ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 11:1 (2017) 17-30

records of natural products

A Comparison of Diosgenin, Phenolics, Fatty Acid Profiles and Mineral Contents with Free Radical Scavenging Activity of *Trigonella* L. Species from Section *Cylindricae*

Selma Ş. Uras Güngör[1], Ahmet İlçim[2] and Gamze Kökdil[1*]

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

(Received May 06, 2016; Revised August 10, 2016; Accepted August 11, 2016)

Abstract: In this study, the fatty acids composition, the total flavonoids, phenolics, and mineral contents of *Trigonella* species belonging to Cylindricae Section (*T. spruneriana*, *T. sibthorpii*, *T. kotschyi*, *T. mesopotamica*, *T. cylindricae*, *T. cilicica*, *T. filipes*, *T. velutina*, *T. strangulata* and *T. smyrnea*) were determined. Diosgenin content in seeds and aerial parts of these species was investigated by RP-HPLC. Furthermore, the phytochemical profiles and radical scavenging activities of the plants were evaluated. The oils contained high proportions of α-linolenic (29.7-42.1 %) and linoleic acids (26.6-40.3 %). All the seed extracts had higher concentrations of total flavonoids and phenolics than those of aerial parts. *T. strangulata* and *T. spruneriana* seeds showed the highest total phenolics (201.35±0.51 and 191.90±0.86 mg GAE/g, respectively) and flavonoids contents (121.84±0.05 and 128.04±0.09 mg RE/g, respectively). For the DPPH radical scavenging assay, the extracts of four species of *Trigonella* showed moderate radical scavenging activity. The seeds contained high concentrations of potassium, calcium and phosphorus while the aerial parts were rich in calcium and potassium. Phytochemical screening tests showed that saponins, flavonoids, coumarins and fixed oils were main secondary metabolites. The highest diosgenin level was detected in *T. cilicica* seed and aerial part extracts (0.52±0.013 and 0.16±0.005 mg/g, respectively).

Keywords: Fabaceae; *Trigonella*; fatty acid; diosgenin; antioxidant activity; chemotaxonomy.

1. Introduction

The genus *Trigonella* L. (Fabaceae) comprises about 135 species in the world. These species are mainly distributed throughout the Mediterranean region, Europe, South and North Africa, South Australia, West Asia and North America [1, 2]. Several species of the genus (*T. foenum-graecum*, *T. corniculata*, *T. occulta* and *T. incise*) are known as a spice or vegetable, and used in traditional systems of medicine [3-5]. *T. foenum-graecum* (fenugreek) are well-known cultivated species as a crop in the Mediterranean region, Indian, North Africa and China [6-10] because of its using as food and natural remedies since antiquity in Greece and Egypt. While the plant was used to increase lactation and to treat painful menstruation in ancient Egypt [1, 5, 6], the seeds have been used for the treatment of fever, epilepsy, gout, diabetes, chronic cough, dropsy, abscess, leprosy, piles, vomiting

* Corresponding author: E-Mail: gkokdil@gmail.com; Phone:+90-324-3412815/2629 *Fax:+90*-324-3413022.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 09/21/2016 EISSN:1307-6167

and as tonic, aphrodisiac and gastric stimulan in Ayurvedic, Chinese and Unani systems of medicine [1, 5, 6, 10-12]. The leaves was used to prevent hair loss, and treatment of burns and swelling in Unani system of medicine [5].

The crushed powder of fenugreek seeds, various extracts of seeds/leaves and its active constituents have been studied for their pharmacological effects and reported to possess hypocholestrolaemic, antidiabetic, anti-inflammatory, antiulcer, analgesic, antipyretic, CNS-stimulant, antioxidant, wound healing and immunomodulatory activity as well as gastroprotective and chemopreventive properties [4-8, 10, 12-15].

T. foenum-graecum seeds contain fixed oil consisting mainly of polyunsaturated fatty acids, alkaloids, coumarins, triterpenic and steroidal types of saponins such as diosgenin, gitogenin, and yamogenin, protein, aminoacids, mucilage, flavonoids, fibers and minerals [3, 6, 8, 14-17]. Additionally the leaves of fenugreek are potential source of minerals, vitamins and flavonoids [6-8, 18]. In the literature, many phytochemical studies on fenugreek have been reported, but there are little information for other species of *Trigonella* [3, 18-20].

Trigonella genus represented by 54 taxa which are divided eight groups and thirteen sections in Turkey and have not been detaily studied [2, 21-24]. The main objectives of the present study were to investigate phytochemical constituents, fatty acid compositions, total phenol and flavonoid contents as well as radical scavenging activity of all species of Section Cylindricae of *Trigonella* genus (*T. spruneriana* Boiss., *T. sibthorpii* Boiss., *T. kotschyi* Fenzl, *T. mesopotamica* Hub.-Mor., *T. cylindracea* Desv., *T. cilicica* Hub.-Mor., *T. filipes* Boiss., *T. velutina* Boiss., *T. strangulata* Boiss., *T. smyrnea* Boiss.) from Turkey, for the first time. The macronutrient and trace elements of all plant parts were also determined by ICP-MS. Furthermore, qualitative and quantitative characterization of diosgenin has been investigated by RP-HPLC method in these species for possible source of diosgenin.

