Received: 25 April 2020 | Revised: 16 July 2020 | Accepted: 15 August 2020

DOI: 10.1111/jfbc.13463

FULL ARTICLE

Journal of Food Biochemistry WILEY

In vitro studies on different extracts of fenugreek (*Trigonella spruneriana* BOISS.): Phytochemical profile, antioxidant activity, and enzyme inhibition potential

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Abstract

The side effects of synthetic antioxidants make it necessary to find a natural alternative. Therefore, the current study investigates the potential of *T. spruneriana* as a new alternative in terms of natural bioactive components. In this context, antioxidant activity, enzyme inhibition, and phenolic compounds of different extracts including ethanol, methanol, ethyl acetate, and aqueous were identified. The results show that the ethyl acetate (113.59 ± 2.73 mg GAE/g) has the highest phenolic content, but ethanol extract has the highest scavenging activity for DPPH and TAC. The ethanol extract showed stronger inhibition on cholinesterase and α-amylase compared to other extracts. Besides, 12 bioactive compounds were characterized in *T. spruneriana* extracts by HPLC-DAD. Our findings support that *T. spruneriana* could be considered as a new source of active phytochemicals, as well as provide remarkable data on biological activities of some main enzymes playing role in the healing of hyperpigmentation, Alzheimer, and diabetes.

Practical applications

This study reports the total content, types and amounts of bioactive compounds and potential beneficial bioactivities of the different extracts of *T. spruneriana*. *Trigonella* is abundant in nature and spread over a wide geographical area, and is used in making cheese, pastries, spices, and sausages in different countries, as well as for antidiabetic purposes. *Trigonella* leaves are a good source of bioactive compounds that contain compounds like quercetin, catechin, cinnamic acid, and coumaric acid, along with it have also a high content of soluble fibers and is suggested for body weight control. Apart from being the first study conducted to point out the potential of *T. spruneriana* as being a natural food additive, this study also demonstrated its medicinal importance by revealing the anti-hyperpigmentation, antidiabetic, neuroprotective, and antioxidant properties of *T. spruneriana*.

KEYWORDS

antidiabetic, anti-hyperpigmentation, antioxidants, bioactive compounds, enzyme inhibition, *Trigonella*

1 | INTRODUCTION

Trigonella species known as fenugreek, belong to the family Fabaceae that contains about 135 species most of which spread in the Mediterranean, Europe, Asia, Africa, and North America (Mabberley, 1997). *Trigonella* species are the most abundant plants in the pasture composition and are an important forage plant with high protein content, especially in animal husbandry areas (Ranjbar & Zahra, 2016). The leaves of *Trigonella* are rich in a wide variety of minerals, vitamins, and especially choline, while the seeds contain high-fiber structures and proteins, as well as neutral lipids, glycolipids, and phospholipids, and also have an aromatic, galactogogue, and antibacterial effect (Shankaracharya, Anandaraman, & Natarajan, 1973; Srinivasan, 2006).

Trigonella species were used as medicinal plants since ancient ages and are rich in calcium, iron, carotenes, and phytochemicals (Bhanger, Bukhari, & Memon, 2008), along with being also a good source of soluble dietary fibers which is suggested for weight control programs (Burton-Freeman, 2000). Besides, the plant has high content of bioactive compounds such as gallic acid, caffeic acid, hydroxybenzoic acid, ferulic acid, myricetin, and kaempferol (Benziane, Acem, Aggad, & Abdali, 2019; Hussain, Suradkar, Javaid, Akram, & Parvez, 2016). Due to these beneficial ingredients and medicinal importance, *Trigonella* species can be a therapeutic agent and a good source of antioxidant dietary supplement. Many countries use this plant as spice and vegetable, particularly in Asian countries (Singh, Singh, Shukla, & Singh, 2010). Furthermore, it is used to add more flavor to cheese in Switzerland, make bread in Egypt, produce artificial maple syrup in Germany, used as an antidiabetic herb in Israel and in traditional pastry and sausage production in Turkey (Altuntaş, Özgöz, & Taşer, 2005; Rajagopalan, 1998).

