

Research Article

## Study of variability in agro-morphological traits, proximate composition, and phenolic compounds of some *Trigonella* L. species in Iran

Ziba Bakhtiar<sup>1</sup>, Mohammad Reza Hassandokht<sup>1,\*</sup>, Mohammad Reza Naghavi<sup>2</sup>, Mohammad Hossein Mirjalili<sup>3</sup>

<sup>1</sup> Department of Horticultural Sciences, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

<sup>2</sup> Division of Biotechnology, Department of Agronomy and Plant Breeding, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

<sup>3</sup> Department of Agriculture, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, 1983969411, Tehran, Iran

---

### ARTICLE INFO

Keywords:

Diversity  
Fabaceae  
Fenugreek  
Herb  
Nutritive value  
Phenol  
Spice

---

### ABSTRACT

**Background:** *Trigonella* L. is a well-known worldwide growing genus of the Fabaceae family. In addition to food, the plants have various medicinal properties, including reducing fat and blood sugar, antibacterial, anti-ulcer, and analgesic. **Objective:** Variability in agro-morphological traits, phenolic compounds, and proximate composition of the aerial parts of some Iranian *Trigonella* and its relative e.g. *Medicago* L. species was studied to introduce the superior species for further use in breeding and exploitation programs. **Methods:** The seed samples of *T. astroides*, *T. elliptica*, *T. filipes*, *T. foenum-graecum*, *T. spruneri*, *M. crassipes* (syn: *T. crassipes*), *M. monantha* (syn: *T. monantha*), *M. monspeliaca* (syn: *T. monspeliaca*), *M. orthoceras* (syn: *T. orthoceras*), and *M. phrygia* (syn: *T. aurantiaca*) were collected from different regions of Iran, and then were cultivated at the same agronomic conditions. Morphological traits were measured by a ruler, digital caliper and scales. Proximate composition (moisture, ash, fibre, protein, fat, and carbohydrates) and phenolic acids of the aerial parts were determined by the AOAC method and HPLC, respectively. **Results:** Significant morphological differences among the studied species were observed. The highest protein content was measured in *M. orthoceras* ( $13.4 \pm 0.4\%$ ), followed by *T. filipes* ( $11.5 \pm 0.8\%$ ), and *T. spruneri* ( $11.1 \pm 0.4\%$ ). Fibre content was ranged from  $0.7 \pm 0.04\%$  to  $4.9 \pm 0.4\%$ . In the extracts of the studied species, catechin, chlorogenic acid, and *p*-coumaric acid were found in abundance ( $0.01 \pm 0.05$  -  $69.32 \pm 0.7$  mg/g DW). **Conclusion:** *Trigonella filipes* and *T. spruneri* could be selected as suitable species for further exploitation in food and pharmaceutical industries.

---

Abbreviations: Syn, Synonym; HPLC, High-performance liquid chromatography; PCA, Principal component analysis

\*Corresponding author: [mrhassan@ut.ac.ir](mailto:mrhassan@ut.ac.ir)

doi: 10.52547/jmp.21.82.1

Received 8 April 2022; Received in revised form 22 May 2022; Accepted 24 May 2022

© 2020. Open access. This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<https://creativecommons.org/licenses/by-nc/4.0/>)

## 1. Introduction

Due to the prevalence of various cardiovascular diseases and diabetes, today's observance of diet and changing consumption patterns in human societies has become prominent, so the consumption of protein has changed from dairy and meat to other types of food such as cereals, legumes and vegetables [1]. Also, people's appetite for fruits, vegetables, grains, and legumes has increased dramatically due to the presence of natural antioxidants containing phenolic compounds in the plants.

The most common phenolic compounds are phenolic acids, flavonoids, anthocyanins, coumarins, and lignins. To date, more than ten thousand phenolic compounds have been isolated and identified from plants. Many biological activities of phenolic compounds including antioxidant, anti-inflammatory, anticancer, and antidiabetic properties have been reported so far [2, 3]. Phenolic compounds are the most abundant in some families such as Lamiaceae, Boraginaceae, Rhamnaceae, Rubiaceae, and Fabaceae [4, 5].

Fabaceae is one of the largest families of flowering plants with 750 genera and about 19,000 species, whose representatives are found on all continents, from the hot and humid regions of Asia to the cold steppes of Siberia; from the savanna of Africa to the mountains of South America [6].

The genus *Trigonella* L., with more than 60 species, is a well-known genus of the Fabaceae family that grows worldwide from Australia to the Canary Islands [7]. The fresh and dried leaves of the plants, especially fenugreek (*Trigonella foenum-graecum* L.), are used as aromatic and spice plants in food recipes and preparations. The plants contain protein, carbohydrates, unsaturated fatty acids, minerals,

and a variety of vitamins [8]. Anticancer, antibacterial, anti-gastric, and anti-diabetic properties of these plants have also been reported [9, 10]. The plant has been cultivated and exploited in different parts of the world for a long time. So, the selection of adequate species for further exploitation in food and pharmaceutical industries is needed. The study of morphological characteristics is one of the first steps in breeding programs to select the desirable characteristics used by plant breeders [11]. A literature survey revealed that the morphological traits and proximate composition of *T. foenum-graecum* have been extensively studied [12-15], however, similar studies especially variations in the phenolic compounds in the other *Trigonella* species are limited [16, 17]. However, the results of studies showed that some species of *Trigonella* have high levels of phenolic compounds and are highly active against free radicals [7, 17]. Due to the increasing trend of cancer in the world, it seems necessary to provide natural antioxidant reserves to replace synthetic antioxidants in the pharmaceutical and food industries. Some *Tigonella* species has now been transferred to the genus *Medicago* L. In the present study, morphological variability, proximate composition, and phenolic compounds of some Iranian *Trigonella* and its relative e.g. *Medicago* species were performed. Our findings can be considered for further use in breeding and exploitation programs.

