



Original Scientific Paper

Phytochemical screening and antioxidant activity of *Trigonella cariensis* seeds

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ABSTRACT:

The genus *Trigonella* (Fabaceae) has been widely used for both culinary and clinical purposes since ancient times. The aim of this study was to investigate the chemical composition and *in vitro* antioxidant activities of *Trigonella cariensis* seeds for the first time. The seeds were collected in the province of Mersin, Turkey. Chemical analyses were performed using chromatographic methods. Fatty acid, tocopherol and sterol analyses were done on hexane extracts and amino acid and mineral analyses were also carried out on the seeds. The total phenolic and flavonoid contents were measured by using the Folin-Ciocalteu and $\text{Al}(\text{NO}_3)_3$ methods and *in vitro* antioxidant activity was evaluated via different chemical assays including 1,1-diphenyl-2-picrylhydrazily (DPPH[•]), cupric reducing antioxidant power (CUPRAC) and 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulphonate (ABTS^{•+}) on its 80% methanolic extract. The seeds were shown to contain mainly linoleic, α -linolenic and oleic acids ($43.74 \pm 0.24\%$, $18.38 \pm 0.45\%$, and $10.89 \pm 0.71\%$, respectively). The seeds showed high α -tocopherol content (233.54 ± 2.48 mg/100 g). The main sterols were β -sitosterol, δ -5-avenasterol and campesterol. Na, K, Ca, P, and Mg were the predominant minerals, while glutamic acid, aspartic acid and lysine (5801 ± 0.15 , 3629 ± 0.12 and 2062 ± 0.03 mg/100 g, respectively) were determined as the major amino acids in the seeds. The total phenolic and flavonoid contents were 3.34 ± 0.02 mg GAE/g seed and 0.96 ± 0.09 mg QE/g seed, respectively. The results of the ABTS^{•+}, DPPH[•] and CUPRAC methods were $43.22 \pm 3.14\%$, $31.15 \pm 1.79\%$ and 9.67 ± 0.78 mM AAE/g, respectively. In conclusion, *T. cariensis* seeds provide nutritional value with a good source of polyunsaturated fatty acids, α -tocopherol, β -sitosterol, some amino acids and minerals and medicinal properties with total phenolic and flavonoid contents and antioxidant activities.

Keywords:

Trigonella cariensis, chemical composition, fatty acid, amino acid, DPPH, CUPRAC

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INTRODUCTION

Plants and humans have nurtured a close relationship since the beginning of this planet. Humans receive valuable offerings from plants, including food, fodder, shelter, and most importantly, medicines to treat various ailments (ALAM 2019). Natural products have tremendous nutritional and health benefits due to their rich content of vitamins, minerals, amino acids, proteins, fibres, phenolic compounds, antioxidants, and bioactive metabolites. Spices and herbs are the largest source of natural

compounds and have also been widely used as food additives in the art of cooking since time immemorial. They have become an integral part of healthcare and traditional herbal products. Their natural pharmacological properties with negligible toxicity help delay the aging process, heart ailments, neurodegenerative diseases and metabolic disorders, and rejuvenate biological tissues (SINGH *et al.* 2022).

The genus *Trigonella* L. belongs to the Fabaceae family, and includes about 135 species widely distributed in dry regions around the Eastern Mediterranean, West

Asia, Southern Europe, North and South Africa, and South Australia (only one species). Certain taxa of the genus are used in food and medicine, and have been known and used for different purposes since ancient times, especially in Greece and Egypt (AKAN *et al.* 2020). *Trigonella foenum-graecum* is well-known for its usage in nutraceuticals and medicine, as well as fenugreek, while some species of the genus including *T. occulta* Del., *T. incise* Royle, *T. corniculata* L., *T. arabica* Delile and *T. berythea* Boiss. & Blanche are used as a spice and/or vegetable and for the treatment of various diseases. (JAIN *et al.* 1996; SRINIVASAN 2006; JARADAT *et al.* 2016; RAO & RAO 2018; SINGH *et al.* 2022).