2. Materials and Methods

2.1. Plant material

The studied *Trigonella* species were obtained from different regions of Turkey (Table 1). The voucher specimens were kept in the Herbarium of Mustafa Kemal University, Faculty of Arts and Sciences (MKU) and identified by Prof. Dr. Ahmet İLÇİM.

2.2. Phytochemical screening

Qualitative phytochemical analysis were carried out using classical methods as described in the literature [25-31]. Flavonoids, tannins, alkaloids, coumarins, cardiac glycosides, cyanogenetic glycosides, anthraquinones, anthocyanins, sugars and starches were detected by precipitation and/or color reactions. Saponins were searched by foam value. Briefly, flavonoids were detected using the cyanidin test and dilute NH_3, $Pb(AC)_2$ and $FeCl_3$ solutions. Tannins were searched using gelatin and $FeCl_3$ solutions, bromine water and Stiasny reagent. Alkaloids were screened by Mayer and Dragendorff reagents. Coumarins were searched with ammoniacal solution in ultraviolet light. Cardiac glycosides and steroids were detected using Keller-Kiliani, Baljet and Liebermann-Burchard tests. Cyanogenetic glycosides were searched using picric acid/sodium carbonate. Anthraquinones were detected with Borntrager's tests. Anthocyanins were searched using dilute H_2SO_4, $Pb(AC)_2$, amyl alcohol and NaOH with HCl. The screening for sugars were carried out by Fehling's solution, Molisch's and Seliwanoff reagents. Starches were detected with iodine solutions.

2.3. Extraction of the seed oils and determination of the fatty acids

The seeds were powdered and extracted with *n*-hexane in a Soxhlet apparatus for 6 h. Evoporation of the solvent was performed under reduced pressure by rotary evaporator. The seed oil compositions were determined using fatty acid methyl esters according to the method of Houghton *et*

al. (1995) [32]. Gas chromatographic separation (GC, Agilent Technologies 7890A) was performed on a DB-Wax capillary column (60 m x 0.25 mm x 0.25 μm), and following oven temperature program:140^{o}C for 5 min, increased at 5^{o}C/min to 165^{o}C, held for 10 min, increased at 5^{o}C/min to 190^{o}C, held for 20 min, increased at 5^{o}C/min to 240^{o}C, held for 20 min. Detector: FID. Injector and detector temperature were 250^{o}C and 260^{o}C, respectively, carrier gas: helium, flow rate 1.5 mL/min, injection volume 20 μL, split ratio 50:1.

GC-MS analyses were carried out on an Agilent Technologies 6890 N Network GC and an Agilent 5973 Network Mass Selective Detector. DB-23 capillary column (60 m x 0.25 mm x 0.25 μm) was used. Oven temperature was kept at 130^{o}C for 1 min, programmed to 170^{o}C at a rate of 6.5^{o}C/min then programmed to 215^{o}C at a rate of 2.75^{o}C/min, kept at 215^{o}C for 12 min, then programmed to 230^{o}C at a rate of 40^{o}C/min and kept at 230^{o}C for 3 min.

The constituents of the oils were identified by comparison of their GC retention times with those of reference methyl esters of the fatty acids and also by comparison of their mass spectra with published spectra (Famedb23.L, NIST02.L).

Table 1. Localities, vouchers numbers and the oil yields of the studied *Trigonella* species.

Species/ Voucher number	Localities	Oil Yield (% dry weight)
T. spruneriana Boiss. MKU1751	C4 Içel:Gülnar, 600-700 m, *Ş.S.Uras Güngör*	2.3
T. sibthorpii Boiss. MKU1752	C5 Içel:Mersin, 0-20 m, *Ş.S.Uras Güngör*	4.1
T. kotschyi Fenzl* MKU1753	C6 Maraş:Kozludere, 1300-1500 m, *A. İlçim*	6.1
T. mesopotamica Hub.-Mor. MKU1754	C6 Maraş:Çağlayancerit, 1300-1500 m, *Ş.S.Uras Güngör, A. İlçim*	3.8
T. cylindracea Desv. MKU1755	C4 Içel:Tömük, 0-20 m, *Ş.S.Uras Güngör*	1.7
T. cilicica Hub.-Mor.* MKU1756	C5 Içel:Tarsus, 800-900 m, *Ş.S.Uras Güngör*	4.9
T. filipes Boiss. MKU1757	C6 Maraş:Büyüknacar, 1300 m, *Ş.S.Uras Güngör, A. İlçim*	6.7
T. velutina Boiss. MKU1758	C4 Içel:Gülnar, 900-1000 m, *Ş.S.Uras Güngör*	2.2
T. strangulata Boiss. MKU1766	C4 Içel:Silifke, 900 m, *Ş.S.Uras Güngör*	5.2
T. smyrnea Boiss.* MKU1782	C2 Antalya:Gömbe, 1150 m, *Ş.S.Uras Güngör*	5.0

2.4. Mineral analysis

Each of dried samples of seeds, stems and leaves of the plants was prepared for digestion according to Başgel and Erdemoğlu methods [33]. An oven CEM MARS 240/50 model with a timer and variable temperature settings were used for microwave-assisted digestion of materials. Mineral analysis were done using an Agilent ICP-MS 7500ce (Tokyo, Japan) equipped with Octopole Reaction System. Experimental conditions are 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^{o}C; Torch: Quartz; Auto sampler: CETAC ASX-520; Read time (s): 30; Delay time (s): 60; Wash time (s): 20.

