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Tocopherol, Sterol and Amino Acid Compositions of *Trigonella strangulata* Boiss. Seeds

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Abstract— *Fenugreek (Trigonella foenum-graecum L.) which is known from ancient times is one of the most promising medicinal herbs and having high nutritional value. Fenugreek contains diverse categories of phytoconstituents such as amino acids, fatty acids, phytosterols, phenolics, alkaloids etc. This study aimed at determining the seeds of T. strangulata for their tocopherol, sterol and amino acid compositions by using HPLC, GC, UFLC techniques. The seed oil contains mainly α-tocopherol (188.78±1.18 mg/100 g). Major phytosterols (%) were β-sitosterol (56.80±0.74), delta-5-avenasterol (13.82±0.46) and delta-5,24-stigmastadienol (10.53±0.57) while high quantities of glutamic acid, lysine, proline, arginine and leucine were observed in seeds (3086±0.08, 2482±0.07, 2334±0.07, 2244±0.08 and 2053±0.07 mg/100 g, respectively). The results indicate that this species are a source of α-tocopherol, β-sitosterol and amino acids.*

Keywords— *Amino acid; Sterol; Tocopherol; Trigonella strangulata Boiss.*

I. INTRODUCTION

Trigonella L. genus has about approximately 260 species worldwide and widely distributed throughout the Mediterranean region, South and North Africa, North America, Europe, West Asia and South Australia [1-3]. Some species of the genus including *T. foenum-graecum* (fenugreek), *T. occulta* Del., *T. incise* Royle, *T. corniculata* L. [4,5], *T. arabica* Delile and *T. berythea* Boiss. & Blanche [6] are well known as a spice and/or vegetable, and traditionally used in several medicinal systems for treatment of diseases [7-9].

Fenugreek (*T. foenum-graecum*) seeds possess a number of medicinal properties and the seeds have been used for treating various ailments from antiquity [10]. Fenugreek plant has multiple use as food. Its seeds are being consumed as spice either whole and ground form in various kinds of spice mix used in cuisines of the Asian continent while green as well as dried leaves are used as vegetable [10]. The seeds contain fixed oil (mainly polyunsaturated fatty acids such as oleic, linoleic, and linolenic acids) [11,12], flavonoids, alkaloids [3,8,13,14], saponins (diosgenin, gitogenin, and yamogenin), mucilage, coumarins [2,9], amino acids, protein, dietary fibers (soluble and insoluble fibers) [12], volatile oil [8], vitamins (A, B1, B2, C, D), and various other functional elements [9,11,13]. Modern pharmacological studies show that fenugreek has many medicinal qualities, such as anti-inflammatory, antiulcer, hypocholestrolaemic, analgesic, antidiabetic, antipyretic, wound healing, CNS-stimulant, immunomodulatory, antifertility, anti cancer, antimicrobial, antioxidant activity, gastroprotective and chemopreventive properties [2,12,14-16]. Nowadays, production and marketing of fenugreek are becoming increasingly important because of medicinal and nutraceutical properties [9,15,17].

Although many *Trigonella* species have been spread throughout the world, most chemical and pharmacological investigations have concentrated on the popular agricultural species *T. foenum-graecum* [18]. The genus *Trigonella* comprises about 54 taxa which are represented by 13 sections and 8 groups in Turkey [1,19,20]. In our previous studies, the Section Cylindricae including 10 species [2] and *T. monspeliaca* L. [16], *T. plicata* (Boiss. & Bal.) Boiss. [21] were investigated for their fatty acid composition, diosgenin, phenolics and mineral contents and antioxidant activities. In this study, *T. strangulata* Boiss. was examined tocopherol, sterol and amino acid compositions by using HPLC, GC and UFLC techniques for the first time.

II. MATERIALS AND METHODS

A. Plant Material

T. strangulata was collected from the Silifke district of Mersin province of Turkey by one of the authors (S. S. Uras Gungor). The authentication of the plant material was confirmed by a botanist, Prof. Dr. Ahmet İLÇİM (Department of Biology, Faculty of Sciences and Arts, Mustafa Kemal University, Antakya, Hatay, TURKEY), stored in the Herbarium of the Faculty of Sciences and Arts, Mustafa Kemal University and assigned the Voucher number MKU1766.

B. Oil Extraction

The oil was extracted by the method based on ISO 659:2009 [22]. Briefly, seeds were extracted with diethylether for 3 h at 50°C by using Soxhlet systems. Then obtained seed oil was put into Brown bottle and stored at room temperature for the further analysis (tocopherols and sterol compositions). The all data was represented by the content in either 1 kg (tocopherols) or 100 g (sterols) of raw oil extracted from the seeds.