In Turkey, the *Trigonella* genus is divided into 10 sections. *T. cariensis* Boiss., which belongs to Sect. *Foenum-graecum* Ser. as *T. foenum-graecum*, is known as Kokuluboncuk in Turkey (AKAN *et al.* 2020). *Trigonella cariensis* is an annual herbaceous plant species common in Turkey, especially in western Anatolia. It has a wide distribution in the natural population. It can be an important ornamental plant with flashy flowers and fruits (AKAN *et al.* 2020). There is no study on the phytochemical and biological properties of *T. cariensis* seeds in the available literature. Therefore, the aim of this study was to investigate the fatty acid, sterol, tocopherol, amino acid and mineral composition of the seeds by using GC, HPLC, UFLC, and ICP-MS techniques to determine the nutritional characteristics and also the total phenolic and total flavonoid contents and *in vitro* antioxidant activities such as DPPH[•], ABTS^{•+} and CUPRAC by using spectrophotometric systems.

MATERIAL AND METHODS

Plant material. *Trigonella cariensis* seeds were collected in 2019, during the seedling season. The plant material was collected in the Mersin province of Turkey (Gül-
nar, situated at an altitude of 705 m, on roadsides). The plant was identified by Prof. Dr. Ahmet İLÇİM (Department of Biology, Faculty of Sciences and Arts, Mustafa Kemal University, Antakya, Hatay, TURKEY) and the voucher specimen stored in the Herbarium of the Faculty of Sciences and Arts, Mustafa Kemal University (MKU1831).

Fatty acid, tocopherol and sterol composition

Oil extraction. The oil extraction procedure was performed according to the method based on ISO 659:2009 (ERTAS *et al.* 2013). Briefly, the seeds were extracted with diethylether for 3 h at 50°C by using the Soxhlet approach. The obtained seed oil was then placed in brown bottles and stored at room temperature for further analysis (fatty acid, tocopherol, and sterol composition). All the data are presented by the content in either 1 kg (tocopherols) or 100 g (fatty acids and sterols) of raw oil extracted from the seeds.

Fatty acid composition. The analysis of the fatty acid methyl esters (FAME) was performed according to the method based on ISO 12966-2:2011 (ERTAS *et al.* 2013).

Tocopherol and sterol composition. The ISO 9936:2006 method was used for the tocopherol analysis (ERTAS *et al.* 2013). A HPLC system (Agilent HPLC Series 1100, Waldbronn, Germany) equipped with a fluorescence detector and a normal-phase column (5 µmLiCrosorb Si60 25 cm × 4.6 mm i.d.) was used for the analysis.

The determination of sterol was carried out according to ISO 12228:1999 method (ERTAS *et al.* 2013). A GC system (Perkin Elmer, Autosystem GLX, Shelton, USA) equipped with a flame ionization detector (FID) and SE-54 column (5%-phenyl-1%-vinylmethylpolysiloxane, 30 m × 0.32 mm × 0.25 µm) was used for the analysis.

Amino acid composition. The determination of the amino acid composition, the alkaline hydrolysis of protein for tryptophan, acid hydrolysis for others and derivatisation were carried out according to the methods described by EROGLU *et al.* (2016) and CEVIKKALP *et al.* (2016). An 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 fluorescence detector was used for separation and detection (CEVIKKALP *et al.* 2016; EROGLU *et al.* 2016).

Mineral analysis. The dried seeds were prepared for digestion according to the methods proposed by BAŞGEL & ERDEMOĞLU (2006). A CEM MARS 240/50 oven model with a timer and variable temperature settings was used for the microwave-assisted digestion of the materials. Mineral analysis was carried out by using an Agilent ICP-MS 7500ce (Tokyo, Japan) equipped with an Octopole Reaction System.

Total phenolic and flavonoid content and antioxidant activities

Preparation of extracts. The method proposed by KOCAZORBAZ *et al.* (2017) was modified with minor changes for the extraction of the *T. cariensis* seeds (KOCAZORBAZ *et al.* 2017). The powdered seeds (3 g) were sonicated twice, each with 40 mL of 80% methanol in a water bath at 40°C. The extract was filtered and the volume was adjusted to 80 mL with aqueous methanol (80%). The extract was stored at 4°C for further investigation.

Total phenolic and flavonoid contents. The total phenolic content of the seeds were quantified using the Folin-Ciocalteu method (KOCAZORBAZ *et al.* 2017). A calibration curve with gallic acid was prepared and the results were expressed as gallic acid equivalents (mg GAE g⁻¹ extract). The amount of flavonoids in the seeds was estimated using an aluminium chloride colorimetric assay as de-

scribed by KOCAZORBAS *et al.* (2017). The flavonoid content was expressed as quercetin equivalents (mg QE g⁻¹ extract).