Today's common lifestyle related diseases like diabetes, cancer, cardiovascular diseases, neurodegenerative diseases, hypertension, hyperlipidemia, and digestive disorders are leading to health problems in many countries. Synthetic food additives are known to have an impact on these health problems due to carcinogenic and toxic effects (Carocho, Barreiro, Morales, & Ferreira, 2014). Recently, the tendency toward the use of natural bioactive components as a food additive has increased because of its rapid recovery feature in the body (da Silva, Barreira, & Oliveira, 2016), and its long-term effects compared to synthetic food additives. In this regard, it has been shown that there is a strong relationship between reducing the risk of developing common diseases and dietary bioactive compounds (Genkinger, Platz, Hoffman, Comstock, & Helzlsouer, 2004; Willett, 2002).

Free radicals are molecules/molecular fragments that have unpaired free electron(s), derived from many different origins such as drug, ultraviolet light, hypoxia, ionizing radiation, cell metabolism (internal source), nutrient deprivation, and heavy metals. Excessive formation of free radicals causes oxidative stress. High levels of oxidative stress in living organisms damage biomolecules like proteins, DNA, and lipids (Chandra, Salman, Mohd, Sweety, & Ali, 2015). Therefore, it causes many diseases to occur, including neurodegenerative (Gandhi & Abramov, 2012), cardiovascular (Rochette et al., 2013), diabetes (Avalos-Soriano, la Cruz-Cordero, Rosado, & Garcia-Gasca, 2016), hypertension, and malignancies (Abas & Naguib, 2019). Antioxidants can prevent and reduce these diseases by inhibiting free radicals oxidating biological molecules (Joana Gil-Chávez et al., 2013).

Although many reports on *Trigonella* have been stated in the literature, yet, there are neither studies conducted on enzymes nor phytochemical compounds obtained from *T. spruneriana*. Studies on *T. spruneriana* are limited and generally focused on geographical distribution, morphology (Martin, Akan, Ekici, & Aytac, 2011), cytogenetics (Ranjbar & Zahra, 2016), and taxonomy (Khandani, Assadi, Nejadsatari, & Mehregan, 2016). The purpose of current study was to investigate amounts of phytochemicals (total phenolics and flavonoids), antioxidant properties, enzyme-inhibiting activities, and main profile of phenolic compounds of different *T. spruneriana* extracts.

2 | MATERIALS AND METHODS

2.1 | Chemicals

All chemicals used to determine antioxidant and enzyme inhibition activities were analytical grade and obtained from Sigma Chemical Co. (Sigma-Aldrich St. Louis, MO, USA).

2.2 | Plant materials

The aerial parts of *T. spruneriana* were collected from Karaoren Village (Aksaray-Turkey) during the vegetation period in February 2015 and were identified by botanist experts Assist. Prof. Dr. Bulent Eskin and Dr. Mustafa Keskin.

2.3 | Preparation of extracts

T. spruneriana samples were pressed and air-dried in the dark at room temperature. Fully dried samples were milled using a commercial blender. Powdered samples were weighed in 15 g each in order to obtain MeOH, EtOH, EA, and AQ extract. The MeOH, EtOH and EA extracts were subjected to extraction using the a Soxhlet apparatus for 6–8 hr, while the AQ extract was performed by steeping in boiling water for 30 min. Then, the extracts were filtered using Whatman filter paper and the solvent was evaporated completely at 40–50°C with a Rotary Evaporator under vacuum (Aktumsek, Zengin, Guler, Cakmak, & Duran, 2013). Stock solutions were prepared from these extracts as 2 mg/ml prior to analysis.

2.4 | Profile of bioactive compounds

The contents of total bioactive substance as phenolics (TPC) and flavonoids (TFC) of different extracts from *T. spruneriana* were

TABLE 1 Total phenolic and flavonoid contents, ferric ion reducing power activity, and total antioxidant capacity of the obtained different extracts from *T. spruneriana*

Extracts	TPC[a] (mg GAE/g extract)	TFC[b] (mg QE/g extract)	FRAP[c] (mg TE/g extract)	TAC[d] (mg TE/g extract)
MeOH	35.41 ± 3.37[e]	89.90 ± 0.53	78.95 ± 1.14	47.00 ± 2.98
EtOH	55.64 ± 1.45	30.08 ± 1.14	77.57 ± 3.83	65.75 ± 3.25
EA	113.59 ± 2.73	8.69 ± 0.68	75.64 ± 4.98	62.62 ± 2.21
AQ	18.59 ± 2.73	19.70 ± 0.88	45.60 ± 0.83	15.04 ± 0.89

[a]TPC expressed as gallic acid equivalent (mg GAE g^{-1} extract).
[b]TFC expressed as quercetin equivalents (mg QE g^{-1} extract).
[c]FRAP expressed as trolox equivalents (mg TE g^{-1} extract).
[d]TAC expressed as trolox equivalents (mg TE g^{-1} extract).
[e]Values expressed are means ± *SD*.

analyzed spectrophotometrically by using Beckman Coulter DU 730, LifeScience UV/VIS Spectrophotometer. The results were calculated as equivalents of gallic acid and quercetin for TPC and TFC, respectively (Uysal et al., 2017).