C. Tocopherol Composition

The tocopherol analysis was performed according to the method based on ISO 9936:2006 [22]. HPLC system (Agilent HPLC Series 1100, Waldbronn, Germany) equipped with a fluorescence detector and a normal-phase column (5 µmLiCrosorb Si60 25 cm x4.6 mm i.d.) was used for the

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analysis. Briefly, the sample (1 g) was dissolved in n-heptane (10 mL) in dark screw capped tube via vortexing for 10 min. for each analysis. Then for obtaining final extract the sample was filtered through at 0.45-µm pore size syringe filter. 3.8 % (v/v) tetrahydrofuran (THF) in n-heptane was used as an isocratic mobile phase and 1 mL/ min. flow rate was chosen for the analysis. Detector wave lengths were kept at 270 nm and 310 nm for excitation and emission, respectively. Experiments were performed triplicate.

D. Sterol Composition

Sterol analysis was performed according to the ISO 12228:1999 method [22]. The seed oil (1 g) was kept into screw-capped glass tube and an internal standard 5α-cholestan-3β-ol and betulin (1,000 ppm) (1 mL) was put into the tube, 1 h later, 0.5 N NaOH was added to saponify the mixture. The saponification product was extracted with n-hexane (3 x 5 mL). Under nitrogen gas, the extract volume was reduced to 10 mL and then anhydrous sodiumthiosulfate was used to dry the sample. For 15 min. each sample (0.5 mL) was silylated at 60 °C and a bis (trimethylsilyl) trifluoroacetamide / trimethylchlorosilane (4:1, byvol) solution (250 µL) and dry pure pyridine (250 µL) were added. Finally, the silylated sample was examined by GC (Perkin Elmer, Autosystem GLX, Shelton, USA) system equipped with a flame ionization detector (FID) using SE-54 (5 %-phenyl- 1 %-vinylmethylpolysiloxane, 30 m x 0.32 mm x 0.25 µm) column. Carrier gas was helium and flow rate was 0.8mL/min, injector temperature; 280^0C, detector temperature; 300^0C, oven temperature program; (1) initial temperature of 60^0C for 2 min and (2) increase upto 220^0C at a rate of 4 C/min, (3) 1 min waiting at 220^0C followed by an increase upto 310^0C at a rate of 5 C/min, (4) holding at 310^0C for 30 min. Each sterol was identified by using relative retention times (RRT) of betulin and 5α-cholestan-3β-ol. All sterols come out to have retention times between those of betulin and 5α-cholestan-3β-ol. Experiments were performed triplicate.

E. Amino Acid Composition

Determination of amino acid composition, the alkaline hydrolysis of protein for tryptophan, an acid hydrolysis for others and derivatization were carried out according to the methods proposed by Eroglu *et al*., 2016 and Cevikkalp *et al*., 2016. Ultra fast liquid chromatography (UFLC) system equipped with a binary pump and UV/VIS detector were chosen for chromatographic analysis and a reversed phase analytical column of HPLC with a fluorescens detector was used for seperation and detection [23,24]. Experiments were performed triplicate.

III. Results And Discussions

A. Tocopherol Composition

Table I presents the tocopherol profile and content of the seed oil. As can be seen in Table I, the seeds were rich in α-tocopherol (188.78±1.18 mg/100 g) followed by γ-tocopherol and β-tocopherol.

TABLE I. Tocopherol composition of *T. strangulata* seed oil.

Tocopherol	**(mg/100 g oil)**
α-tocopherol	188.78±1.18
β-tocopherol	0.98±0.04
γ-tocopherol	1.49±0.17

Data presented as means±SD (n=3)

Tocopherol composition were in accordance with previous studies [15,25,26]. The levels of α-tocopherol found in the *T. foenum-graecum* seed oil was from 620 mg/kg to 910 mg/kg by Çiftçi *et al*., (2011) while Aljuhaimi *et al*., (2016) reported α-tocopherol content as 100.1 mg/100 g in fenugreek seeds from Turkey [15,26].

Tocopherols are one of the important lipid soluble ingredients which shows antioxidant effects. It was reported that α-tocopherol shows the highest biological activity (i.e. effect on cholesterol metabolism, anti-inflammatory and oxidative stability of vegetable oils) among the other homologs [15,27,28]. This species could be recommended for human nutrition because of its tocopherol content together with unsaturated fatty acid composition of its oil that reported earlier by Uras Gungor *et al*., (2017) [2].