In vitro antioxidant activities

DPPH[•] free radical scavenging assay. The antioxidant activity of the extract was measured to assess the scavenging capacity of the *T. cariensis* extract based on the ability to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH[•]) radical as described by PAVITHRA & VADIVUKKARASI (2015). The antioxidant activity was expressed as gallic acid equivalents.

ABTS^{•+} radical-cation scavenging assay. The ABTS^{•+} (2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)) assay was used with a minor modification to the protocol of APAK *et al.* (2008). The absorbance of the resulting solution was measured at 734 nm. The antioxidant capacity was expressed as micromoles/g seed.

CUPRAC (cupric ion reducing antioxidant capacity) assay. A slightly modified version of the CUPRAC method (APAK *et al.* 2008) was used to measure the copper (II) or cupric ion reducing ability of the *T. cariensis* seeds. The absorbance of the resulting solution was measured at 450 nm. The antioxidant capacity was expressed as micromoles of ascorbic acid equivalents.

RESULTS AND DISCUSSION

Fatty acid composition. The oil yield of the *T. cariensis* seeds was 2.01%. The fatty acid composition of the oil is shown in Table 1. Twenty one fatty acids were found in the oil. The oil contains high amounts of polyunsaturated fatty acids (PUFA, 62.52%). Linoleic and α -linolenic acids were the dominant fatty acids at 43.74±0.24%, and 18.38±0.45%, respectively. Palmitic and oleic acids were the other major fatty acids.

According to the literature, *T. foenum-graecum* seeds contain fixed oils consisting mainly of unsaturated fatty acids such as linoleic (22-70%), linolenic (4-23%) and oleic (9-35%) acids (SRINIVASAN 2006; SULIEMAN *et al.* 2008; CIFTCI *et al.* 2011; KOCAK *et al.* 2011; SHAKUNTALA *et al.* 2011; AL-JASSAS & AL-JASSER 2012). The quantitative differences in these fatty acids can be explained by different geographical origin or different cultivation conditions (SULIEMAN *et al.* 2008; BIENKOWSKI *et al.* 2017).

Several chemical studies on other *Trigonella* species were found in the literature. BAGCI *et al.* (2004) recorded that *T. cretica* contained 49.9% oleic and 24.2% linoleic acids (BAGCI *et al.* 2004). Ten species of *Trigonella* genus were studied to determine fatty acid composition by URAS GÜNGÖR *et al.* (2017). These species were found to contain high quantities of polyunsaturated fatty acids and the sum of linoleic and linolenic acids ranged from

Table 1. Fatty acid composition of *Trigonella cariensis* seeds

| Fatty acid | % |
|---|------------|
| Saturated | |
| C _{4:0} (butiric acid) | 0.02±0.01 |
| C _{8:0} (caprylic acid) | 0.44±0.07 |
| C _{12:0} (lauric acid) | 0.01±0.00 |
| C _{14:0} (myristic acid) | 0.19±0.00 |
| C _{15:0} (pentadecanoic acid) | 0.19±0.03 |
| C _{16:0} (palmitic acid) | 14.16±0.38 |
| C _{17:0} (heptadecanoic acid) | 0.46±0.07 |
| C _{18:0} (stearic acid) | 4.78±0.42 |
| C _{20:0} (arachidic acid) | 2.34±0.34 |
| C _{21:0} (heneicosanoic acid) | 0.44±0.07 |
| C _{22:0} (behenic acid) | 0.89±0.13 |
| C _{23:0} (tricosanoic acid) | 0.05±0.03 |
| C _{24:0} (lignoseriic acid) | 0.66±0.07 |
| TSFA | 24.63 |
| Unsaturated | |
| C _{16:1n7} (palmitoleic acid) | 0.08±0.00 |
| C _{18:1n9} (oleic acid) | 10.89±0.71 |
| C _{20:1n9} (11-eicosenoic acid) | 0.16±0.12 |
| C _{18:3n3} (α -linolenic acid) | 18.38±0.45 |
| C _{18:2n6} (linoleic acid) | 43.74±0.24 |
| C _{20:2n6} (11, 14- eicosadienoic acid) | 0.06±0.01 |
| C _{20:3n3} (11, 14, 17- eicosatrienoic acid) | 0.08±0.02 |
| C _{20:4n6} (arachidonic acid) | 0.26±0.01 |
| TUFA | 73.65 |

