Pak. J. Agri. Sci., Vol. 53(4), 851-862; 2016
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/16.5559
http://www.pakjas.com.pk

DIVERSITY IN PHYTOCHEMICAL COMPOSITION OF OMANI FENUGREEK (*Trigonella foenum - graecum* L.) ACCESSIONS

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Fenugreek (*Trigonella foenum-graecum* L.) has enormous nutritional and medicinal values. Its herbaceous leaves and seeds are being used for human consumption since centuries. This study evaluated the diversity of certain phytochemicals (total phenolic contents, flavonoids, saponins and tannins,) in seeds of twenty Omani fenugreek accessions. The fenugreek plant material used in this study was obtained from the Germplasm Collection Centre, Ministry of Agriculture and Fisheries (MAF), Oman. The seeds were planted and morphologically evaluated using biodiversity international passport and then the seeds obtained from the first and second season harvests were used for their phytochemical analysis. Significant ($P<0.05$) variability was observed in the mean values for total phenolic contents (TPC), saponins, flavonoids and tannins, in seeds of various fenugreek accessions. The values in various accessions ranged for TPC from 107.88 to 216.47 mg GAE/100g; for saponins from 7.27 to 17.03g/100g; for flavonoids 8.46 to 32.81 mg CAE/100g and for tannins from 30.21to 74.54 mg CAE/100g, respectively. The results indicated that accession #49 had the highest TPC (216.47 mg GAE/100g), saponins (17.03g/100g) and flavonoids (32.81 mg CAE/100g), while the highest tannin content was recorded in accession #209 (74.54 mg CAE/100g). Accession #63 exhibited the maximum ferric reducing antioxidant power (FRAP) value (0.259 mMoles/L) compared to all other accessions. Our findings showed that a broader diversity exists in the seeds of various fenugreek accessions with respect to their phytochemical composition and antioxidant activities. The selection of potential fenugreek accessions for future dissemination should therefore be based on their phytochemical composition and antioxidant potential properties to harvest the maximum health benefits.
Keywords: Fenugreek, phytochemicals, genetic diversity, antioxidants, seeds, health benefits

INTRODUCTION

Trigonella foenum-graecum L. is an annual dicotyledonous plant, which belongs to the subfamily *Papilionaceae* of the *Leguminaceae* family (Acharya *et al*., 2006b). Historically, it has always been known as a medicinal herb for over two thousand years in various parts of the world (Srinivasan, 2006; Raja and Kudesia, 2012). Due to its distinct smell, it was described as a 'malodorous' plant (Pour *et al*., 2012; Lust, 2014). It imparts a pleasant flavour, colour and aroma to foods that make it a highly desirable supplement to be used in culinary practices. Srinivasan (2006) documented that fenugreek is one of the important ingredients in making up of curry powders. Fenugreek seeds contain significant amounts of galactomannans also called fenugreek gum, which are the main polysaccharide in fenugreek seeds and may contain up to about 50% of seed weight (Reid *et al*., 2003). They are used as industrial thickener and food emulsifier and for various medicinal purposes (Pandya *et al*., 1991; McCormick *et al*., 2006; Youssef *et al*., 2009, Kumar *et al*., 2009b; Raghuram *et al*., 1994; Raja and Kudesia, 2012). Its green leaves are rich in calcium, iron, beta-carotene and other vitamins (Sinha *et al*., 2007). In Ethiopia and Egypt, it is also used to supplement the maize and wheat flours for bread-making (Al-Habori and Raman, 2002).

The medicinal value of fenugreek seeds is well documented (Mishra *et al*., 2004; Lubbe and Verpoorte, 2011; Rajor *et al*., 2012). It is used as a carminative to improve digestion and prevention of flatulence and treating gastric ulcers, as a galactagogue and an aphrodisiac agent (Mir *et al*., 1998; Tiran, 2003, Srinivasan, 2006). It is reported to have antipyretic, antinociceptive (Prabha *et al*., 2010), anthelmintic (Babu *et al*., 2010), hypolipidemic and hypocholesterolemic effects, anti-diabetic and anti-cancer properties (Bhatti *et al*., 1996; Mishra *et al*., 2004, Edison, 1995; Yadav *et al*., 2007). Fenugreek seeds contain diosgenin, a steroidal saponin, which is used to make synthetic estrogen and can induce the apoptosis in a variety of tumor cells (Edison, 1995). Because of the presence of a small cysteine-rich peptide in the fenugreek seeds they show anti-microbial and antifungal activities (Bhatti *et al*., 1996;

Al-Habori and Raman, 2002; Olli and Kirti 2006; Evidente *et al*., 2007; Haouala *et al*., 2008; Karim *et al*., 2011).