2.5 | Antioxidant activity

The antioxidant activities of the obtained plant extracts were determined by DPPH radical-scavenging activity, ferric ion reducing antioxidant power (FRAP), and total antioxidant capacity (TAC) (by phosphomolybdate assay) analyzes (Uysal et al., 2017). The findings are expressed as trolox equivalent for both FRAP and TAC. Also, in DPPH analysis, IC_{50} values which are scavenging concentration to 50% of free radicals were calculated using concentration-inhibition graphics for each extract.

2.6 | HPLC-DAD analysis of phenolic compounds

The phenolic compounds of *T. spruneriana* extracts were analyzed in HPLC (Agilent 1,290 Infinity) equipped with a C18 column and diode-array detector (DAD) as detailed in an earlier study (Caponio, Alloggio, & Gomes, 1999). We used as mobile phase 3% of acetic acid (A) and MeOH (B). The following gradient program was applied: 95% A/5% B for 3 min, 80% A/20% B for 15 min, isocratic for 2 min, 60% A/40% B for 10 min, 50% A/50% B for 10 min and until the finish 100% B (10 min). The *T. spruneriana* extracts were prepared in methanol with a concentration of 20 mg/ml and the injection volumes were 10 μl.

2.7 | In vitro enzyme inhibition activities

Potential enzyme inhibition activities of obtained extracts from *T. spruneriana* with different solvents were successfully tested on α-amylase, α-glucosidase, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase enzymes. The potential antienzymatic capabilities of *T. spruneriana* extracts were performed as previously reported by Uysal et al. (2017). Enzyme inhibition abilities of extracts were denoted as the equivalent of galantamine (GALAE) for AChE and BChE, acarbose (ACAE) for α-amylase, α-glucosidase, and kojic acid (KAE) for tyrosinase. The explanation of enzyme inhibition assays is given in detail in our previous study (Uysal et al., 2017).

2.8 | Statistical analysis

All assays were carried out in three replicates and the obtained results were denoted as mean ± standard deviation (*SD*). The obtained data were analyzed using SPPS 26 software (Chicago, IL, USA) and Analysis-it software Excel Tool Pak (Leeds, LSE 1HS, UK). One-way analysis of variance was conducted to see whether there is a statistical significance. $p < .05$ was considered as significant. Also, Pearson and Spearman correlation coefficients were calculated to ascertain the relationship between total bioactive contents and biological activities of extracts.

3 | RESULTS AND DISCUSSION

3.1 | TPC

Phenolics are among the most important biologically active compounds that contain phenol rings in their structures and are natural compounds with anticancer, antidiabetic, antioxidant, and antimicrobial effects. According to the results given in Table 1, the TPC of *T. spruneriana* extracts ranged from 18.59 to 113.59 mg GAE/g extract, and maximum TPC was found in EA extract (113.59 mg GAE/g extract) followed by EtOH, MeOH, and AQ extracts, respectively. It is known that different solvents used for extraction have a significant effect on the tested plant extracts. The recovery of phenolic compounds of the *T. spruneriana* plant was recorded in the best EA solvent and this indicates that the TFC of the *T. spruneriana* plant was extracted better in semi-polar solvents such as EA.

