mg/100 g. The extract was more effective than BHA. Luteolin, apigenin, kaempferol, gallic acid and caffeic acid are the phenolics detected in the extract. It has been reported that these phenolics have antioxidant properties. Various phenolic compounds respond differently in DPPH assay, depending on the number of phenolic groups they have [21]. In the present study, a correlation between radical scavenging activity and amounts of phenolic compounds of the extracts was observed. The plant has been found to be as potent antioxidant activity. Furthermore studies in isolation and quantification of individual phenolic compound to elucidate their antioxidant mechanisms and the existence of possible synergism.

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References

[1]. Bown, D. Encyclopedia of Herbs & Their Uses, 1st edition, Darling Kindersley Limited, 2002.
[2]. Evans, W. C. Trease and Evans Pharmacognosy, 15th edition, University of Nottingham, 2002.
[3]. Townsend, C. C. Leguminosae. In Flora of Iraq, The University Press, 1974.
[4]. Basch, E.; Ulbricht, C.; Kuo, G.; Szapary, P.; Smith, M. *Altern. Med. Rev.* **2003**, *8*, 20-26.
[5]. Srinivasan, K. *Food Rev. Int.* **2006**, *22*, 203-224.
[6]. Pandian, R. S.; Anuradha, C. V.; Viswanathan, P. *J. Ethnopharmacol*. **2002**, *81*, 393-397.
[7]. Toppo, F. A.; Akhand, R.; Pathak A. K. *Asian J. Pharm. Clin. Res.* **2009**, *2(4)*, 28-38.
[8]. Yaday, R.; Kaushik, R.; Gupta, D. *Inter. J. Eng. Res. Appl.* **2011**, *1*, 32-35.
[9]. Bown Yuldashev, M. P. *Chem. Nat. Compd*. **2002**, *38*, 291-292.
[10]. Kawashyt, S. A.; Abdalla, M. F.; El Din, E. M. G.; Saleh, N. A. M. *Biochem. Syst. Ecol.* 1**998**, *26*, 851-856.
[11]. Huber-Morath, A. *Trigonella* L. in: Davis P. H. (ed.), Flora of Turkey and The East Aegean Island, Edinburgh University Press, 1969.
[12]. Davis, P. H.; Mill, R. R.; Tan, K. Flora of Turkey and The East Aegean Island, Edinburgh University Press, 1988.
[13]. Guner, A.; Ozhatay, N.; Ekim, T.; Baser, K. H. C. Leguminosae. Flora of Turkey and The East Aegean Islands (Supplement 2), Edinburgh University Press, 2000.
[14]. Gikas, E.; Bazoti, F. N.; Papadopoulos, N.; Alesta, A.; Economou, G.; Tsarbopoulos, A. *Anal. Lett.* **2011**, *44*, 1463-1472.
[15]. Kim, D. O.; Jeong, S. W.; Lee, C. Y. *Food Chem.* **2003**, *81*, 321-326.
[16]. Sunhasree, B.; Baskar, R.; Keerthana, R. L.; Susan, R. L.; Rajasekaran, P. *Food Chem.* **2009**, *115*, 1213-1220.
[17]. Wang, S.; Di, D.; Liu, X.; Jiang, S. *J. Liq. Chromatogr. Rel. Technol.* **2007**, *30*, 1991-1999.
[18]. Khoschsorur, G. A.; Erwa, W. *J. Chromatogr. B* **2004**, *799*, 355-360.
[19]. Yen, G. C.; Duht, P. D. *J. Agr. Food Chem.* **1994**, *42*, 629-632.
[20]. Mariod, A. A.; Matthaus, B.; Hussein I. H. *Int. J. Food Sci. Tech.* **2008**, *43*, 921-926.
[21]. Zheng, C. D.; Li, G.; Li, H. Q.; Xu, X. J.; Gao, J. M.; Zhang, A. L. *Nat. Prod. Commun.* **2010**, *5*, 1759-1765.