Due to its anecdotal traits, fenugreek is considered as one of the leading functional food (Liu *et al*., (2012). The seeds of fenugreek provide a good source of dietary fiber, protein, essential oils and minerals as well as essential amino acid lysine (El-Soud *et al*., 2007, Ali *et al*., 2015). Broca *et al*. (2004) and Kassaian *et al*. (2009) reported that the seeds of fenugreek contain Isoleucine, which can be used as a metabolic precursor of 4-hydroxyisoleucine (4-HIL) that regulates insulin secretion in animals. The 4-HIL has been reported to decrease the hepatic glucose production and glucose/insulin ratios which in turn decrease the overall glucose levels. The 4-HIL is an important indicator of increased glucose utilization, hepatic damage, total cholesterol, high-density lipoprotein and triglycerides (Zafar and Gao 2016). It has been suggested that the hypoglycaemic effects of fenugreek seeds are caused by isoleucine together with the gastro-intestinal effects of dietary fibre (Germano *et al*., 2006). The bioactive compounds in fenugreek have been shown to have immune-modulatory effects (Sarada *et al*., 2008; Al-Tabbal and Al-Fraihat, 2012) and hepatoprotective properties (Jani *et al*., 2009; Goswami, 2012) and play role in the prevention of oxidative stress, cardiovascular diseases and other health related disorders (Germano *et al*., 2006; Srinivasan, 2006; Mishra *et al*., 2004; Kaviarasan *et al*., 2007; Bukhari *et al*., 2008).

Fenugreek can be used as a flavoring agent in ruminant and swine feed (Fotopoulos, 2002). The inclusion of fenugreek plant, seeds or seed meal in animal feeds not only improved the palatability due to its flavoring agents (Smith, 2003; Pearson and Aghakhani, 2010) but also increased the growth in beef cattle and milk flow in dairy cows through its natural steroidal properties (Shah and Mir, 2004, Lust, 2014). Although the Omani fenugreek is well adapted to the local farming system, their genetic diversity in terms of phytochemical composition has not been fully explored yet. We have reported earlier about the proximate and phytochemical composition of various native Omani legume species (Ali *et al*., 2013) and various land races of Omani fenugreek seeds (Ali *et al*., 2015; Al-Saady *et al*., 2014). It is anticipated that due to extensive trade links between Oman and the Indian sub-continent, fenugreek has also been introduced from regional countries like other crops in the country (Al-Sadi *et al*., 2012b). The objective of the study was to evaluate the diversity in phytochemical composition and antioxidant potential of twenty Omani fenugreek accessions. The information generated will be helpful in the selection of potential fenugreek accessions for future propagation and crop improvement based on their phytochemical composition and antioxidant potential to harvest the maximum health benefits.

MATERIALS AND METHODS

***Plant material*:** The seeds of Omani fenugreek accessions were obtained from the Germplasm Collection Centre, Ministry of Agriculture and Fisheries (MAF), Oman. The detail of fenugreek accession collection sites is given in Table 1.

Table 1. Plant material collection site of *Trigonella foenum-graecum* L. accessions from Oman.

S. No.	Acces. No.	Region	District	Village
1	312	Batinah North	Sohar	Al-Ghudafa
2	63	Batinah South	Rustaq	Haat
3	136	Batinah South	Rustaq	WadiBaniAouf
4	153	Batinah South	Rustaq	WadiBaniGhafer
5	160	Batinah South	Rustaq	Aldhahir
6	209	Batinah South	Rustaq	WadibaniGhafer
7	235	Buraimi	Buraimi	Al-Hail
8	240	Buraimi	Muhadha	Al-Khabeen
9	122	Dhahira	Yanqul	Al-Bouwerdah
10	97	Dhahira	Ibri	Asubal
11	2	Dakiliya	Nizwa	Tanuf
12	17	Dakiliya	Manah	Al-Blaad
13	31	Dakiliya	Adam	Al-Belad
14	35	Dakiliya	Bahla	Al-Khatwa
15	49	Dakiliya	Al-Hamra	Al-Qlaah
16	212	Dakiliya	Bidbid	Al-Buwareed
17	246	Sharqiya North	Al-Qabel	Bateen
18	260	Sharqiya North	Ibra	Al-Haimah
19	274	Sharqiya North	Mudhaibi	WadiEndam
20	304	Sharqiya North	WadiBani Khaled	Halfah