Previous studies on various plant species have also reported that phenolic levels differed based on the use of different solvents (Alothman, Bhat, & Karim, 2009; Rusak, Komes, Likić, Horžić, & Kovač, 2008). As of the current date, there is only one study in the literature on the TPC of *T. spruneriana* and the TPC in MeOH

extract was found as 83.46 mg GAE/g extract (Uras Güngör, İlçim, & Kökdil, 2017). In the present study, the TPC of MeOH extract was measured as 35.41 mg GAE/g extract. In our study, different values of TPC can be attributed to various factors such as environmental and genetic (Fratianni, Tucci, De Palma, Pepe, & Nazzaro, 2007). In a study on *Trigonella monantha* (Esmaeili, Rashidi, & Rezazadeh, 2012), the TPC of the plant was found lower in MeOH extract (22.36 mg GAE/g) compared to our findings. Chatterjee, Variyar, and Sharma (2009) investigated the TPC of *Trigonella foenum* and demonstrated that the phenolic content was in the range of 81–84 mg GAE equivalent per dry weight. Many studies indicate that phenolic compounds could make significant contributions to antioxidant action and, thus, plants having high amounts of phenolic compounds may be used as natural antioxidant sources. The results of this work support that the TPC of *T. spruneriana* extracts was found to vary significantly based on the type of solvent used.

3.2 | TFC

Flavonoids are common compounds found in herbal food, derived from metabolites such as carbohydrates and amino acids, needed for the vital activities of plants. Furthermore, flavonoids have many pharmacological features, and they have many positive effects on health (CF Bodewes et al., 2011). In the results given in Table 1, the highest TFC was seen in MeOH extract (89.90 mg QE/g), while the lowest flavonoid content was determined in EA extract (8.69 mg QE/g). Studies on the TFC of *Trigonella* species are quite limited in the literature. Uras Güngör et al. (2017) researched the TFC of different *Trigonella* species and reported the TFC value of *T. spruneriana* as 62.85 mg RE/g in MeOH extract. The results obtained in this study were found to be in the same range as of the results obtained in other studies on different *Trigonella* species (Esmaeili et al., 2012; Güngör, Güzel, İlçim, & Kökdil, 2014; Jaradat, Shawahna, Hussein, & Al-Lahham, 2016). As in the TPC, the TFC values were also significantly affected depending on the type of the solvents used. In the correlation analysis on the content of total bioactive substance of *T. spruneriana* extracts, a moderately negative correlation ($r = -.460$) was found between TPC and TFC.

3.3 | DPPH assay

Measurement of free radical-scavenging activity of *T. spruneriana* was detected using the DPPH assay. The obtained results are given in Figure 1. Accordingly, MeOH and EtOH extracts showed higher inhibition rates compared to other extracts and DPPH radical scavenging activity between both fractions is not significantly different. The minimum activity was determined in the AQ extract. This may be due to the low phenolic content of the AQ extract. In a previously reported study, *T. spruneriana* showed 21.11% inhibition of DPPH radical in MeOH extract (Uras Güngör et al., 2017). According to the reported results by Jaradat et al. (2016), it was measured that MeOH extracts of *Trigonella arabica* and *Trigonella berythea* showed 23.2% and 48.3% inhibition, respectively. Additionally, in the statistical analysis applied to explain the relationship between DPPH radical-scavenging activity of total bioactive compounds, there is a direct relationship between TPC and DPPH radical scavenging antioxidant activity at 200 μg/ml ($p < .05$). Interestingly, no direct relationship was found between TPC and DPPH at concentrations of 500 and 1,000 μg/ml. Contrarily, there was no significant relationship between TFC and DPPH in the studied concentrations.

In this method, IC_{50} values were also calculated for each extract and standard substance butylated hydroxytoluene (BHT), which is the concentration that removes 50% of the DPPH radical. Based on these values, the highest activity was observed in EtOH extract with 3.87 ± 0.09 mg/ml. The IC_{50} value of BHT is 0.03 ± 0.38 mg/ml. MeOH, EA and AQ extracts were determined 4.00 ± 0.07 mg/ml, 4.51 ± 0.27 mg/ml, and 5.28 ± 0.58 mg/ml, respectively. The IC_{50} values obtained in the present study were found moderate or relatively higher in free radical-scavenging activity compared to previous studies on *Trigonella* species (Jaradat et al., 2016; Kenny, Smyth, Hewage, & Brunton, 2013).