2.5. Extraction and determination of diosgenin

The plant materials were defatted in a Soxhlet apparatus for 6 h with petroleum ether. Defatted plant materials were extracted with 80 % ethanol at 80^{o}C for 3 h., filtrates were evaporated to dryness. These extracts were hydrolyzed in isopropanol (70 %) solution containing 1 M sulfuric acid at 100^{o}C for 2 h. The isopropanol was evaporated using rotary evaporator. Extracts were dissolved in *n*-hexane

and washed three times with 4 N NaOH solution and distilled water, respectively. *n*-hexane was evaporated and the residue was dissolved in methanol [17, 34].

These extracts were analyzed for their diosgenin content by HPLC. A μBondapak C18 column (250 mm x 4.6 mm, 10 μm) was used. The mobile phase was a mixture of methanol:water (85:15, v/v), isocratic, with a flow rate of 1 mL/min. Diosgenin was detected on a DAD detector at 200 nm [35].

2.6. Determination of total phenolics and flavonoids contents and DPPH radical scavenging activity

Powdered aerial part and seed of each species were extracted with aqueous methanol (80 %) at 30^{o}C for 60 min. The extracts were filtered and the volumes were adjusted to 100 mL with aqueous methanol (80 %). These extracts were used to determine total phenolic and flavonoid contents of the studied *Trigonella* species [36].

Total phenolic contents of the extracts were carried out by Folin-Ciocalteu method [37, 38]. The results were expressed as mg of gallic acid equivalents (GAE)/g for total phenolics. Total flavonoids content in the extracts of studied *Trigonella* species was measured as previously reported method by Kim et al. 2003 [37] and Subhasree et al. 2009 [38]. The total flavonoids content was expressed as mg of rutin equivalents (RE)/g.

DPPH scavenging activity of the extracts was carried out by the method of Kroyer 2004 and Koleva et al. 2002 [39, 40]. The absorbance of the mixtures solutions was measured at 517 nm. BHA (25-200 mg/L) was used as standard for comparison. All the analysis were carried out in triplicate.

3. Results and Discussion

3.1. Phytochemical screening

Saponins, flavonoids and coumarins and sugars were detected in the seeds and aerial parts of all the studied plants. Four species (*T. filipes*, *T. velutina*, *T. strangulata* and *T. smyrnea*) contained alkaloid. The extracts of *T. cilicica*, *T. velutina*, *T. strangulata* and *T. smyrnea* seeds gave positive result for anthocyanosid tests. Tannins were present in the seeds of *T. spruneriana*, *T. sibthorpii*, *T. kotschyi*, *T. mesopotamica*, *T. cilicica* and *T. filipes*. Cardiac glycosides, cyanogenetic glycosides, anthracenosids and starch were not detected in the seeds and aerial parts of all the investigated plants.

The phytochemical screening, total phenolics, flavonoids and DPPH free radical scavenging activity of ten *Trigonella* species reported for the first time in this study. Studies cited in the literature are usually focused on *T. foenum-graecum* because of its use as a spice, nutritional value for human, and its important pharmacological activity. Chemical constituents of fenugreek are well-known but there are few studies can be found about phytochemistry of the other species [3, 18, 19, 41]. The main chemical constituents of studied *Trigonella* species identified by phytochemical screening tests. The results showed that these species contained similar chemical constituents with *T. foenum-graecum*.

3.2. Oil content and Fatty acid composition

The oil yields of the studied *Trigonella* seeds were given in Table 1. The yields ranged from 1.7 to 6.7 %. The highest yields were obtained from *T. filipes* and *T. kotschyi* (6.7 % and 6.1 %, respectively). Fatty acid composition of the oils was presented in Table 2. Thirteen fatty acids were detected in the oils. All samples were characterized by high amounts of unsaturated fatty acids (75.9-80.9 %). Linoleic and α-linolenic acids were the main polyunsaturated fatty acids. The level of these fatty acids ranged from 26.6 to 42.1 %. The seed oils of *T. filipes*, *T. cilicica*, *T. mesopotamica* and *T. velutina* showed high concentrations of α-linolenic acid (40.5 - 42.1 %) that are greater than other species (29.7-35.5 %). The oils also contain oleic acid but in low amounts. Palmitic acid (11.4-14.1 %) was the main saturated fatty acid in the oils.