TSFA: total saturated fatty acids, TUFA: total unsaturated fatty acids. Data presented as mean±SD (n=3)

Table 2. Tocopherol and sterol composition of *Trigonella cariensis* seeds

| Tocopherol and sterol | (mg/100 g) |
|--------------------------------|-------------|
| α -tocopherol | 233.54±2.48 |
| β -tocopherol | 36.29±1.52 |
| γ -tocopherol | 0.78±0.08 |
| Campesterol | 11.24±1.21 |
| Stigmasterol | 2.88±0.22 |
| Chlerosterol | 0.58±0.01 |
| β -sitosterol | 62.65±0.53 |
| δ -5-avenasterol | 18.40±0.31 |
| δ -5,24-stigmastadienol | 3.93±0.42 |
| δ -7-stigmastenol | 0.32±0.01 |

Data presented as mean±SD (n=3)

Table 3. Amino acid composition of *Trigonella cariensis* seeds

| Amino acid | Symbol | (mg/100 g) |
|---------------------------|--------|------------|
| Essential amino acids | | |
| Histidine | HIS | 1019±0.02 |
| Isoleucine | ILE | 1399±0.04 |
| Leucine | LEU | 1997±0.03 |
| Lysine | LYS | 2062±0.03 |
| Methionine | MET | 211±0.00 |
| Phenylalanine | PHE | 1270±0.01 |
| Threonine | THR | 1144±0.03 |
| Valine | VAL | 1279±0.02 |
| Non-essential amino acids | | |
| Arginine | ARG | 1700±0.03 |
| Alanine | ALA | 1307±0.02 |
| Aspartic acid | ASP | 3629±0.12 |
| Glycine | GLY | 1776±0.03 |
| Glutamic acid | GLU | 5801±0.15 |
| Proline | PRO | 1557±0.04 |
| Serine | SER | 1520±0.02 |
| Tyrosine | TYR | 876±0.01 |
| Tryptophan | TRP | 311±0.00 |

Data presented as mean±SD (n=3)

66.7% to 73.6%. In our previous study, linoleic and linolenic acids were the most dominant fatty acids in the seeds of *T. rhytidocarpa* (GÜNGÖR *et al.* 2021).

The present results for *T. cariensis* seed oil are similar to the findings of other authors who recorded that linoleic and linolenic acids were the most dominant fatty acids in the seeds of *Trigonella* species (CİFTÇİ *et al.* 2011; ALJUHAİMİ *et al.* 2018). This study showed that *T. cariensis* with a 2.38 ratio of omega 6/omega 3 fatty acids provides a valuable oil for nutraceutical purposes.

Tocopherol and sterol composition. The tocopherol and sterol profiles are given in Table 2. The results indicated that the seeds were rich in α -tocopherol (233.54±2.48 mg/100 g) and β -sitosterol 62.65±0.53 mg/100 g).

BAGCI *et al.* (2004) found that α -tocopherol (89.4%) was the major tocopherol in *T. cretica* (BAGCI *et al.* 2004). In another study, the content of α -tocopherol varied from 620 mg/kg to 910 mg/kg in *T. foenum-graecum* seeds (CİFTÇİ *et al.* 2011). ALJUHAİMİ *et al.* (2016) showed that fenugreek seeds also contained α -tocopherol (100.1 mg/100 g). PANT *et al.* (2017) determined that the α -tocopherol content of fenugreek seeds was 171.918 μ g/g. URAS GÜNGÖR & KÖKDİL (2018a) reported that *T. strangulata* seeds contained 188.78 mg/100 g of α -tocopherol and our group (GÜNGÖR *et al.* 2021) also

Table 4. Mineral content of *Trigonella cariensis* seeds

| Minerals | Symbol | μ g/g |
|--------------------------|--------|----------------|
| Macro minerals | | |
| Sodium | Na | 44540±3.09 |
| Magnesium | Mg | 1217.10±27.41 |
| Phosphorus | P | 3584.51±11.02 |
| Potassium | K | 14876.43±22.34 |
| Calcium | Ca | 4527.31±11.21 |
| Essential trace minerals | | |
| Chromium | Cr | 27.2±0.71 |
| Manganese | Mn | 11.63±0.11 |
| Iron | Fe | 174.21±2.44 |
| Nickel | Ni | 4.42±0.32 |
| Zinc | Zn | 43.57±2.25 |
| Copper | Cu | 7.82±0.06 |
| Selenium | Se | 8.12±0.07 |