3.4 | FRAP assay

Because the antioxidant activity of a substance is generally related to its reducing capacity the FRAP analysis ensures a fast and reliable way to evaluate the antioxidant activity of the compounds. To this end, the

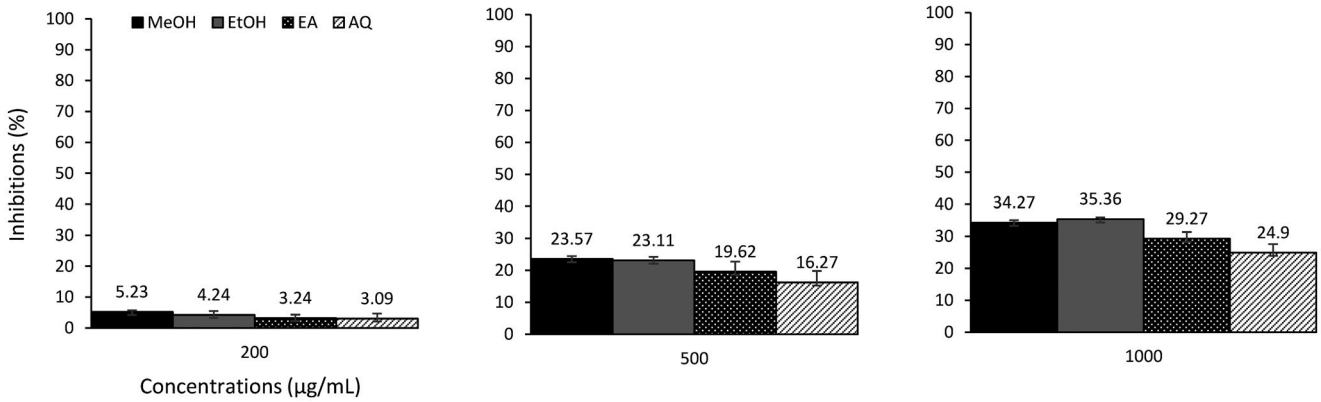

FIGURE 1 DPPH radical scavenging activity of MeOH, EtOH, EA and AQ extract of *T. spruneriana* in different concentrations

FRAP ($Fe^{3+} \rightarrow Fe^{2+}$) analysis was performed to evaluate the electron donor power of *T. spruneriana* extracts. In FRAP analysis, antioxidant substances in the plant cause a reducing of $TPTZ/Fe^{3+}$ – $TPTZ/Fe^{2+}$ and the formed iron complex can be observed by quantifying blue color at 593 nm (Huang, Ou, & Prior, 2005). The reduction activities of MeOH, EtOH, EA, and AQ extracts of *T. spruneriana* are given in Table 1. A trend similar to DPPH radical scavenging analysis of different extracts appeared in FRAP analysis. Accordingly, the highest reducing power activity was determined in MeOH (78.95 mg TE/g extract) and EtOH (77.57 mg TE/g extract) having a value very close to it. These were followed by EA (75.64 mg TE/g extract), and the lowest value was determined in AQ extract (45.60 mg TE/g extract).

Considering FRAP and also DPPH results, the polar components of *T. spruneriana* extracts exhibited higher antioxidant activity in polar solvents such as MeOH and EtOH. This may be due to the dissolution of antioxidant compounds with different polarities in different solvents (Gong et al., 2012; Sultana, Anwar, & Ashraf, 2009). Therefore, when the antioxidant capacity of plant extracts is determined, the used solvents are an important factor and this may need to be taken into consideration. The results of the current study were higher than those obtained by Premanath, Sudisha, Lakshmi Devi, and Aradhya (2011) on *Trigonella foenum graecum* L. Given the FRAP results shown in Table 1 demonstrated that extracts in terms of higher the TFC values showed high ferric reduction activity, generally. In the correlation analysis performed, a strong correlation was observed between TFC and FRAP. This situation has been stated in previous studies that there is a relationship between TFC and TPC and reducing power (Sathisha, Lingaraju, & Prasad, 2011; Smeriglio et al., 2016).

3.5 | TAC

In the phosphomolybdenum determination of four different extracts of *T. spruneriana*, total antioxidant activities varied between 65.75 and 15.04 mg TE/g extract. The maximum action was observed in EtOH extract followed by EA, MeOH, and AQ extracts, respectively (Table 1). Gupta and Prakash (2009) emphasized the TAC of *Trigonella graecum* as 1.294.78 μmol ascorbic acid/g sample. In another study, the TAC of *Trigonella hamosa* (IC_{50} = 25.82 μg/mL) was determined (Shahat, Ibrahim, & Alsaid, 2015). In addition, some authors have reported a poor relation between TPC and TFC and phosphomolybdenum analysis (Nićiforović et al., 2010; Sarikurkcu, Uren, Uren, Tepe, Cengiz, & Kocak, 2015). Similarly, a poor correlation was obtained between TFC and phosphomolybdenum assay statistically. On the contrary, a significant positive correlation was found between methods of TPC and TAC.