## 2. Materials and Methods

### 2.1. Chemical material and reagents

All standards with 96 % purity were purchased from Sigma Aldrich Company (Buchs, Switzerland). HPLC grade methanol and *n*-hexane were purchased from Merck

Company (Darmstadt, Germany). Deionized water was obtained from the Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA).

## 2.2. Plant material

Seeds of the ten *Trigonella* or *Medicago* species including *T. astroides*, *T. elliptica*, *T. filipes*, *T. foenum-graecum*, *T. sprunneriana*, *M. crassipes* (syn: *T. crassipes*), *M. monantha* (syn: *T. monantha*), *M. monspeliaca* (syn: *T. monspeliaca*), *M. orthoceras* (syn: *T. orthoceras*), and *M. phrygia* were collected from Khuzistan-Mollasani, East Azerbaijan-Mianeh, Ilam-Salehabad, Razavi Khorasan-Mashhad, Fars-Dehbozorgi, Hamedan-Kabudarahang, Kerman-Jiroft, Golestan-Gonbad, Sistan va Baluchestan-Kaslouch, and Isfahan-Khour va Biabanak, respectively.

The seeds were then sown in the field research at the Horticultural Research Station at the University of Tehran, Karaj, Iran ( $N35^{\circ} 46' 47''$ ,  $E50^{\circ} 55' 2''$  at an altitude of 1320 m) in a randomized complete block design in May 2020. The minimum and maximum yearly temperatures were -20 and 42 °C, respectively, and the average yearly precipitation was equal to 247.3 mm. Agronomical practices including weed management and irrigation were carried out for all the studied species. Voucher specimens of *T. astroides* (HIAK-6567), *T. elliptica* (HIAK-6529), *T. filipes* (HIAK-6531), *T. foenum-graecum* (HIAK-6512), *T. sprunneriana* (HIAK-6582), *M. crassipes* (HIAK-6528), *M. monantha* (HIAK-6578), *M. monspeliaca* (HIAK-6580), *M. orthoceras* (HIAK-6581), and *M. phrygia* (HIAK-6571) have been deposited at the Herbarium of College of Agriculture and Natural Resources (Herbarium Agronomici Keredjensis) (HIAK), University of Tehran, Karaj, Iran.

## 2.3. Phenotypic analysis

The plants were harvested at the vegetative stage (50 days after planting). Twelve morphological traits such as plant height, canopy width, stem diameter, internode length, petiole diameter, petiole length, leaf length, and leaf width were determined from fresh materials. Dimensions (length, width and diameter) for leaves were measured by a digital caliper. Morphological evaluations were based on 36 replicates and the mean values were used.

## 2.4. Proximate analysis

Fresh leaves of ten studied *Trigonella* or *Medicago* species were examined for moisture, ash, fibre, protein, fat, and carbohydrate content using the methods described by AOAC [18]. To measure moisture and ash content, the samples were dried in the oven at 105 °C for 3 h and burnt to ashes in a muffle furnace at 550 °C for 24 h, respectively. To obtain fibre, leave samples were boiled sequentially with dilute acid and alkali, and then sequentially washed with ethanol and diethyl ether, and the residue was subtracted by its ash. The nitrogen content of the samples was determined by the micro-Kjeldahl method. Fat was extracted from powdered samples in a soxhlet extractor with *n*-hexane for 6 hours at 80 °C. The carbohydrate content was determined by calculation using the different methods:

$$\% \text{ Total Carbohydrate} = [100 - \% (\text{Moisture} + \text{Ash} + \text{Fibre} + \text{Protein} + \text{Fat})]$$

The values of proximate composition were on a fresh matter basis and expressed as percentages (%).

## 2.5. Extraction and quantification of phenolic compounds

For the phytochemical analysis, the plants were harvested at vegetative stage (50 days after

planting). Phenolic compounds extraction was performed as described previously with minor modifications [17]. Briefly, 500 mg of dried leaves of each sample was drenched in 20 ml methanol for 24 h at room temperature. The samples were filtered using a disposable syringe filter with 0.22  $\mu$ m and 20  $\mu$ l were used for determination of phenolic compounds including apigenin, caffeic acid, catechin, chlorogenic acid, ellagic acid, ferulic acid, gallic acid, kaempferol, *p*-coumaric acid, quercetin, rosmarinic acid, and syringic acid. Phenolic compounds analysis was performed using a high-performance liquid chromatography (HPLC) equipped with a photodiode array detector (DAD) with a C<sub>18</sub> column (150  $\times$  4.6 mm, 3.5  $\mu$ m) and a UV detector (Waters 2487). The mobile phase contained methanol and 1 % formic acid (90:10). High-performance liquid chromatography assay was carried out using an isocratic system with a flow rate of 0.5 ml/min at 25 °C. The detection was performed using calibration curves drew with standard solutions. The data were expressed as mg/g dry weight (DW). All the experiments were done with three replications.

## 2.6. Statistical analysis

All the measurements were performed in triplicate. The results were expressed as mean  $\pm$

SD. The data were subjected to one-way analysis of variance (ANOVA) following Duncan's test ( $P \leq 0.05$ ) using SPSS software version 16.0 (SPSS Inc., Chicago, Illinois, USA). Heatmap and biplot graphs based on studied parameters were drawn by Origin 2021 software.

## 3. Results

### 3.1. Morphological assessments

The analysis of variance showed that the plant species had significant differences ( $P \leq 0.05$ ) for the studied morphological traits. Petiole diameter had the highest coefficient of variation ( $CV = 139.6\ %$ ) (Table 1). All of the studied morphological traits showed coefficient of variations greater than 20.00 %, which indicated a high variability among the species studied. The highest plant height ( $26.4 \pm 1.9$  cm) and canopy width ( $36.1 \pm 1.5$  cm) were in *T. foenum-graecum*. The mean value for stem diameter was  $1.6 \pm 0.1$  mm. Internode length varied from  $0.71 \pm 0.0$  to  $15.0 \pm 0.6$  cm. The range of petiole diameter and length were  $0.1 \pm 0.01$  -  $1.2 \pm 0.02$  mm and  $9.2 \pm 0.3$  -  $49.3 \pm 0.7$  mm, respectively. In addition, leaf length varied from  $9.9 \pm 0.2$  -  $30.8 \pm 1.5$  mm and leaf width ranged from  $7.0 \pm 0.1$  -  $36.4 \pm 2.2$  mm.