They were studied for their germination characteristics under laboratory conditions (25±2°C) using three replications containing 20 seeds per replication. The fenugreek seeds were then planted twice during winter season of 2010-2011 and 2011-2012. The temperatures during the season ranged between 19°C and 26°C with an average temperature of 23 ± 2°C. The experiment was conducted under open-field conditions using a randomized complete block design (RCBD) with three replications at the Masarat Al Andhar, Royal Court Affairs (RCA) site in Al Suwaiq District (RCA site is located at 23° 48.28 longitude and 57° 17.33.4 latitude with an altitude of 80 m above sea level). The total area of plot taken for experimentation was 50m x 30m, which was further divided into three blocks. The distance between each block was kept 2m and the accessions were separated by keeping 1m distance throughout. Each block contained 20 accessions with three replications containing 12 plants in each replicate. The distance between the plants was 50cm. All fenugreek accessions were assigned randomly to plots. The fenugreek field was fertilized initially with recommended doses of 100 kg P_2O_5/ha and 50 kg

K_2O/ha in the form of triple-super-phosphate and potassium sulphate, respectively (Akhtar and Nadaf, 2001). The plants were uniformly irrigated with light irrigation corresponding to the need of the plants till their germination and later thrice a week till one week prior to harvest. Plants were monitored for pest and disease infestation and appropriate protective measures were taken whenever necessary as described by Akhtar and Nadaf (2001). The plants were morphologically evaluated using biodiversity international passport. The seeds obtained from the first and second season's harvests were then collected and stored at 5±1°C in the College of Agricultural and Marine Sciences seed storage facility until their phytochemical analysis for total phenolic contents (TPC), tannins, flavonoids, saponins and ferric reducing antioxidant power.

***Extraction of samples for phytochemical analysis*:** Randomly collected fenugreek seeds from the first and second season's harvests for each accession were grouped together. The collected seeds were grounded well in a grinder and sieved by using a 60 mesh sieve. About 0.5 g of the finely grounded seed sample was placed in a centrifuge tube along with 5mL mixture of acetone, water and methanol (50:30:20, v/v). The tubes were incubated at room temperature in an orbital shaker for 3 h at 300 rpm. The tubes were incubated in dark room overnight for approximately 12 h. The samples were centrifuged at 3000 rpm for 10 m and the residues were re-extracted with same solvent for another 12 h. The combined extracts were stored at 4±1°C for further analysis (AOAC, 1990).

***Determination of total phenolic content (TPC)*:** The TPC were determined by Folin-Ciocalteu assay (Singleton and Rossi, 1965). The sample extract (50 µl) was mixed with 250 µl of Folin-Ciocalteu's reagent, 3ml distilled water and 750 µl of saturated $NaCO_3$ and thoroughly mixed. It was then incubated at room temperature (25±2°C) for 8 minutes. After incubation 950 µl of distilled water was added to the mixture and again incubated at room temperature (25±2°C) for 2 hours. The color produced by the mixture was measured in a spectrophotometer at 765 nm with water as a blank. For estimation of total phenolic contents (TPC) of samples, a standard calibration curve of containing different concentrations of gallic acid was prepared as mg of Gallic Acid Equivalents (mg GAE/100 g sample). A stock solution of gallic acid was prepared using 0.5 g gallic acid which was dissolved in 10% ethanol. Further dilutions of this stock solution were made containing 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml of this stock solution and the volume was made to 10 ml for the preparation standard curve.

***Measurement of condensed tannins*:** The Broadhurst and Jones (1978) protocol was followed for the estimation of condensed tannin contents (CTC). About 50 µl of sample extract was mixed with 1.5 ml concentrated hydrochloric acid and 3ml of 4% methanol-vanillin solution. The mixture was incubated at room temperature for 15 minutes and the color intensity of the mixture was measured in a spectrophotometer at 500 nm against methanol as a blank. The quantity of condensed tannins was calculated using the standard calibration curve prepared with different concentrations of pure (+)-catechin. The standard was prepared by using 100 mg of pure catechin dissolved in 100 ml of methanol. Further dilutions of 0.1, 0.2, 0.4 and 0.6 ml were prepared and the volume was made to 10 ml. The results are expressed as mg of (+)-catechin equivalents (mg CAE/100 g sample).