3.6 | HPLC-DAD analysis of *T. spruneriana*

The Folin method is a rapid and reliable one which is frequently used in comparing the same samples that gives basic information about phytochemicals. However, this method does not provide a complete information on the quantity nor quality of bioactive components in mixed test material. Hence, the identification of phenolic acids and flavonoids of different fractions (MeOH, EtOH, EA, and AQ) of *T. spruneriana* was determined using HPLC-DAD and the obtained results are given in Table 2. The obtained results from the analysis of *T. spruneriana* extracts exposed the existence of 12 compounds, including caffeic acid, gallic acid, epicatechin, and quercetin.

Quercetin was the most abundant component in *T. spruneriana* extracts. The presence of quercetin has been demonstrated in many studies and has been used in traditional medicine for many diseases like cancer (Murakami, Ashida, & Terao, 2008), chronic inflammation (García-Mediavilla et al., 2007), cardiovascular (Shankar, Singh, & Srivastava, 2007), and obesity (Yang et al., 2008) due to its therapeutic features. Some authors have claimed that quercetin is an antioxidant (Karbarz & Malyszko, 2008). On the contrary, some authors have reported that quercetin in plant plays a role in nutrient uptake, mycorrhizal network formation, and defense mechanism (Gholami, De Geyter, Pollier, Goormachtig, & Goossens, 2014). After quercetin, the most abundant compounds in the plant were catechin, hydroxybenzoic acid, and cinnamic acid, respectively. Additionally, coumaric, cinnamic, and chlorogenic acid were observed in all tested extracts. Chlorogenic acid, a dietary polyphenol found in all extracts, has been shown to have a powerful role in the arrangement of the body's blood sugar homeostasis and dermal hyperpigmentation in previous studies (Fukushima et al., 2015).

In this study, chlorogenic acid, coumaric acid, gallic acid, caffeic acid, syringic acid, and t-ferulic acid were identified, and also EtOH extract can be interpreted as the richest extract in terms of bioactive compounds. But it was not so in terms of total phenolic and flavonoid content. Although many studies in the literature report that the high antioxidant activity of foods, beverages, or different plant extracts are directly related to their high phenolic content (Benabdallah, Rahmoune, Boumendjel, Aissi, & Messaoud, 2016; Do et al., 2014), there are also reports stating that this is not always the case (Al-Musayeib, Perveen, Fatima, Nasir, & Hussain, 2011; Aryal et al., 2019; Kähkönen et al., 1999). In the Folin–Ciocalteu assay, phenolic compounds are known to exhibit different antioxidant responses depending on their molecular structure. The hydrogen atoms of adjacent hydroxyl groups (o-diphenol) at different positions of the rings (A, B, and C) in the compound, the double bonds of the benzene ring and the double bond of the oxo functional group (-C=O) of some flavonoids ensure them with high antioxidant activity and this situation can be seen clearly in quercetin and catechin (Figure 2) (Minatel et al., 2017). Quercetin and catechin have similar hydroxyl groups at the same positions, but quercetin has a 2,3-double bond the C ring and the 4-oxo function, which allows quercetin to have higher antioxidant activity compared to the saturated heterocyclic ring of catechin (Rice-Evans, Miller, & Paganga, 1996). Considering the results of HPLC in our study, catechin (6774.73 μg/g extract), and quercetin (3144.15 μg/g extract) were found in EtOH extract with the highest values. The EtOH extract had a lower TPC value compared to MeOH and EA extracts but showed higher antioxidant

TABLE 2 Polyphenolic compounds in different extracts of *T. spruneriana* (μg/g extract)

Phenolic components	Extracts			
	MeOH	EtOH	EA	AQ
Gallic acid	18.51	48.03	nd	34.59
Catechin	nd	6774.73	4460.25	201.66
Chlorogenic Acid	49.33	261.65	98.27	33.94
Caffeic acid	nd	nd	nd	63.68
Hydroxybenzoic acid	nd	nd	nd	1118.79
Epicatechin	nd	73.99	nd	nd
Syringic acid	53.18	36.49	nd	nd
Coumaric acid	97.47	206.54	171.69	138.35
Ferulic acid	nd	416.56	199.09	nd
Sinapic acid	241.50	nd	nd	nd
Cinnamic acid	192.93	283.09	156.43	84.65
Quercetin	1489.86	3144.15	1800.01	nd

Abbreviation: nd, not detected.