**Table 1.** Variation of the morphological traits among some *Trigonella* and its relative e.g. *Medicago* species from Iran.

Morphological trait	Range	Mean	SD <sup>a</sup>	CV <sup>b</sup>
Plant height (cm)	1.6 - 26.4	9.7	6.2	63.5
Canopy width (cm)	13.4 - 36.1	26.4	6.8	25.8
Stem diameter (mm)	0.98 - 4.2	1.6	0.9	56.4
Internode length (cm)	0.71 - 15.0	4.8	4.1	83.9
Petiole diameter (mm)	0.1 - 1.2	0.2	0.3	139.6
Petiole length (mm)	9.2 - 49.3	24.1	11.7	48.1
Leaf length (mm)	9.9 - 30.8	15.6	5.8	37.2
Leaf width (mm)	7.0 - 36.4	15.0	7.9	51.7

<sup>a</sup> Standard deviation; <sup>b</sup> Coefficient of variation (%).

### 3.2. Proximate data

The proximate composition of the ten studied *Trigonella* species is given in Table 2. The result revealed significant differences ( $P \leq 0.05$ ) in proximate composition among the species. Moisture content varied widely between different species ( $73.2 \pm 0.4$  -  $89.2 \pm 0.3$  %). The lowest and highest ash content of the plant leaf were measured in the aerial parts of *T. sprunneriana* ( $0.7 \pm 0.02$  %) and *M. monantha*

( $1.7 \pm 0.05$  %), respectively. The maximum content of fibre was found in *M. orthoceras* ( $4.9 \pm 0.4$  %), while it was ranged from  $0.7 \pm 0.04$  to  $1.8 \pm 0.05$  % in the other species. The highest protein content was determined in *M. orthoceras* ( $13.4 \pm 0.4$  %), followed by *T. filipes* ( $11.5 \pm 0.8$  %) and *T. sprunneriana* ( $11.1 \pm 0.6$  %). Fat content was less than 2.0 % in all samples studied. Carbohydrate content ranged from  $5.1 \pm 0.5$  to  $6.6 \pm 0.1$  %.

**Table 2.** Proximate composition of some *Trigonella* and its relative e.g. *Medicago* species from Iran

Species	Content (%)					
	Moisture	Ash	Fibre	Protein	Fat	Carbohydrate
<i>Trigonella astroides</i>	$87.3 \pm 1.1^{ab}$	$1.3 \pm 0.08^c$	$0.7 \pm 0.04^{de}$	$3.1 \pm 0.3^d$	$1.2 \pm 0.02^{ab}$	$6.4 \pm 0.2^a$
<i>T. elliptica</i>	$88.9 \pm 0.3^a$	$1.2 \pm 0.10^c$	$1.2 \pm 0.1^c$	$2.4 \pm 0.1^e$	$1.1 \pm 0.06^b$	$5.2 \pm 0.4^{bc}$
<i>T. filipes</i>	$77.3 \pm 0.5^d$	$1.6 \pm 0.09^a$	$1.8 \pm 0.05^b$	$11.5 \pm 0.8^b$	$1.3 \pm 0.09^a$	$6.5 \pm 0.7^a$
<i>T. foenum-graecum</i>	$87.3 \pm 0.5^{ab}$	$0.8 \pm 0.03^e$	$1.2 \pm 0.06^c$	$4.3 \pm 0.3^{cd}$	$1.0 \pm 0.01^{bc}$	$5.4 \pm 0.2^b$
<i>T. sprunneriana</i>	$81.4 \pm 0.8^c$	$0.7 \pm 0.02^e$	$0.8 \pm 0.03^d$	$11.1 \pm 0.6^b$	$0.9 \pm 0.05^c$	$5.1 \pm 0.5^c$
<i>Medicago crassipes</i>	$85.1 \pm 0.7^{bc}$	$1.4 \pm 0.11^b$	$0.8 \pm 0.04^d$	$5.2 \pm 0.2^c$	$0.8 \pm 0.05^{cd}$	$6.6 \pm 0.1^a$
<i>M. monantha</i>	$88.3 \pm 1.1^a$	$1.7 \pm 0.05^a$	$1.3 \pm 0.01^c$	$2.7 \pm 0.1^e$	$0.7 \pm 0.08^{de}$	$5.3 \pm 0.5^{bc}$
<i>M. monspeliaca</i>	$89.2 \pm 0.3^a$	$1.0 \pm 0.00^d$	$0.9 \pm 0.00^{cd}$	$2.3 \pm 0.0^e$	$1.1 \pm 0.06^b$	$5.6 \pm 0.4^b$
<i>M. orthoceras</i>	$73.2 \pm 0.4^e$	$1.4 \pm 0.07^b$	$4.9 \pm 0.4^a$	$13.4 \pm 0.4^a$	$0.9 \pm 0.03^c$	$6.2 \pm 0.7^a$
<i>M. phrygia</i>	$88.0 \pm 0.9^a$	$0.8 \pm 0.04^e$	$0.8 \pm 0.01^d$	$4.4 \pm 0.0^{cd}$	$0.8 \pm 0.01^{de}$	$5.4 \pm 0.3^b$

Data expressed as mean  $\pm$  standard deviation (SD) of three replicates.

Different letters in column indicate statistically different means at  $P \leq 0.05$ .