***Measurement of total flavonoids*:** The colorimetric method was used to determine total flavonoid contents in the sample (Heimler *et al*., 2005). Approximately 250 µl of fenugreek extract was thoroughly mixed with 1.25 ml of distilled water and 75µl of 5% $NaNO_2$ solution in 5 ml test tube. Approximately 150µl of 10% $AlCl_3.6H_2O$ solution was added after 6 minutes and incubated for 5 minutes. After incubation about 0.5 ml of 1M NaOH solution was added to the mixture and the volume was made up to 2.5 ml using distilled water. The color intensity of the mixture was immediately determined in UV-Visible Spectrophotometer at 510 nm (Thermospectronic 9423, England). The results were calculated by using the standard calibration curve of (+)- catechin and expressed as milligrams of (+)- catechin equivalents (mg of CAE/100g sample). For standard preparation, about 10 mg of catechin was dissolved in 100 ml of methanol. Further dilutions of 0.2, 0.4, 0.6, 0.8 and 1.0 ml of this stock solution were made and the volume was made up to 10 ml.

***Determination of saponins*:** The method of AOAC (1990) was followed for the determination of saponins. Two different solvents were used for saponins extraction. Acetone was used to extract crude lipid from the samples, followed by methanol to extract saponins. Around 2 g of the finely grounded sample was put into a thimble and placed in a Soxhlet's extraction unit. The extraction was carried using acetone and methanol for 2 h. To determine change in weight, the flask was weighed before and after each extraction. At the end of extraction, methanol was recovered by distillation and the flask was oven-dried. The weight of flask was then again measured after cooling in a desiccator. Saponins were calculated as g/100g of sample.

***Ferric reducing antioxidant power assay*:** Benzie and Strain (1996) protocol was used to carry out FRAP assay. FRAP reagent was prepared using 300 mM acetate and glacial acetic acid buffer (pH 3.6), 20 mM ferric chloride and 10 mM 4,6-tripryridyls- triazine (TPTZ) and made up in 40 mMHCl. All solutions were mixed in a ratio of 10:1:1. For each sample, 1 ml of distilled water, 25 µl of sample and 1 ml of FRAP reagent was added and incubated at 37°C for 6 minutes. The absorbance of the extract samples was measured at 593 nm and compared to a reagent blank. Ferrous sulphate was used as a standard reagent.

***Statistical analysis*:** The collected data was analyzed by

using one way analysis of variance (ANOVA) and results are presented as means ± standard deviation (SD) associated with the Tukey's test to determine the statistical significance ($P<0.05$). The means were compared as described by Snedecor and Cochran (1989) using the least significant difference (LSD). All the statistical analyses were carried using SPSS v.16.0 software. The principal component analysis of the data was done using the computer software system of PAST (PAST version 2.08, 2011).

RESULTS

The data on the mean values for total phenolic contents (TPC), saponins, flavonoids and tannins, in seeds of various fenugreek accessions is presented in figures 1-4. The mean values in various accessions of Omani fenugreek seeds ranged for TPC from 107.88 to 216.47 mg GAE/100g; for saponins from 7.27 to 17.03 g/100g; for flavonoids 8.46 to 32.81 mg CAE/100g and for tannins from 30.21 to 74.54 mg CAE/100g, respectively. Results indicated that accession #49 has the highest TPC (216.47 mg GAE/100g), saponins (17.03 mg/100g) and flavonoids (32.81 mg CAE/100g), while the highest tannin content was recorded in accession #209 (74.54 mg CAE/100g).

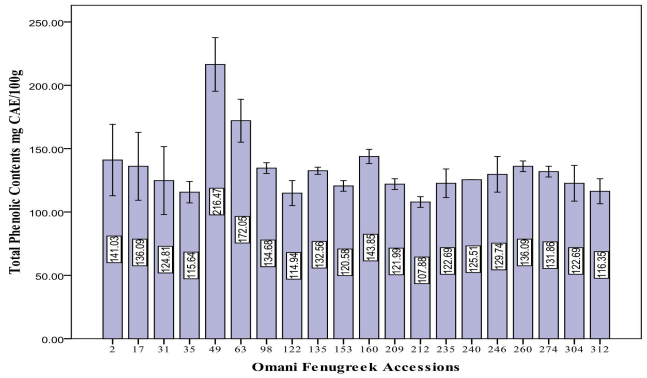


Figure 1. Mean total phenolic contents of Omani fenugreek accessions.