The fatty acid composition of the oil of *T. foenum-graecum* cultivated in different regions of the world has been extensively reported. According to the literature, its yield of fixed oil ranged from 5.8 to 15.2 % [6, 9, 42, 43]. In general, the fatty acid composition of this species has been reported to be rich in linoleic (21.5-69.5 %), linolenic (3.5-23.2 %), oleic (9-35 %) and palmitic (8.6-17.5 %) acids [6, 9, 13, 42-46]. On the other hand *T. foenum-graecum* fixed oil is rich in unsaturated fatty acids (67.1-84.5 % TUFA) [13, 42, 43, 46]. Furthermore, the ratio of n-6 to n-3 fatty acids in fenugreek seed lipids was reported as 2.1-2.7 by Ciftci et al. (2011) [42]. Bagcı et al. (2004) reported that oleic (46.9 %) and linoleic acid (24.2 %) were the major fatty acids in *T. cretica* seed oil [47]. The fixed oils from all studied *Trigonella* species in this study are rich in polyunsaturated fatty acids which play an important role in human health. The results are in agreement with the reported literature values for *T. foenum-graecum* fixed oil. The composition of fatty acids of studied species is similar to each other but there are some quantitative differences. The oils of *T. filipes*, *T. cilicica*, *T. mesopotamica* and *T. velutina* showed high concentrations of α-linolenic acid (40.5 - 42.1 %). The ratios of n-6 to n-3 fatty acids of the oils from seven *Trigonella* species (*T. spruneriana*, *T. mesopotamica*, *T. cilicica*, *T. filipes*, *T. velutina*, *T. strangulata* and *T. smyrnea*) were between 0.66 and 1.00 (Table 2). These oils with a lower ratio of omega-6/omega-3 fatty acids are a good source of essential fatty acids. The optimal ratio of n-6 to n-3 is presumed to be between 1 and 5. Increased levels of omega-3 PUFA play a role in reducing the risk of many of the chronic disease [48]. This study showed that seven species mentioned above were valuable source of essential fatty acids.

3.3. Mineral analysis

The results for element compositions of examined seeds are presented in Table 3. Four macro minerals, namely potassium (14727.67-11041.67 µg/g), calcium, phosphorus and magnesium were relatively high in all seeds. Of the other elements determined in the present study, Cr, Mn, Zn, Al, Ni, Co, Mo, Se, Cd and Pb were in low amount in the seeds, but Na and Fe were present in moderate quantity. Table 4 shows the concentrations of the elements in aerial parts of examined *Trigonella* species. Calcium is the most abundant element in the samples, followed by potassium, phosphorus and magnesium. The other elements, in descending order by quantity, were Na, Cr, Fe, Zn, Mn, Se, Pb, Cu, Ni, Cd and Co.

In the literature, there is no information about mineral content of *Trigonella* species except fenugreek. Gupta et al. (2003), 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 [49]. Kan et al. (2005) 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 [50]. Srinivasan (2006) reported that *T. foenum-graecum* seeds were rich in K (530 mg/100 g), P (370 mg/100 g), Ca and Mg (160 mg/100 g). Cu, Na and Fe were determined in moderate concentrations in that study [6]. Shakuntala et al. (2011) 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 [43]. It was reported by Naidu et al. (2012) that low humidity air dried fenugreek greens had maximum content of calcium (56.3 mg/100 g), but K and Fe were present amounts of 4.7 and 3.5 mg/100 g, respectively. They were found that other minerals like Zn, Mn and Cu were present in trace amounts [51]. Data of mineral analysis in this study revealed that the seeds contain highest amount of potassium than fenugreek seeds. *T. kotschyi*, *T. cilicica*, *T. velutina*, *T. strangulata* and *T. symrnea* seeds also contain calcium as well as fenugreek seeds.

We also studied the mineral content of the aerial parts of ten *Trigonella* species. Ca and Zn concentrations in the leaves of fenugreek were reported by Yadav and Sehgal (1999) [52]. These authors indicated that the Ca content ranged from 940-970 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. (1989) [53]. Srinivasan (2006) reported 395 mg Ca, 76 mg Na and 67 mg Mg per 100g in fresh fenugreek leaves [6]. Marzougui et al. (2007) measured six mineral elements in 38 Tunisian cultivars of *T. foenum-graecum*. They reported that K content was less than 1 % of the leaves weight

in 65.8 % of the studied cultivars and Na content higher than 0.2 % and Mg content higher than 0.05 % in 34.2 of cultivars. It was also reported that P content was higher than 0.3 % in 23.7 % of cultivars, Ca content over 0.3 % in 39.5 % of cultivars [54]. Present results showed that the leaves content of calcium was found to be in agreement with these reported by Gupta et al. (1989) [53] and Yadav and Sehgal (1999) [52] for fenugreek leaves. Additionally, aerial parts of ten *Trigonella* species have high content of selenium (41.72±0.32-37.73±0.15 µg/g) as compared to seeds of the species. Selenium is a nutritionally essential element to the life of humans and animals [55]. To our knowledge, we report selenium content as well as other minerals for the first time from the studied *Trigonella* species.

3.4. Diosgenin analysis

Table 5 indicates the content of diosgenin in the studied *Trigonella* species. The amounts of diosgenin in seed extracts were ranged from 0.06 to 0.52 mg/g while the amounts were ranged from 0.03 to 0.16 mg/g in aerial parts extracts. The highest diosgenin content were determined in seeds of *T. cilicica*. Among the ten *Trigonella* species, diosgenin content of *T. velutina*, *T. mesopotamica* and *T. spruneriana* seeds ranging between 0.15-0.22 mg/g. Only one species *T. strangulata* did not contain diosgenin in any parts. Additionally *T. kotschyi*, *T. mesopotamica*, *T. filipes* and *T. strangulata* did not contain diosgenin in their aerial parts.