Data presented as mean±SD (n=3)

Table 5. Antioxidant activity of *Trigonella cariensis* seeds

| Sample | DPPH [•] (%) | ABTS ^{•+} (%) | CUPRAC (mM AAE/g) |
|--------------------------|-----------------------|------------------------|-------------------|
| <i>T. cariensis</i> seed | 31.15±1.79 | 43.22±3.14 | 9.67±0.78 |

Values are expressed as the mean standard deviation (\pm SD) where (n=3)

found that α -tocopherol (3498.53 mg/kg) was the major tocopherol in *T. rhytidocarpa* seeds.

Tocopherols are one of the most effective antioxidants which prevent cardiovascular diseases, especially arteriosclerosis and cancer, through the protection of cell membranes from oxidation (CİFTÇİ *et al.* 2011; DİABY *et al.* 2016). Our results are in agreement with those mentioned above. However, *T. cariensis* had a higher content of α -tocopherol than *T. foenum-graecum*. This species can be recommended for human nutrition due to its high tocopherol content as well as its oil rich in unsaturated fatty acids.

Several studies are available on the phytosterol composition of *Trigonella* species. CİFTÇİ *et al.* (2011) found that the main sterol in fenugreek seeds was β -sitosterol in the range of 5.989 mg/kg to 6.684 mg/kg followed by campesterol. KIRALAN *et al.* (2017) reported that β -sitosterol was the major component of sterols in fenugreek seeds, followed by campesterol and δ -5-avenasterol. In our previous studies, β -sitosterol was found to be the main sterol in the seeds of *T. strangulata* (56.8±0.74%) and *T. rhytidocarpa* (36.53±0.31%) (URAS GÜNGÖR & KÖKDİL 2018a; GÜNGÖR *et al.* 2021).

Previous studies have shown that the main sterol in *Trigonella* species is β -sitosterol, which is compatible with the current result for *T. cariensis*. β -sitosterol exhibits certain biological activities such as promoting apoptosis, inhibiting cancer cell proliferation and exerting antioxidant effects by means of different mechanisms (BASKAR *et al.* 2010).

Amino acid composition. The presence of seventeen essential and non-essential amino acids were detected in the seeds and the results of the amino acid composition are given in Table 3. According to our analysis, glutamic and aspartic acids were determined as the major amino acids present in large amounts in the *T. cariensis* seeds. Lysine and leucine were the highest in essential amino acids with values of 2062 ± 0.03 and 1997 ± 0.03 mg/100 g, respectively. Among the non-essential amino acids detected in the seeds, glycine and arginine were also found in large amounts in addition to glutamic and aspartic acids.

In the literature on the amino acid composition of *Trigonella* species, it has been reported that the seeds of *T. foenum-graecum* are rich in glutamic and aspartic acids. There is a quantitative difference in these amino acids depending on the geographical source (FEYZI *et al.* 2015; ALJUHAIMI *et al.* 2018).

Trigonella strangulata and *T. rhytidocarpa* seeds from other *Trigonella* species have been reported to contain mainly glutamic acid, lysine, proline and arginine, while *T. kotschyi*, *T. cilicica* and *T. filipes* seeds are rich in glutamic and aspartic acids, lysine and leucine (URAS GÜNGÖR & KÖKDİL 2018a, b; GÜNGÖR *et al.* 2021).

The present results are similar to the findings previously reported for *T. foenum-graecum*, *T. strangulata*, *T. kotschyi*, *T. cilicica* and *T. filipes* seeds with some quantitative differences. Consequently, the results of this study are in line with the literature and *T. cariensis* seeds have the nutritional value required by the FAO for adults, especially in terms of the amount of lysine, leucine, isoleucine, valine and threonine (FEYZI *et al.* 2015).

Mineral analysis. The mineral analysis results showed that the seeds of *T. cariensis* contain macro minerals such as Na, K, Ca, P, and Mg, as well as essential trace minerals such as Fe, Zn, Cr, and Mn. The results are given in Table 4. Na and K were the dominant macro minerals followed by Ca, P and Mg. The seeds contain the highest amount of Fe with the value of 174.21 ± 2.44 μ g/g as an essential trace mineral.