### 3.3. HPLC analysis of phenolic compound

Phytochemical analysis revealed that the content of phenolic compounds was significantly ( $P \leq 0.05$ ) varied among the ten Iranian *Trigonella* or *Medicago* species. A heat map was generated based on the studied samples and the twelve phenolic compounds (Fig. 1). The heat map indicated that studied species can be divided into two groups based on changes in their phenolic compounds. The more abundant phenolic compounds (mg/g DW) in

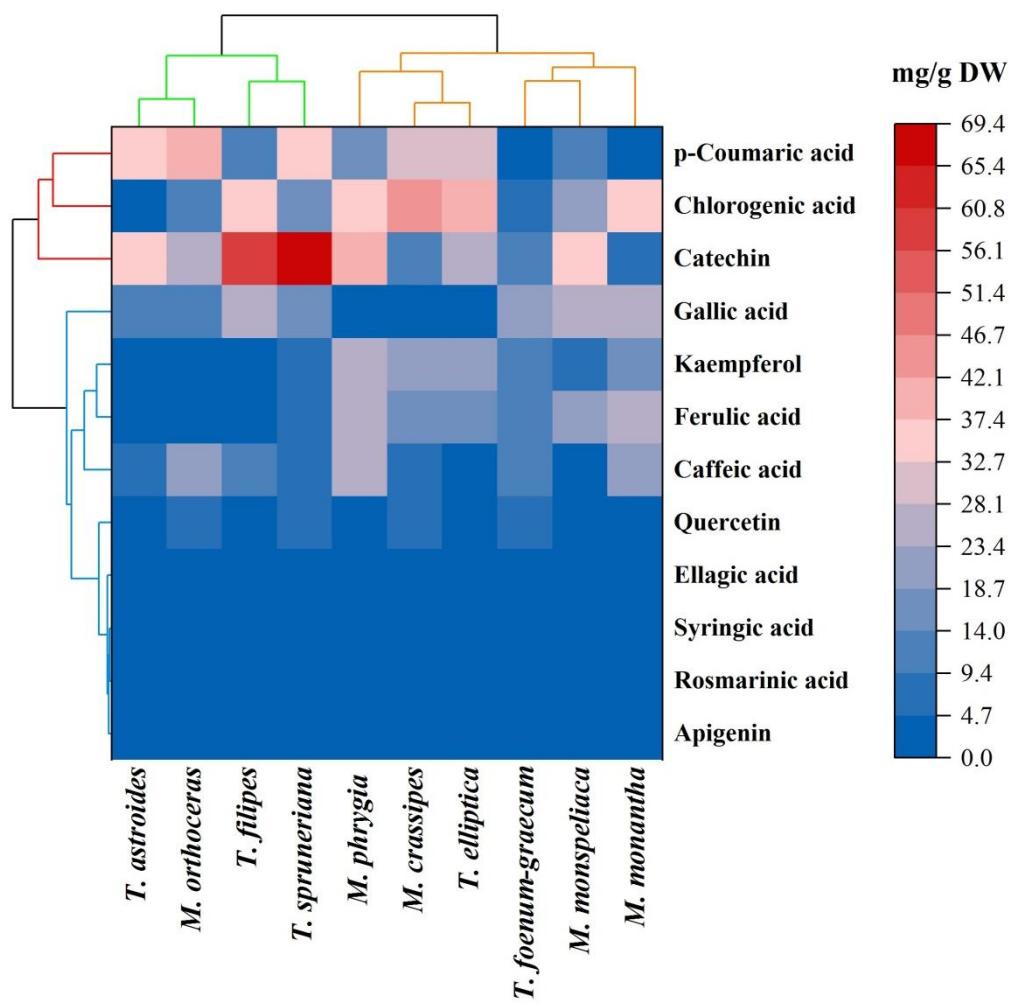
the leaves of the studied species were catechin ( $6.5 \pm 0.05$  -  $69.3 \pm 2.5$ ), chlorogenic acid ( $0.11 \pm 0.01$  -  $43.3 \pm 1.7$ ), and *p*-coumaric acid ( $0.1 \pm 0.0$  -  $39.3 \pm 1.6$ ). Also, gallic acid ranged from  $0.12 \pm 0.01$  mg/g DW in *M. crassipes* to  $25.8 \pm 0.8$  mg/g DW in *M. monantha* with an average of  $14.7 \pm 0.05$  mg/g DW. The highest and lowest caffeic acid (mg/g DW) were measured in the leaves of *M. phrygia* ( $25.5 \pm 1.1$ ) and *T. elliptica* ( $2.5 \pm 0.03$ ), respectively. The plant

samples were also contained (mg/g DW) kaempferol (0.0 ± 0.0 - 26.4 ± 1.2), ferulic acid (0.0 ± 0.0 - 25.8 ± 1.5), quercetin (1.4 ± 0.02 - 8.3 ± 0.5), ellagic acid (0.10 ± 0.0 - 4.2 ± 0.07), apigenin (0.0 ± 0.0 - 2.5 ± 0.08), rosmarinic acid (0.1 ± 0.0 - 1.6 ± 0.02), and syringic acid (0.0 ± 0.0 - 1.6 ± 0.02).