Table 5. Diosgenin content in the studied *Trigonella* species (mg/g)*.

Species	Diosgenin content	
	Seeds	**Aerial Parts**
T. spruneriana	0.18±0.005	0.03±0.005
T. sibthorpii	0.06±0.005	0.10±0.005
T. kotschyi	0.07±0.005	-
T. mesopotamica	0.18±0.008	-
T. cylindracea	-	0.10±0.005
T. cilicica	0.52±0.013	0.16±0.005
T. filipes	0.07±0.005	-
T. velutina	0.22±0.005	0.07±0.005
T. strangulata	-	-
T. smyrnea	0.15±0.005	0.06±0.000

*Values are in mean ± standart deviation (n=3)

Some steroidal sapogenins such as diosgenin and hecogenin, and steroidal alkaloids were used to manufacture of steroidal drugs (glucocorticoids) and hormones (testosterone, progesterone etc.). Plant materials containing these steroidal compounds were main source as starting materials for the production of synthetic steroids in the pharmaceutical industry [29, 30]. *Dioscorea* species are the main raw materials with higher level of diosgenin (1-2 %) [30]. Another important source is fenugreek seeds whose content of diosgenin ranging from 0.8 to 2.2 % as well as *Costus speciosus* and *Balanites aegyptica* in different geographical regions [30]. Ortuno et al. (1998) reported the diosgenin content of fenugreek seed, leaves and stems to be 5.65, 9.56 and 6.55 mg/g, respectively [56]. Oncina et al. (2000) studied cell and callus cultures of fenugreek and reported the diosgenin content of leaves and stems were 2.2 and 0.74 mg/g, respectively [57]. Taylor et al. studied diosgenin content in fenugreek seed by GC and the content ranged from 0.28 to 0.92 % [58]. Arivalagan et al. (2013) investigated steroidal saponin content in 46 fenugreek genotypes whose steroidal saponin content ranging from 1.34 to 5.19 g/100g [59]. Abdelkarim et al., reported that fenugreek seeds from Sudan and Indian had 0.12 and 0.069 (% w/w) diosgenin content [60]. Although *T. foenum-graecum* were investigated for diosgenin content in seeds, leaves and stems with above mentioned studies, only one study had been

Table 2. Fatty acid composition of the studied *Trigonella* seed oils.

Fatty acid	*T. spruneriana*	*T. sibthorpii*	*T. kotschyi*	*T. mesopotamica*	*T. cylindracea*	*T. cilicica*	*T. filipes*	*T. velutina*	*T. strangulata*	*T. smyrnea*
Myristic acid ($C_{14:0}$)	0.2±0.0	0.2±0.0	0.2±0.0	0.1±0.0	0.2±0.0	0.1±0.0	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0
Palmitic acid ($C_{16:0}$)	12.9±0.7	12.5±0.8	13.5±0.7	12.1±0.7	12.5±0.3	12.1±0.4	11.4±0.4	11.7±0.6	14.1±0.3	13.1±0.1
Stearic acid ($C_{18:0}$)	3.6±0.6	3.8±0.5	4.8±0.7	2.8±0.3	3.7±0.6	3.3±0.8	2.6±0.3	3.5±0.3	3.3±0.2	3.2±0.2
Arachidic acid ($C_{20:0}$)	3.6±1.4	3.1±0.8	2.7±0.6	2.0±0.7	2.7±0.6	2.2±0.7	2.4±0.8	1.9±0.3	2.4±0.8	2.2±0.6
Behenic acid ($C_{22:0}$)	1.7±0.1	1.4±0.1	1.4±0.2	1.2±0.2	1.4±0.1	1.1±0.1	1.2±0.2	0.5±0.2	0.8±0.1	1.2±0.1
Palmitoleic acid ($C_{16:1n7}$)	0.1±0.0	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0	0.1±0.0
Oleic acid ($C_{18:1n9}$)	5.5±0.5	9.1±0.4	8.2±0.6	7.7±0.7	6.7±0.5	5.2±0.3	7.0±0.1	12.9±0.6	7.6±0.1	7.5±0.1
11-eicosenoic acid ($C_{20:1n9}$)	0.6±0.1	0.8±0.1	0.5±0.0	0.5±0.1	0.7±0.1	0.5±0.0	0.5±0.0	0.5±0.1	1.1±0.2	0.5±0.1
α- linolenic acid ($C_{18:3n3}$)	35.5±0.6	29.7±0.9	33.2±0.9	40.5±0.8	30.2±0.5	40.9±0.6	42.1±0.3	40.5±0.9	35.1±0.8	35.1±0.1
Linoleic acid ($C_{18:2n6}$)	34.8±0.7	37.0±1.3	33.5±1.0	30.8±0.1	40.3±0.9	32.7±0.9	30.6±0.8	26.6±0.8	33.5±1.2	34.9±0.3
γ- linolenic acid ($C_{18:3n6}$)	0.1±0.0	0.2±0.0	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
11, 14- eicosadienoic acid ($C_{20:2n6}$)	0.2±0.1	0.1±0.0	0.1±0.0	0.08±0.01	0.2±0.1	0.1±0.0	0.07±0.01	0.05±0.0	0.08±0.01	0.1±0.0
Arachidonic acid ($C_{20:4n6}$)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
Total fatty acids	98.9	98.2	98.5	98.1	98.9	98.5	98.3	98.7	98.6	98.3
Saturated FAs	22.0	21.0	22.6	18.2	20.5	18.8	17.7	17.8	20.8	19.9
Unsaturated FAs	76.9	77.2	75.9	79.9	78.4	79.7	80.6	80.9	77.8	78.4
MUFA	6.2	10.1	8.8	8.3	7.5	5.8	7.6	13.5	8.9	8.1
PUFA	70.7	67.1	67.1	71.6	70.9	73.9	73.0	67.4	68.9	70.3