While there are many studies on the mineral composition of fenugreek seeds in the literature, there are fewer studies on other *Trigonella* species. In recent research on fenugreek seed mineral composition, it was determined that the seeds contain potassium, calcium, phosphorus, magnesium and iron as the main minerals (SHAKUNTALA *et al.* 2011; AL-JASASS & AL-JASSER 2012; SINGH *et al.* 2013a, b; BIENKOWSKI *et al.* 2017; RAO & RAO 2018).

In another study, the mineral composition of different *Trigonella* species seeds was investigated and high potassium and calcium were detected in all of these species. The K and Ca amounts ranged from 11041.67 to 14727.67 μ g/g, and 2751.33 to 4925.67 μ g/g, respectively, while the amounts of phosphorus and magnesium were between 2335.33 and 4574.67, and 2477.33 and 3745.33 μ g/g, respectively. Phosphorus, magnesium and iron were present in descending order (URAS GÜNGÖR *et al.* 2017).

Trigonella cariensis seeds contain higher amounts of K and Mg than those reported for fenugreek seeds by SHAKUNTALA *et al.* (2011) and AL-JASASS & AL-JASSER (2012). In addition, in this study it was determined that the quantities of calcium and potassium in the seeds are compatible with those in other *Trigonella* species (URAS GÜNGÖR *et al.* 2017).

Total phenolic and total flavonoid contents and *in vitro* antioxidant activities

Total phenolic and total flavonoid contents. The total phenolic and flavonoid contents of the seeds were determined as 3.34 ± 0.02 mg GAE/g seed and 0.96 ± 0.09 mg QE/g seed, respectively. The total phenolic and flavonoid contents of 80% methanolic extracts of different *Trigonella* species (*T. spruneriana*, *T. sibthorpii*, *T. kotschyi*, *T. mesopotamica*, *T. cylindricea*, *T. cilicica*, *T. filipes*, *T. velutina*, *T. strangulata*, *T. smyrnea*, and *T. rhytidocarpa*) from Turkey have been studied previously and the results indicated that the total phenolic and flavonoid contents ranged from 35.47 to 201.35 mg GAE/g and 2.57–128.04 mg RE/g, respectively (URAS GÜNGÖR *et al.* 2017; GÜNGÖR *et al.* 2021) The total phenolic and flavonoid contents of methanolic extracts of *T. foenum-graecum* seeds from different geographical origins were in the range from 36.20–48.76 mg GAE/g and 2.73–4.76 mg QE/g, respectively (PANT *et al.* 2017; BABA *et al.* 2018).

In the current study, the total phenolic and flavonoid contents indicated that *T. cariensis* seeds contain phenolic and flavonoid compounds which enhance their antioxidant capacity.

***In vitro* antioxidant activities.** The DPPH scavenging assay showed that the extract had moderate scavenging activity at a concentration of 3 mg/mL with a value of $31.15 \pm 1.79\%$. The antioxidant activity was also determined using the ABTS and CUPRAC assays. The antioxidant capacity of the seed extract by employing the ABTS assay ($43.22 \pm 3.14\%$) was higher than that by the DPPH and CUPRAC methods (Table 5).

In recent years, the antioxidant activities of *T. foenum-graecum* and other species of the genus using DPPH, ABTS and/or CUPRAC assays have been reported in several studies (NORZIAH *et al.* 2015; URAS GÜNGÖR *et al.* 2017; BABA *et al.* 2018; HAMEED *et al.*

2019; GÜNGÖR *et al.* 2021). These studies revealed that the seeds of *Trigonella* species have different degrees of antioxidant activity. NORZIAH *et al.* (2015) investigated the antioxidant activity in fenugreek extracts prepared with different extraction solvents by the DPPH method and found that the 75% methanol extract showed 64.04% inhibition at 0.5 mg/mL concentration. In another study, with the exception of fenugreek, 80% methanol extracts of ten *Trigonella* species seeds were investigated by the DPPH method for antioxidant effects where it was determined that the species showed varying rates of inhibition 27.4-76.2% (URAS GÜNGÖR *et al.* 2017). The antioxidant activity of fenugreek seeds with methanol extract was measured using the DPPH method by BABA *et al.* (2018). Inhibition activity in the range of 26.7-39.8% was found in that study. HAMEED *et al.* (2019) determined the antioxidant activity of 70% extract of fenugreek seeds with DPPH (IC₅₀: 56.4 µg/mL) and ABTS (55.7 µmol TE/g). DPPH, CUPRAC and ABTS assays (85.88%, 401.6 µmolAAE/g seed and 420 µmol/g seed, respectively) of *T. rhytidocarpa* seeds were evaluated for the first time by our group (GÜNGÖR *et al.* 2021).