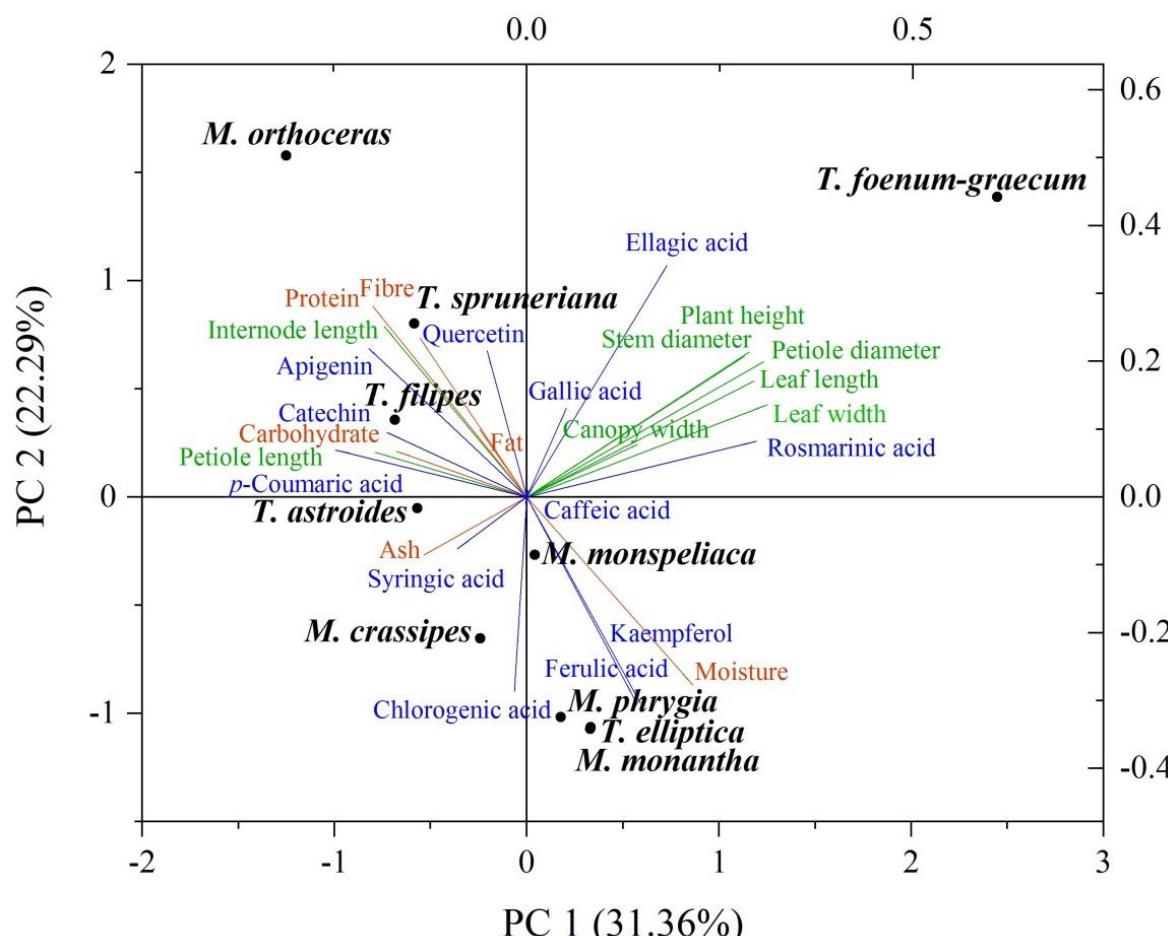
### 3.4. Principal component analysis (PCA)

The PCA analysis of the studied characters of *Trigonella* or *Medicago* species is presented in Fig. 2. The first axis explained 31.36 % of the variability. The second axis explained 22.29 %

of the variability. The species were grouped in four groups. The first group contains *T. foenum-graecum* which had high amounts of most of the morphological traits. The species of *M. monspeliaca*, *T. elliptica*, *M. phrygia*, and *M. monantha* formed the second group. Two species (*T. astroides* and *M. crassipes*) formed third group and were associated with chlorogenic acid and syringic acid. The fourth group was including *T. filipes*, *T. spruneriiana*, and *M. orthoceras* species and characterized by high values in catechin, *p*-coumaric acid, quercetin, apigenin, protein and carbohydrate.



**Fig. 1.** Heatmap of the phenolic compounds of some *Trigonella* and its relative e.g. *Medicago* species. From low in blue to high in red



**Fig. 2.** Bi-plot graph of quantified morphological traits, proximate composition and phytochemical traits.

### 3.5. Multiple regression analysis

Multiple regression analysis revealed that proximate and phytochemical compositions as dependent variable, showed significant correlation and in associated with some morphological traits as independent variables (Table 3).

Leaf length and canopy width showed association ( $P \leq 0.01$ ,  $P \leq 0.05$ ) with ash with a combination accounted of the variation of 0.68. Variables ratio of internode length was found to be associated with fibre content ( $R^2 = 0.54$ ,  $P \leq 0.05$ ). There was strongly significant correlation between internode length ( $R^2 = 0.76$ ,  $P \leq 0.01$ ) and petiole length ( $R^2 = 0.87$ ,

$P \leq 0.05$ ) with apigenin. Canopy width showed correlation with catechin ( $R^2 = 0.45$ ,  $P \leq 0.05$ ). Two variables (internode length and leaf length) showed strong correlation ( $P \leq 0.01$ ,  $P \leq 0.05$ ) with chlorogenic acid with a combination accounted of the variation of 0.71. Similarly, stem diameter was associated with ellagic acid ( $R^2 = 0.71$ ,  $P \leq 0.01$ ). Besides, canopy width was associated with *p*-coumaric acid ( $R^2 = 0.42$ ,  $P \leq 0.05$ ). In addition, leaf width had association with rosmarinic acid ( $R^2 = 0.62$ ,  $P \leq 0.01$ ). There was a negative significant correlation between internode length and kaempferol ( $R^2 = 0.41$ ,  $P \leq 0.05$ ).

**Table 3.** Morphological characters associated with proximate and phytochemical compositions in ten studied *Trigonella* and its relative e.g. *Medicago* species.

Proximate and phytochemical compositions	Morphological trait	r	R <sup>2</sup>	S $\beta$ <sup>a</sup>	t value	p value
Ash	Leaf length	0.69 <sup>a</sup>	0.41	-0.72	-3.77	0.00
	Canopy width	0.86 <sup>b</sup>	0.68	0.59	2.78	0.03
Fibre	Internode length	0.73 <sup>a</sup>	0.54	0.73	3.04	0.02
Apigenin	Internode length	0.87 <sup>a</sup>	0.76	0.77	5.37	0.00
	Petiole length	0.93 <sup>b</sup>	0.87	0.34	2.37	0.05
Catechin	Canopy width	0.67 <sup>a</sup>	0.45	-0.67	-2.57	0.03
Chlorogenic acid	Internode length	0.71 <sup>a</sup>	0.52	-0.84	-4.51	0.00
	Leaf length	0.88 <sup>b</sup>	0.71	-0.53	-2.85	0.02
Ellagic acid	Stem diameter	0.84 <sup>a</sup>	0.71	0.84	4.42	0.00
Kampferol	Internode length	0.64 <sup>a</sup>	0.41	-0.64	-2.36	0.04
p-Coumaric acid	Canopy width	0.64 <sup>a</sup>	0.42	-0.64	-2.39	0.04
Rosmarinic acid	Leaf width	0.79 <sup>a</sup>	0.62	0.79	3.62	0.01