FA, fatty acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Data presented as mean±standart deviation, (n=3)

Table 3. Mineral contents of seeds of the *Trigonella* species from Section *Cylindricae* (µg/g of dry samples, mean±SD).

Minerals	*T. spruneriana*	*T. sibthorpii*	*T. kotschyi*	*T. mesopotamica*	*T. cylindracea*	*T. cilicica*	*T. filipes*	*T. velutina*	*T. strangulata*	*T. smyrnea*
Macro minerals										
Sodium (Na)	705.33±9.07	325.65±0.49	451.8±2.36	338.93±0.21	1185.23±9.31	567.77±35.17	531.13±1.33	503.83±0.25	290.54±0.23	460.57±1.39
Magnesium (Mg)	3625.67±7.64	3277±8.19	2477.33±7.64	3676.67±5.03	3463.33±108.83	3534.67±70.32	2911.66±18.56	2834.33±13.2	2925.67±10.69	3745.33±9.45
Phosphorus (P)	3231.67±14.84	3586.67±9.45	2335.33±12.06	4574.67±9.29	2739.33±37.87	3723.67±53.63	3738.66±13.01	3965.67±59.5	3358.33±15.63	3624.67±11.02
Potassium (K)	12806.33±5.51	11041.67±20.31	11781.33±10.97	14727.67±10.6	11098±80.73	12623.33±63.88	12886±9.17	14469.67±4.04	12257±7.55	13824.67±7.02
Calcium (Ca)	3565±24.64	2961±11.79	4922.33±9.61	3089.67±12.01	2751.33±38.03	4804.66±59.41	3442.33±11.93	4732.67±4.73	4925.67±7.77	4181.33±3.79
Essential trace minerals										
Chromium (Cr)	28.24±0.06	35.51±0.52	27.73±0.1	28.54±0.09	27.9±0.4	33.02±1.71	28.86±0.22	37.75±0.06	26.77±0.25	28.61±0.04
Manganese (Mn)	17.57±0.27	9.32±0.09	14.52±0.11	18.74±0.06	15.2±0.37	11.05±0.06	14.74±0.06	15.42±0.04	12.38±0.14	17.55±0.08
Iron (Fe)	306.32±0.47	125.41±0.49	119.67±0.59	125.67±0.42	130.8±0.92	149.43±2.82	141.59±0.6	172.2±0.23	143.3±0.36	276.4±0.27
Nickel (Ni)	15.35±0.32	3.84±0.07	2.55±0.27	4.46±0.23	23.54±0.27	3±0.12	2.49±0.1	6.07±0.15	1.74±0.09	10±0.11
Zinc (Zn)	31.25±0.12	52.58±0.61	42.68±0.24	55.43±0.08	31.82±0.27	49.58±1.04	45.83±0.19	59.38±0.05	34.76±0.09	47.2±0.3
Copper (Cu)	10.04±0.12	10.55±0.07	9.78±0.1	14.48±0.15	9.67±0.15	14.07±0.13	14.92±0.04	14.03±0.11	7.24±0.07	7.47±0.09
Selenium (Se)	7.85±0.07	7.75±0.07	7.94±0.07	8.18±0.04	8.05±0.06	7.26±0.57	8.34±0.07	7.76±0.06	7.84±0.11	8.02±0.1
Other elements										
Aluminium (Al)	70.73±0.1	33.56±0.07	89.74±0.09	29.82±0.05	42.53±0.48	103.93±1.57	47.32±0.12	35.54±0.44	86.17±0.35	182.86±0.23
Cobalt (Co)	1.93±0.09	0.62±0.05	0.46±0.07	0.77±0.09	1.85±0.06	0.59±0.01	0.61±0.02	0.75±0.06	0.74±0.09	1.18±0.08
Molibden (Mo)	8.14±0.09	8.5±0.17	11.36±0.08	8.28±0.04	9.59±0.23	12.29±0.71	23.98±0.07	12.87±0.07	7.72±0.25	9.5±0.13
Cadmium (Cd)	1.18±0.03	1.12±0.06	1.32±0.08	1.27±0.09	1.19±0.06	1.23±0.09	1.3±0.01	1.23±0.06	1.25±0.09	1.26±0.11
Lead (Pb)	3.4±0.11	2.84±0.13	3.65±0.36	3.11±0.03	5.48±1.19	3.52±0.49	7.43±0.05	3.57±0.1	19.02±0.1	6.67±0.08

Table 4. Mineral contents of aerial parts of the *Trigonella* species from Section *Cylindricae* (µg/g of dry samples, mean±SD).