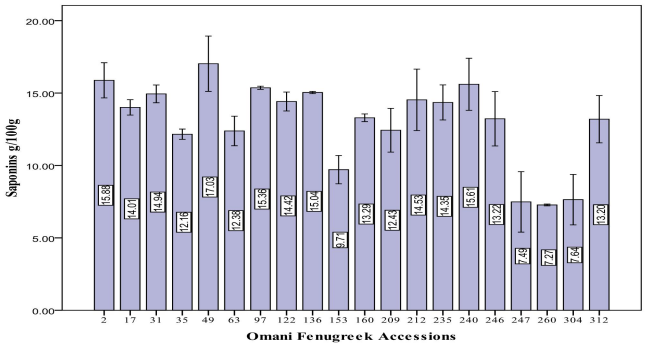


Figure 2. Mean saponins contents in Omani fenugreek accessions.

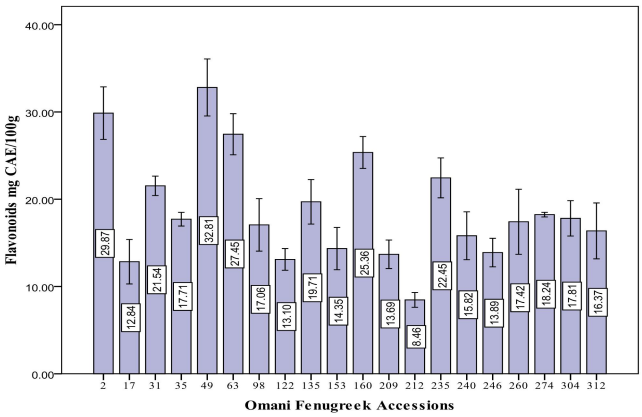


Figure 3. Mean flavonoids contents of Omani fenugreek accessions

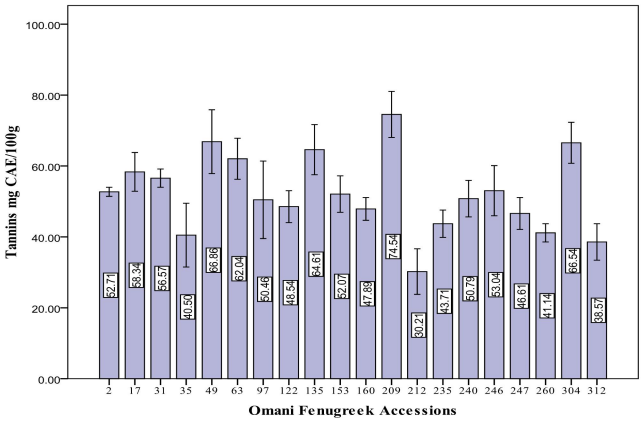
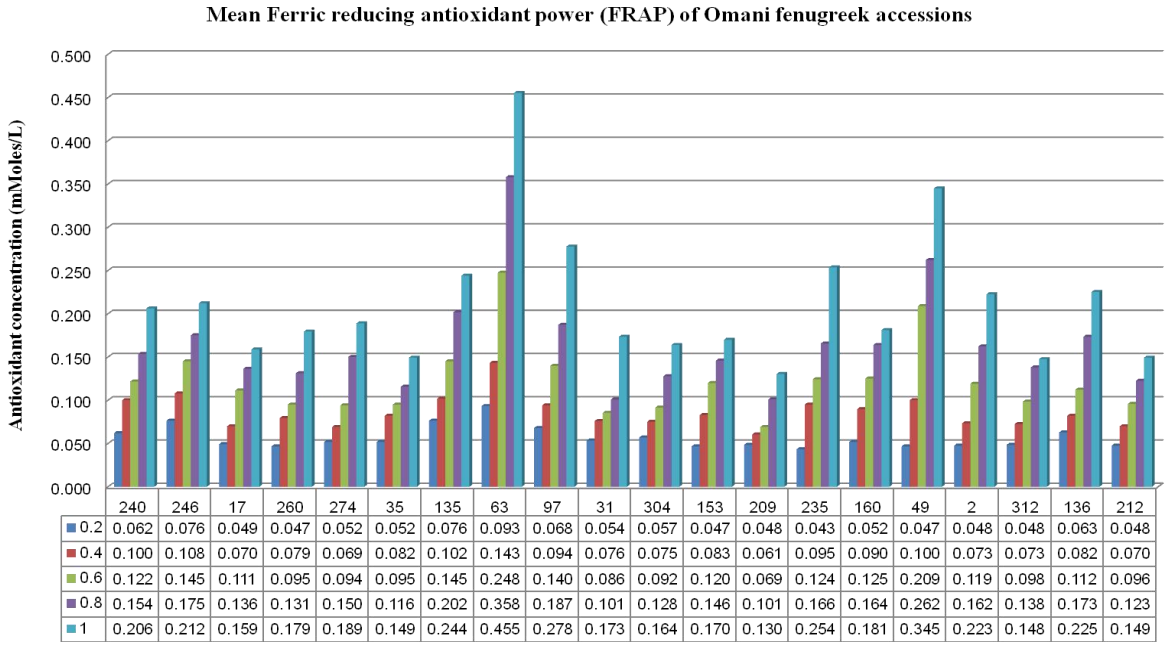