<sup>a</sup>S $\beta$ : Standardized  $\beta$  coefficients

#### 4. Discussion

The studied parameters have a considerable diversity and there is a significant difference between species in terms of morphological traits, proximate and phenolic compositions. The chemical composition of medicinal plants and their biological activities are related to genetic and environmental factors [11]. According to the study of different plant species and their cultivation in the same environmental conditions, the effect of genetics on the occurrence of morphological diversity is very significant and visible. Therefore, the selection of superior traits will be useful for their genetic improvement [19].

Genetic diversity of some morphological traits and proximate composition in native populations of Iranian fenugreek (*T. foenum-graecum*) has been previously reported [15]. Mean leaf length and width, petiole length, and internode length were 23.59, 0.14, 33.03, 43.65 mm, respectively. Also, the mean content of ash and protein of leaf in the studied populations

were 0.98 and 11.39, respectively, which morphological data and ash percentage are similar to the present study. However, the leaf protein content of *T. foenum-graecum* in the present study was  $4.3 \pm 0.3$  %, which may be due to differences in the origin of the species and the geographical conditions of the cultivation site. In another study, the genetic diversity of some native populations of Iranian fenugreek (*T. foenum-graecum*) based on morphological traits was reported that are consistent with our results obtained [13]. Karamian and Haji Moradi [20] studied morphological traits of *T. elliptica* collected from Ghorveh, Kurdistan Province. In this study, the plant internode and leaf lengths were  $2.7 \pm 0.5$  cm and  $10.5 \pm 0.2$  mm, respectively, while in our study these traits were  $0.7 \pm 0.0$  cm and  $15.4 \pm 0.6$  mm. In the present study, *T. foenum-graecum* was distinguished as superior species in terms of leaf dimensions and canopy width, which is the most well-known species for

planting and consumption in food and pharmaceutical industries around the world.

The leaf proximate composition of *T. elliptica* and *T. tehranica* has also been reported [21]. Protein content in the leaves of these species were 10.07 and 14.76 %, respectively. Also *T. elliptica* had the highest content of fat (21.00 %), fibre (35.93 %) and ash (11.35 %). In the other studies [10, 22], the leaf proximate composition of *T. foenum-graecum* was similar to our results obtained.

Phenols are one of the major classes of phytochemical compounds [3]. Catechin and chlorogenic acid have been previously reported as the predominant phenolic compounds of the leaf extracts of six *Trigonella* or *Medicago* species *i.e.* *T. foenum graecum*, *T. persica*, *T. uncata*, *M. monantha*, *T. elliptica*, *T. coeruleascens* [17]. Catechin and chlorogenic acid are effective compounds in the treatment of many cancers and have antioxidant and antibacterial activities. These compounds have been extensively extracted from many plants, including green tea, coffee, and cocoa [2]. In another study, chlorogenic acid was the major phenol identified in fenugreek [23]. *P*-coumaric acid is another compound identified in the present study with high interspecific diversity. Anti-inflammatory, antibacterial, and neuroprotective effects of *p*-coumaric acid have been reported so far [24].

A wide range of phenolic compounds including cinnamic acid derivatives, flavonols, flavones, and coumarines have been reported from the vegetative parts of *Trigonella* species [7, 8]. The aerial parts of *T. foenum-graecum* contain quercetin, vitexin, luteolin, trigocoumarin and kaempferol. Fresh flowers of

*T. corniculata* have been identified as compounds of kaempferol, quercetin, myricetin, and scopoletin. Also, coumarin was obtained from the shoot of *T. coerulea*. Aesculetin is derived from the vegetative organs of *T. calliceras* and *T. cretica* [7]. Rutin and hesperetin compounds have been reported from the leaves of *T. persica* and *T. foenum-graecum* [17]. Vitexin and isovitexin have been reported as the major phenolic constituents of fenugreek germinated seeds [25]. However, further analysis is needed to determine the composition of the fenugreek [8]. In current study, the levels of kaempferol, gallic acid, ferulic acid, and caffeic acid were relatively low. Quercetin, ellagic acid, rosmarinic acid, apigenin, and syringic acid were also detected in trace amounts.

Based on multiple regression analysis, morphological traits were related to the content of a number of proximate composition and phenolic compounds. Some morphological variables including leaf length, canopy width, and internode length were associated with more than one proximate and phytochemical composition. The positive correlation of leaf morphological variables with phytochemicals compounds have also been reported in the other plant species [11, 19]. Riasat et al. [16] reported the relation of phenolic compounds including quercetin with morphological characteristics of *T. foenum-graecum*. This information can help the plant breeders to select superior variables for further hybridization.

## 5. Conclusion

The results of this study indicate the great biological potential of the studied species. Due

to the observation of a wide range of morphological and phytochemical diversity, species can be selected, domesticated and cultivated in agricultural systems. *T. filipes*, *T. sprunneriana*, and *M. orthoceras* are the most promising species for further exploitation to extract proximate composition including protein and phenolic compounds such as catechin, chlorogenic acid, *p*-coumaric acid, gallic acid, and caffeic acid. These species can be used as candidate parents in fenugreek breeding programs. In addition, the plants have a short growth period which can be well adapted to the climate of each region. In the present study, due to cultivation in the same climatic conditions, the diversity of the studied traits is related to genetic factors. The observed morphological and phytochemical diversity highlights the potential of the studied species as a medicinal plant that can finally be used on a commercial scale to produce bioactive compounds and functional foods.