Minerals	*T. spruneriana*	*T. sibthorpii*	*T. kotschyi*	*T. mesopotamica*	*T. cylindracea*	*T. cilicica*	*T. filipes*	*T. velutina*	*T. strangulata*	*T. smyrnea*
Macro minerals										
Sodium (Na)	512.33±0.31	321.4±0.4	357.33±0.42	387.27±0.12	539±0.8	573.93±18.52	379.59±0.18	274.4±0.2	472.73±0.12	203.33±0.23
Magnesium (Mg)	1133.8±3.86	1076.8±2.78	1129.4±0.2	897.7±0.1	1346.6±12.6	1368.53±14.6	1278.47±11.11	978.32±0.3	1104.33±0.31	209.4±0.2
Phosphorus (P)	2818.33±8.39	3115.33±4.16	2116±5.29	3835.33±5.03	2046±21.63	3541.33±23.35	3958±9.17	3855±5.57	2762±14.42	3257±30.05
Potassium (K)	5444.33±3.51	5225±7.94	6638±24.58	5822.67±3.06	6218±13.12	13327.33±29.69	9651.33±11.37	5808.37±0.34	5378.67±17.47	5377.67±5.13
Calcium (Ca)	13754±33.29	12744.33±6.03	9750.67±6.66	7832.67±20.03	12422.67±69.64	9870±83.21	12822.67±11.02	7466.61±0.03	13272.67±30.09	13052.67±26.63
Essential trace minerals										
Chromium (Cr)	186.31±0.16	191.33±0.31	200.76±0.14	206.49±0.34	182.43±0.38	193.17±1.59	201.6±0.35	165.33±0.3	188.62±0.12	178.69±0.12
Manganese (Mn)	95.78±0.05	128.83±0.04	69.54±0.28	97.83±0.27	100.72±0.11	58.57±0.96	132.73±0.6	55.58±0.2	90.68±0.07	119.36±0.34
Iron (Fe)	162.63±0.41	235.5±0.27	59.57±0.23	120.54±0.16	173.78±0.43	74.37±0.21	163.57±0.32	35.43±0.04	139.73±0.06	285.36±0.32
Nickel (Ni)	7.87±0.2	23.51±0.11	12.57±0.33	14.26±0.14	22.47±0.44	8.53±0.12	17.7±0.24	7.06±0.08	12.65±0.31	17.61±0.17
Zinc (Zn)	222.4±0.2	236.37±0.15	173.29±0.16	310.37±0.45	210.6±0.2	247.22±2.08	232.2±0.2	228.53±0.31	360.4±0.2	408.21±0.21
Copper (Cu)	23.57±0.16	30.47±0.05	23.62±0.33	30.45±0.25	27.55±0.18	39.49±0.46	32.8±0.12	26.54±0.25	22.72±0.23	35.23±0.05
Selenium (Se)	38.66±0.11	37.73±0.15	38.63±0.29	38.37±0.17	37.92±0.49	39.77±0.31	38.57±0.3	41.72±0.32	39.64±0.02	39.54±0.11
Other elements										
Aluminium (Al)	2244.67±21.94	2270±5.29	1418.8±1	1678.5±0.1	2048±26.15	849.87±20.97	2034.67±22.3	688.52±0.28	2417.33±15.54	3148.67±7.02
Cobalt (Co)	5.61±0.38	6.23±0.05	4.81±0.14	5.05±0.14	5.32±0.29	5.39±0.09	5.33±0.25	4.28±0.17	4.81±0.08	2.05±0.02
Molibden (Mo)	26.77±0.14	25.55±0.13	29.76±0.07	25.61±0.18	28.79±0.29	26.21±0.12	25.47±0.29	33.58±0.14	24.61±0.3	22.55±0.15
Cadmium (Cd)	11.33±0.3	11.36±0.19	11.58±0.26	10.48±0.2	11.43±0.11	11.64±0.06	11.62±0.16	11.21±0.18	12.46±0.31	12.58±0.02
Lead (Pb)	51.21±0.1	60.96±0.16	65.27±0.08	58.59±0.22	45.73±0.22	44.31±0.16	51.19±0.06	47.48±0.23	50.03±0.12	74.7±0.24

done with 15 *Trigonella* species to compare *T. foenum-graecum*. Dangi et al., determined diosgenin content in 15 *Trigonella* species which showed higher levels of diosgenin in aerial parts in *T. anguina* and *T. caerulea* as compared to fenugreek [61]. In this study, it was observed that diosgenin content ranged from 0.07 to 0.52 mg/g in seeds of ten *Trigonella* species. The lower values (0.03-0.16 mg/g) were detected in leaves and stems of the studied *Trigonella* species. *T. cilicica* seeds showed the highest levels of diosgenin among ten *Trigonella* species. This species could be used to search for callus/cell suspension cultures for alternative diosgenin source.