B. Sterol Composition

Sterol composition of fixed oil was given in Table II. *β-sitosterol*, was the main sterol (56.80±0.74 %). *Delta*-5-avenasterol (13.82±0.46 %), delta-5,24-stigmastadienol (10.53±0.57 %), and campesterol (8.88±0.38 %) were also detected in significant concentrations.

TABLE II. Sterol composition of *T. strangulata* seeds.

Sterol	**(%)**
Campesterol	8.88±0.38
Stigmasterol	6.99±0.26
Chlerosterol	0.67±0.17
β-sitosterol	56.80±0.74
Delta-5-avenasterol	13.82±0.46
Delta-5,24-stigmastadienol	10.53±0.57
Delta-7-avenasterol	0.53±0.13
Delta-7-stigmastenol	1.78±0.21
Total sterol	2154.18±0.64 (mg/100g)

Data are expressed as means ±SD (n=3)

The main sterol of *T. strangulata* were similar to that found for *T. foenum-graecum* by Ciftci *et al*., (2011) and Kıralan *et al*., (2017) [15,29]. Phytosterols in *Trigonella* genus were investigated in only *T. foenum-graecum* by two different authors. *β-sitosterol* was found to be main sterol ranged from 31.8 % to 43.2 % followed by campesterol and cycloartenol [15]. Kıralan *et al*., (2017) found that *β-sitosterol* (from 59.94 % to 68.24 %) was the major component of sterols in fenugreek seed and it was followed by campesterol (11.78 % to 16.14 %) and *delta-5-avenasterol* [29].

Phytosterols are of interest due to prevention of some chronic diseases (i.e. cardiovascular problems) and to have anticancer, anti-inflammatory effect [30]. Phytosterols in medicinal plants have been studied comprehensively by some authors [31,32]. *β-sitosterol*, campesterol and stigmasterol are membrane constituents of herbs [33]. It was known that *β-sitosterol* have some biological activity such as promoting apoptosis, inhibiting cancer cell proliferation and antioxidant

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effect by different mechanisms [31]. Our result indicate that *T. strangulate* is a rich source of phytosterols.

C. Amino Acid Composition

Table III presents an overview of the presence of seventeen essential and non-essential amino acids in the seeds. According to the results, amount of lysine, arginine and leucine were higher than other essential amino acids found in the seeds with 2482±0.07, 2244±0.08, and 2053±0.07 mg/100g values, respectively. Leucine was followed by other essential amino acids including phenylalanine, isoleucine, valine, histidine, threonine, and methionine in descending order. Additionally, amount of glutamic acid and proline were higher than other non-essential amino acids found in the seeds with 3086±0.08 and 2334±0.07 mg/100 g values, respectively. Aspartic acid, glycine, alanine, serine and tyrosine were the other non-essential amino acids in significant amounts.

TABLE III. Amino acid composition of *T. strangulata* seeds.

Amino acid	Symbol	(mg/100 g)
Essential amino acids		
Histidine	HIS	770±0.03
Isoleucine	ILE	1594±0.03
Leucine	LEU	2053±0.07
Lysine	LYS	2482±0.07
Methionine	MET	165±0.02
Phenylalanine	PHE	1655±0.06
Threonine	THR	701±0.02
Valine	VAL	1414±0.04
Arginine	ARG	2244±0.08
Non-essential amino acids		
Alanine	ALA	1150±0.03
Aspartic acid	ASP	1875±0.04
Glycine	GLY	1248±0.05
Glutamic acid	GLU	3086±0.08
Proline	PRO	2334±0.07
Serine	SER	1002±0.03
Tyrosine	TYR	947±0.04
Tryptophan	TRP	226±0.01

Data presented as means±SD (n=3)

The amino acid composition of *T. foenum-graecum* seed were investigated extensively because of interesting composition. Fenugreek seeds were rich in glutamic acid, aspartic acid, leucine, threonine and arginine, glycine, lysine and isoleucine in different levels depending on the geographical source [12,34].

The present results are similar to previously reported on *T. foenum-graecum* seeds that those of some quantitative differences. In particular, *T. strangulata* seeds are promising source of glutamic acid, lysine, proline, arginine and leucine.

IV. CONCLUSIONS

The present study measured the tocopherol, sterol and amino acid compositions of *T. strangulata* seed extracts *via* HPLC, GC and UFLC techniques for the first time. Results indicates that tocopherol, sterol and amino acid compositions of *T. strangulata* revealed that the seeds are a source of α-tocopherol, β-sitosterol and amino acids. Therefore, *T. strangulata* seeds can be evaluated in food industry for its nutritional properties and further detailed chemical and biological activity studies could be done because of medicinal potential of this species.

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