## References

1. Millar KA, Gallagher E, Burke R, McCarthy S and Barry-Ryan C. Proximate composition and anti-nutritional factors of fava-bean (*Vicia faba*), green-pea and yellow-pea (*Pisum sativum*) flour. *J. Food Compost. Anal.* 2019; 82: 103233. doi: 10.1016/j.jfca.2019.103233.
2. Zhang Y, Cai P, Cheng G and Zhang Y. A brief review of phenolic compounds identified from plants: Their extraction, analysis, and biological activity. *Nat. Prod. Commun.* 2022; 17: 1934578X211069721. doi: 10.1177/1934578X211069721.
3. Teodoro GR, Ellepola K, Seneviratne CJ and Koga-Ito CY. Potential use of phenolic acids as anti-candida agents: A Review. *Front. Microbiol.* 2015; 6: 1420. doi: 10.3389/fmicb.2015.01420.
4. Dresler S, Szymczak G and Wójcik M. Comparison of some secondary metabolite content in the seventeen species of the Boraginaceae family. *Pharm. Biol.* 2017; 55: 691-695. doi: 10.1080/13880209.2016.1265986.
5. Tsimogiannis D and Oreopoulou V. Classification of phenolic compounds in plants. In Polyphenols in plants. Academic Press. 2019; 263-284. doi: 10.1016/B978-0-12-813768-0.00026-8.
6. Deshpande Usha S and Deshpande SS. "Legumes." Foods of plant origin. Springer, Boston, MA, 1991; 137-300. doi: 10.1007/978-1-4615-2039-9\_3.

## Author contributions

Z.B.: contributed to the conception of the study, plants cultivation, data collection and analysis, interpretation of data, drafting the manuscript. M.R.H.: supervised the study, formal analysis, reviewing and editing the manuscript. M.R.N.: helped in seed samples collection, statistical analysis, reviewing and editing the manuscript. M.H.M.: supervised the study, contributed in phytochemical analysis, data curation, writing and editing the manuscript.

## Conflicts of interest

The authors confirm that there are no conflicts of interest.

## Acknowledgments

The authors thank the Research Council of the University of Tehran for their financial support. We also wish to thank Mr. Hamid Ahadi and Miss. Azita Rezapour for their kind help in the extraction and HPLC analysis.

**7.** Petropoulos GA. Fenugreek, The genus *Trigonella*, First ed. Taylor & Francis. London and New York. 2002.

**8.** Wijekoon C Acharya SN Siow YL Sura S Thandapilly S and Sabra A. Canadian sainfoin and fenugreek as forage and functional foods. *Crop Sci.* 2021; 61: 1-20. doi: 10.1002/csc2.20280.

**9.** Chaudhary S Chaudhary PS Chikara SK Sharma MC and Iriti M. Review on fenugreek (*Trigonella foenum-graecum* L.) and its important secondary metabolite diosgenin. *Not. Bot. Horti Agrobot. Cluj Napoca.* 2018; 46: 22-31. doi: 10.1002/csc2.20280.

**10.** Singh A Singh S and Sharma R. Nutritional potentials and nutrient profile of fenugreek (*Trigonella foenum-graecum* L.). *Int. J. Curr. Microbiol. App. Sci.* 2020; 9: 3606-3615.

**11.** Khadivi-Khub A, Karimi E and Hadian J. Population genetic structure and trait associations in forest savory using molecular, morphological and phytochemical markers. *Gene.* 2014; 546: 297-308. doi: 10.1016/j.gene.2014.05.062.

**12.** Al-Maamari IT, Khan MM, Al-Sadi AM, Iqbal Q and Al-Saady N. Morphological characterization and genetic diversity of fenugreek (*Trigonella foenum-graecum* L.) accessions in Oman. *Bulg. J. Agric. Sci.* 2020; 26: 375-383.

**13.** Hasani Jifroudi H and Mohebodini M. Evaluation of genetic diversity and classification of some Iranian indigenous fenugreek (*Trigonella foenum-graecum*) populations using multivariate statistical methods. *Journal of Vegetables Sciences.* 2016; 2: 21-35. doi: 10.22034/iuvs.2016.32879.

**14.** Pasricha V and Gupta RK. Nutraceutical potential of Methi (*Trigonella foenum-graecum* L.) and Kasuri methi (*Trigonella corniculata* L.). *J. pharmacogn. phytochem.* 2014; 3: 47-57.

**15.** Moradi P Hassandokht MR and Kashi AK. Genetic diversity in some characteristics of Iranian fenugreek (*Trigonella foenum graceum* L.). *Journal of Crop Ecophysiol.* 2011; 4: 55-70.

**16.** Riasat M, Pakniyat H, Heidari B and Jafari AA. Variations in phytophenol compounds in association with morphological traits in *Trigonella* spp. Accessions. *Annu. res. rev.* 2018; 25: 1-16. doi: 10.9734/ARRB/2018/39533.

**17.** Riasat M, Heidari B, Pakniyat H and Jafari AA. Assessment of variability in secondary metabolites and expected response to genotype selection in fenugreek (*Trigonella* spp.). *Ind. Crops Prod.* 2018; 123: 221-231. doi: 10.1016/j.indcrop.2018.06.068.

**18.** AOAC. Official methods of analysis of the AOAC (18<sup>th</sup> ed.). Washington, DC: Author. 2000.

**19.** Hadian J, Nejad Ebrahimi S, Salehi P. Variability of morphological and phytochemical characteristics among *Satureja hortensis* L. accessions of Iran. *Ind. Crops Prod.* 2010; 32: 62-69. doi: 10.1016/j.indcrop.2010.03.006.

**20.** Karamian R and Hajmoradi Z. Comparative study on the morphology, palynology and essential oil composition of three *Trigonella* L. species (Fabaceae) from Iran. *Nova. Biol. Rep.* 2016; 3: 134-144. doi: 10.21859/acadpub.nbr.3.2.136.

**21.** Riasat M and Nasirzadeh AR. Evaluation of 2 perennial *Trigonella* (*T. elliptica* and *T. tehranica*) for forage quality improvement. *IJRFPBGR.* 2007; 14: 230-240.