The fact that different methods and solvents were used in the extraction, and the results of these studies were presented in different units, makes it difficult to compare the results. Our findings are consistent with previous studies showing the antioxidant potential of *Trigonella* species.

CONCLUSIONS

This is the first report describing the fatty acid, tocopherol, sterol, amino acid and mineral contents, total phenolic and flavonoid contents, as well as the antioxidant activities of *T. cariensis* seeds. In conclusion, the results of the current study suggest that *T. cariensis* seeds have good nutritional value with high amounts of polyunsaturated fatty acids, α -tocopherol, β -sitosterol, some amino acids, minerals and total phenolic and flavonoid contents with antioxidant activities. The increasing use of natural compounds as antioxidant food stabilizers means that naturally abundant *T. cariensis* seeds can be a promising resource for the development of new therapeutic agents and food supplements for various industries. In addition, it can be considered as a notable species for further research in terms of its other chemical components and various biological activities.

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REZIME

Fitohemijski skrining i antioksidativna aktivnost semena vrste *Trigonella cariensis*

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Rod *Trigonella* (Fabaceae) se od davnina koristi u kulinarske i kliničke svrhe. Cilj ovog rada bio je da se po prvi put ispita hemijski sastav i *in vitro* antioksidativna aktivnost semena *Trigonella cariensis*. Seme je sakupljeno u provinciji Mersin, Turska. Hemijske analize su vršene hromatografskim metodama. Analize masnih kiselina, tokoferola i sterola su ispitivane na ekstraktu heksana, a takođe su ispitivane analize aminokiselina i minerala na semenima. Ukupan sadržaj fenola i flavonoida je meren pomoću Folin-Ciocalteu i $\text{Al}(\text{NO}_3)_3$ metoda, a *in vitro* antioksidativna aktivnost je ispitana različitim hemijskim testovima uključujući 1,1-difenil-2-pikrilhidrazil (DPPH[•]), Cu redukujuću antioksidativnu moć (CUPRAC) i 2,2'-azinobis(3-etilbenzotiazolin)-6-sulfonat (ABTS^{•+}) na svom 80% metanolnom ekstraktu. Pokazano je da semena sadrže uglavnom linoleinsku, α -linoleinsku i oleinsku kiselinu ($43.74 \pm 0.24\%$, $18.38 \pm 0.45\%$, i $10.89 \pm 0.71\%$, respektivno). Semena su pokazala visok sadržaj α -tokoferola (233.54 ± 2.48 mg/100 g). Glavni steroli su bili β -sitosterol, *delta*-5-avenasterol i kampesterol. Na, K, Ca, P, i Mg su bili predominantni minerali, dok su glutaminska kiselina, asparaginska kiselina i lizin (5801 ± 0.15 , 3629 ± 0.12 i 2062 ± 0.03 mg/100 g, respektivno) određene kao glavne aminokiseline u semenu. Ukupni sadržaj fenola i flavonoida bio je 3.34 ± 0.02 mg GAE/g semena i 0.96 ± 0.09 mg QE/g semena, respektivno. Rezultati ABTS^{•+}, DPPH[•] i CUPRAC analiza su: $43.22 \pm 3.14\%$, $31.15 \pm 1.79\%$ i 9.67 ± 0.78 mM AAE/g, respektivno. U zaključku, seme *T. cariensis* pruža hranljivu vrednost sa dobrim izvorom polinezasićenih masnih kiselina, α -tokoferola, β -sitosterola, nekih aminokiselina i minerala i lekovitih svojstava sa ukupnim sadržajem fenola i flavonoida i antioksidativnim delovanjem.

Ključne reči: *Trigonella cariensis*, hemijski sastav, masne kiseline, amino kiseline, DPPH, CUPRAC