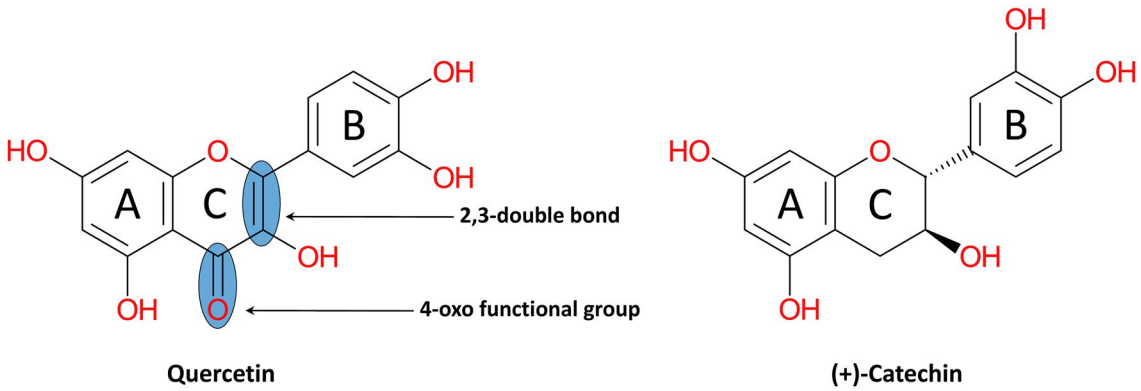

FIGURE 2 2,3-Double bond in the C ring and the 4-oxo function

TABLE 3 Enzyme inhibitory properties of the tested extracts

Extracts	Cholinesterases inhibition		Antidiabetic assay		Hyperpigmentation
	AChE Inhibition (mg GALAE/g)	BChE Inhibition (mg GALAE/g)	α-amylase inhibition (mmol ACAE/g)	α-glucosidase inhibition (mmol ACAE/g)	Tyrosinase inhibition (mg KAE/g)
MeOH	4.04 ± 0.18	5.67 ± 0.70	0.93 ± 0.01	1.81 ± 0.04	124.66 ± 0.53
EtOH	5.22 ± 0.01	17.22 ± 0.31	0.98 ± 0.04	1.63 ± 0.08	122.72 ± 1.81
EA	3.69 ± 0.34	12.30 ± 1.00	0.96 ± 0.02	1.94 ± 0.01	117.74 ± 1.52
AQ	na	na	0.20 ± 0.01	0.07 ± 0.01	na

Note: Values expressed are means ± *SD* of three parallel measurements.

Abbreviations: ACAE, Acarbose equivalent; GALAE, Galantamine equivalent; KAE, Kojic acid equivalent; na, not active.

activity. This can be attributed to the variety and amount of phenolic compounds such as quercetin and catechin, which are found in EtOH extract and can show higher antioxidant activity.

In previous studies on *Trigonella* species, the presence of these compounds has been mentioned and this confirms our current study (Hussain et al., 2016; Kenny et al., 2013). According to the results obtained in our study, different phenolic compounds were determined in variable amounts in MeOH, EtOH, EA, and AQ extracts of *T. spruneriana*, and it was observed that the use of different extracts affected the quantity of phenolic compounds.

3.7 | In vitro enzyme inhibitory properties

Inhibition of certain enzymes is considered as an important therapeutic tactic to struggle with many health problems and is important in controlling inappropriate body functions (Zengin et al., 2019). Cholinesterases are significant enzymes responsible for serious chronic diseases like Alzheimer's disease (AD). AChE inhibition play an important role for the control of AD because AChE inhibitors, which can increase cholinergic transmission by precluding the degradation of acetylcholine, are used to mitigate symptoms of people with AD (Nisa et al., 2017). According to the results given in Table 3, all extracts except AQ showed good inhibitory effect against both AChE and BChE. The EtOH extract exhibited maximum inhibition (17.22 ± 0.31 mg GALAE/g) against BChE, followed by EA extract (12.30 ± 1.00 mg GALAE/g). Enzyme inhibition activities of these extracts may be based on their bioactive content as reported in previous studies (Kennedy & Wightman, 2011; Mazlan et al., 2013). According to the data provided by Satheeshkumar, Mukherjee, Bhadra, and Saha (2010), *Trigonella foenum graecum* L. was able to inhibit AChE with an IC_{50} equal to 53.00 ± 17.33 μg/mL.