**22.** Montgomery J. The potential of fenugreek (*Trigonella foenum-graecum*) as a forage for dairy herds in central Alberta. 2009. doi: 10.7939/R35T3G98H.

**23.** Chughtai MFJ, Pasha I, Shabbir MA, Haider MA, Afzal B, Ahmad S, and Manzoor MS. Biochemical evaluation of *Trigonella foenum*

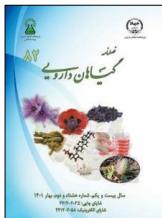
*graecum* (Fenugreek) with special reference to phenolic acids. *Biological Sciences-PJSIR*. 2017; 60: 154-161. doi: 10.52763/PJSIR.BIOL.SCI.60.3.2017.154.161.

**24.** Ferreira PS, Victorelli FD, Fonseca-Santos B and Chorilli M. A review of analytical methods for *p*-coumaric acid in plant-based products, beverages, and biological matrices. *Crit. Rev. Anal. Chem.* 2019; 49: 21-31. doi: 10.1080/10408347.2018.1459173.

**25.** Khole S, Chatterjee S, Variyar P, Sharma A, Devasagayam TPA and Ghaskadbi S. Bioactive constituents of germinated fenugreek seeds with

strong antioxidant potential. *J. Funct. Foods*. 2014; 6: 270-279. doi: 10.1016/j.jff.2013.10.016.

How to cite this article: Bakhtiar Z, Hassandokht MR, Naghavi MR, Mirjalili MH. Study of variability in agro-morphological traits, proximate composition, and phenolic compounds of some *Trigonella* L. species in Iran. *Journal of Medicinal Plants* 2022; 21(82): 1-12.  
doi: 10.52547/jmp.21.82.1



## مقاله تحقیقاتی

## مطالعه تنوع در صفات اگرو-مورفولوژیکی، ترکیبات تقریبی و ترکیبات فنلی برخی گونه‌های شبیله در ایران

زیبا بختیار<sup>۱</sup>، محمد رضا حسندخت<sup>۱\*</sup>، محمد رضا نقوی<sup>۲</sup>، محمد حسین میر جلیلی<sup>۳</sup><sup>۱</sup> گروه مهندسی علوم باگبانی و فضای سبز، پردیس کشاورزی و منابع طبیعی، دانشگاه تهران، کرج، ایران<sup>۲</sup> گروه مهندسی زراعت و اصلاح نباتات، پردیس کشاورزی و منابع طبیعی، دانشگاه تهران، کرج، ایران<sup>۳</sup> گروه کشاورزی، پژوهشکده گیاهان و مواد اولیه دارویی، دانشگاه شهید بهشتی، تهران، ایران

## اطلاعات مقاله

## چکیده

مقدمه: جنس شبیله (*Trigonella L.*) یکی از جنس‌های شناخته شده خانواده بقولات است که در سراسر جهان می‌رویند. این گیاهان علاوه بر مصارف غذایی دارای خواص دارویی از جمله کاهش دهنده چربی و قند خون، ضد باکتری، ضد زخم و ضد درد می‌باشند. هدف: مطالعه تنوع صفات اگرو-مورفولوژیکی، ترکیبات تقریبی و ترکیبات فنولیک اندام هوایی برخی گونه‌ها و خویشاوندان جنس شبیله بومی ایران برای معرفی گونه برتر جهت استفاده در برنامه‌های اصلاحی انجام شده است. روش بررسی: نمونه‌های بذر *T. filipes* *T. elliptica* *T. astroides* *M. monspeliaca* *M. monantha* *M. crassipes* *T. sprunneriana* *T. foenum-graecum* *M. orthoceras* و *M. phrygia* از مناطق مختلف ایران جمع‌آوری و در شرایط زراعی یکسان کشت شدند. صفات مورفولوژیکی با خط کش، کولیس دیجیتال و ترازو اندازه‌گیری شد. ترکیبات تقریبی (رطوبت، خاکستر، فیبر، پروتئین، چربی و کربوهیدرات) و اسیدهای فنولیک اندام هوایی به ترتیب با روش AOAC و HPLC تعیین شد. نتایج: تفاوت‌های مورفولوژیکی معنی داری بین گونه‌های مورد مطالعه مشاهده شد. بیشترین محتوای پروتئین در *T. sprunneriana* (۱۳/۴ ± ۰/۴٪) و پس از آن در *T. filipes* (۱۱/۵ ± ۰/۸٪)، و *M. orthoceras* (۱۱/۱٪) اندازه‌گیری شد. محتوای فیبر بین ۰/۰۴ ± ۰/۰۷٪ تا ۰/۰۴٪ بود. در عصاره گونه‌های مورد مطالعه، ترکیبات کاتچین، کلروژنیک اسید، پارا کوماریک اسید به وفور (۶۹/۳۲ ± ۰/۰۵٪ تا ۰/۰۱ ± ۰/۰۵٪ میلی‌گرم بر گرم وزن خشک) یافت شدند. نتیجه‌گیری: دو گونه *T. sprunneriana* و *T. filipes* می‌توانند به عنوان گونه‌های مناسب برای بهره‌برداری در سیستم‌های کشاورزی، صنایع غذایی و دارویی انتخاب شوند.

گل و ازگان:

تنوع

بقولات

شبیله

بخش رویشی

ارزش غذایی

فنول

ادویه

مخفف‌ها: Syn، مترادف؛ HPLC، کروماتوگرافی مایع با کارابی بالا؛ PCA، تجزیه و تحلیل مؤلفه اصلی  
\* نویسنده مسؤول: [mrhassan@ut.ac.ir](mailto:mrhassan@ut.ac.ir)

تاریخ دریافت: ۱۹ فوریه ۱۴۰۱؛ تاریخ دریافت اصلاحات: ۱ خرداد ۱۴۰۱؛ تاریخ پذیرش: ۳ خرداد ۱۴۰۱

doi: 10.52547/jmp.21.82.1

© 2020. Open access. This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<https://creativecommons.org/licenses/by-nc/4.0/>)