3.5. Total phenolics and flavonoids contents and DPPH radical scavenging activity

The total phenolic and flavonoid contents of studied *Trigonella* species were shown in Table 6. The total phenolic contents of seeds were ranged from 94.83 to 201.35 mg gallic acid equivalents (GAE)/g while the total flavonoid contents of seeds were ranged from 84.00 to 128.04 mg rutin equivalents (RE)/g. In aerial parts of the ten *Trigonella* species, total phenolic and total flavonoid contents were ranged from 79.90 to 139.88 mg/g and from 61.51 to 110.31 mg/g, respectively. Seeds of *T. strangulata, T. spruneriana* and aerial parts of *T. velutina*, *T. cilicica and T. filipes* exhibited higher total phenolic content than other species. The highest total flavonoids content was determined in the seeds of *T. spruneriana* and the aerial parts of *T. velutina*.

Table 6. Total phenolics and flavonoids contents of the studied *Trigonella* species.

Species	Seeds		Aerial Parts	
	Total phenolics (mg/g)	Total flavonoids (mg/g)	Total phenolics (mg/g)	Total flavonoids (mg/g)
T. spruneriana	191.90±0.86	128.04±0.09	83.46±0.24	62.85±0.03
T. sibthorpii	137.34±0.34	104.27±0.09	113.32±1.13	73.15±0.07
T. kotschyi	166.06±1.45	109.75±0.05	97.30±0.43	86.29±0.13
T. mesopotamica	94.83±0.08	84.00±0.93	107.16±0.52	82.95±0.13
T. cylindracea	125.80±0.85	109.21±0.12	90.91±0.94	73.72±0.04
T. cilicica	158.86±1.75	118.89±0.03	139.35±0.36	64.01±0.17
T. filipes	109.79±1.08	92.72±0.03	138.69±1.15	107.25±0.04
T. velutina	138.95±0.55	121.21±0.11	139.88±0.38	110.31±0.09
T. strangulata	201.35±0.51	121.84±0.05	84.99±0.58	71.64±0.04
T. smyrnea	145.33±0.83	112.22±0.20	79.90±0.38	61.51±0.06

The results were presented as the mean±standard deviations of triplicate determinations.
The data were expressed as mg gallic acid equivalents (GAE)/g for total phenolics and mg rutin equivalents (RE)/g for total flavonoids.

The DPPH radical scavenging activity of the plant materials are presented in Table 7. The extract obtained from seeds of *T. smyrnea* showed the high antioxidant activity (76.18 % inhibition). The DPPH scavenging effect, in decreasing order, was determined for the extracts of the aerial parts of *T. filipes* >*T. velutina*, and the seed extracts of *T. spruneriana, T. velutina* and *T. cilicica*.

The results showed that total phenolic contents of the seeds were ranged from 94.83-201.35 mg/g and the total flavonoid contents were ranged from 84.00-128.04 mg/g. Total phenolic contents obtained from aerial parts were ranged from 79.90-139.88 mg/g and the total flavonoid contents were ranged from 61.51-110.31 mg/g. In the literature, there are many scientific studies on the total phenol and antioxidant activities of extracts prepared from fenugreek using different solvents. When total phenolic content and antioxidant activity in the seeds of all the investigated plants were compared with Naidu et al. 2011, it is clearly showed that it had similar or a slightly higher value of phenolic content (65.81-85.88 mg GAE/g) and antioxidant activity (50-70 % inhibition) [62]. Aqil et. al (2006) reported that the total phenolic content of the extract of fenugreek leaves had 74.33 mg GAE/g total phenolics and showed 57.45 % inhibition [63]. 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 % [64]. When total phenolic and flavonoid contents of seed and aerial part extracts are compared with fenugreek in the literature, there are a correlation between the present study and previous ones. The antioxidant activity of the studied *Trigonella* species was measured using the DPPH method. The extracts of the aerial parts showed an inhibition % of 17.37 - 60.16 % while the seeds showed 27.42 - 76.18. This study revealed that the seeds and the aerial parts extracts of ten *Trigonella* species showed similar value of antioxidant activity in comparison with fenugreek.

Table 7. DPPḢ• radical scavenging activity (inhibition%) of the extracts obtained from the studied *Trigonella* species.

Species	Inhibition %	
	Seeds	Aerial Parts
T. spruneriana	54.28±0.09	21.11±0.03
T. sibthorpii	36.42±0.16	32.69±0.04
T. kotschyi	28.29±0.03	31.38±0.06
T. mesopotamica	27.42±0.08	34.16±0.07
T. cylindracea	32.30±0.17	42.24±0.05
T. cilicica	45.39±0.02	21.92±0.06
T. filipes	34.52±0.10	60.16±0.06
T. velutina	47.13±0.02	57.43±0.05
T. strangulata	41.81±0.06	27.46±0.04
T. smyrnea	76.18±0.03	17.37±0.02

In Turkey, 54 *Trigonella* species divided into 13 sections. *Cylindricae* Boiss. with ten species is one of the large sections of genus *Trigonella* and two species are endemic to Turkey (21). No detailed phytochemical studies can be found in the literature for this group. The results of the present studies revealed that all species are important source of essential fatty acids, minerals and phenolics. The fatty acid compositions showed that the oils would be named α-linolenic-linoleic type, and their ratios were important as a chemotaxonomic marker in *Cylindricae* section of *Trigonella* species. Among the studied *Trigonella* species, especially *T. strangulata*, *T. smyrnea* and *T. cilicica* could be used in food and pharmaceutical industries.

Acknowledgments

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

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