Controlling hyperglycemia aims to reduce the levels of blood glucose by inhibitory properties of the two main enzymes α-amylase and α-glucosidase in the carbohydrate metabolism (Kumar, Mehta, Satija, & Garg, 2013). All *Trigonella* extracts exhibited the inhibitory properties for the enzymes of α-amylase and α-glucosidase. Although the plant extracts showed significant inhibition against α-glucosidase (1.94 ± 0.01, 1.81 ± 0.04, and 1.63 ± 0.08 mmol ACAE/g for EA, MeOH, and EtOH, respectively), a slight inhibition against α-amylase (0.96 ± 0.02, 0.93 ± 0.01, and 0.98 ± 0.04 mmol ACAE/g for EA, MeOH, and EtOH, respectively). In addition, the AQ extract showed poor inhibition for both enzymes. Studies on *T. foenum graecum* from the same genus with *T. spruneriana* have been reported to have low levels of the α-amylase inhibitor (Kumar et al., 2013; Narkhede, 2012). The existence of phenolic compounds in *T. spruneriana* may be responsible for α-amylase and α-glucosidase enzyme inhibitory activity, and this situation has mentioned in the previous studies (Kennedy & Wightman, 2011; Shang et al., 1998). At the same time, both α-amylase and α-glucosidase enzyme inhibitory activities were found to be statistically significant ($p < .05$) with TPC and TFC.

Inhibition of tyrosinase, which is associated with skin hyperpigmentation disorders, has become a strategy to prevent excess production/accumulation of melanin in the skin. Due to the negative impacts of synthetic anti-tyrosinase agents, investigation of naturally derived tyrosinase inhibitors is required (Sarikurkcu, Zengin, et al., 2015). In this study, the inhibition of *T. spruneriana* extracts against tyrosinase enzyme was tested. Based on the results given in Table 3, maximum inhibition activity against tyrosinase enzyme was determined in MeOH extract (124.66 ± 0.53 mg KAE/g), followed by EtOH (122.72 ± 1.81 mg KAE/g), and EA extract (117.74 ± 1.52 mg KAE/g), respectively, yet, the AQ extract showed no inhibitory activity. On the contrary, Vaibhav and Lakshaman (2012) reported that leaves and seeds of *T. foenum graceum* MeOH and AQ extracts had anti-tyrosinase activity between 4.60% and 16.15%.

4 | CONCLUSION

This study reports the phytochemical composition and antioxidant activities of the different extracts of the *T. spruneriana* plant. The study also is the first endeavor to investigate the enzyme inhibition activities that are closely related to common diseases such as AD, diabetes and hyperpigmentation. Based on our findings, the used different solvents like MeOH, EtOH, EA, and AQ for extraction were found to have a significant effect on total phenolic and flavonoid contents. Also, the bioactive compounds present in *T. spruneriana* generally showed higher antioxidant activity in polar solvents like EtOH and MeOH. It was highlighted that an extract with the highest total phenolic content does not always exhibit the highest biological activity, and this may be due to the variety of phenolic compounds and their quantities. Besides, *T. spruneriana* had a significant effect on all tested enzymes, and hence, can be seen as a potential source for future phyto-pharmaceuticals studies.

ACKNOWLEDGMENTS

We would like to thank botanist experts Dr. Mustafa Keskin for identifying the *T. spruneriana* plant.

AUTHOR CONTRIBUTION

Volkan Aylanc: Investigation; Methodology; Writing-original draft; Writing-review & editing. **Bulent Eskin:** Formal analysis; Resources; Writing-original draft. **Gokhan Zengin:** Conceptualization; Formal analysis; Investigation; Methodology; Writing-original draft; Writing-review & editing. **Mehmet Dursun:** Formal analysis; Methodology; Visualization; Writing-original draft. **Yavuz Selim Cakmak:** Formal analysis; Investigation; Methodology; Project administration; Resources; Supervision; Writing-original draft; Writing-review & editing.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Aylanc V, Eskin B, Zengin G, Dursun M, Cakmak YS. In vitro studies on different extracts of fenugreek (*Trigonella spruneriana* BOISS.): Phytochemical profile, antioxidant activity, and enzyme inhibition potential. *J Food Biochem*. 2020;00:e13463. https://doi.org/10.1111/jfbc.13463