Figure 4. Mean tannins contents of Omani fenugreek accessions.

The results revealed significant differences ($P<0.05$) among the phytochemical composition of different Omani fenugreek accessions (Table 2). The results indicated that accession #49 produced the highest total phenolic contents (216.47 mg GAE/100g), followed by accession 63 (172.05 mg GAE/100g) and accession 160 (143.84 mg GAE/100g). The lowest value for total phenolic contents was recorded in accession 212 (107.88 mg GAE/100g) (Fig. 1). The highest saponin content was noted in accession #49 (17.02 mg/100g), followed by accession #2 (15.88 mg/100g) and accession #240 (15.60 mg/100g) (Fig. 2). Accession # 260 showed significantly the lowest saponin content (7.48 mg/100g) among the fenugreek accessions (Table 2). The accession #49 was also superior in the terms of total flavonoids (32.81 mg CAE/100g) among all other Omani fenugreek accessions. It was followed by accession #2 (29.86 mg CAE/100g) and accession #63 (27.45 mg CAE/100g) (Fig. 3). The accession # 212 showed significantly the lowest total flavonoids content (8.46 mg CAE/100g), (Fig. 3 and Table 2). The highest mean contents of tannin were found in accession #209 (74.54 mg CAE/100g),

Table 2. Analysis of variance of phytochemical composition of Omani fenugreek accessions.

	Interaction	Sum of squares	df	Mean square	F	Sig.
Replication	Between groups	0.000	19	0.000	0.000	1.000
	Within groups	10.000	20	0.500		
	Total	10.000	39			
Total phenolic contents	Between groups	21706.563	19	1142.451	11.70	0.000
	Within groups	1952.030	20	97.601		
	Total	23658.593	39			
Tannins	Between groups	4592.787	19	241.726	13.40	0.000
	Within groups	360.788	20	18.039		
	Total	4953.575	39			
Flavonoids	Between groups	1450.654	19	76.350	28.54	0.000
	Within groups	53.509	20	2.675		
	Total	1504.163	39			
Saponins	Between groups	312.684	19	16.457	19.65	0.000
	Within groups	16.755	20	.838		
	Total	329.438	39			
Ferric Reducing Antioxidant Power	Between groups	0.154	19	0.008	1.742	0.046
	Within groups	0.372	80	0.005		
	Total	0.526	99			



	240	246	17	260	274	35	135	63	97	31	304	153	209	235	160	49	2	312	136	212
0.2	0.062	0.076	0.049	0.047	0.052	0.052	0.076	0.093	0.068	0.054	0.057	0.047	0.048	0.043	0.052	0.047	0.048	0.048	0.063	0.048
0.4	0.100	0.108	0.070	0.079	0.069	0.082	0.102	0.143	0.094	0.076	0.075	0.083	0.061	0.095	0.090	0.100	0.073	0.073	0.082	0.070
0.6	0.122	0.145	0.111	0.095	0.094	0.095	0.145	0.248	0.140	0.086	0.092	0.120	0.069	0.124	0.125	0.209	0.119	0.098	0.112	0.096
0.8	0.154	0.175	0.136	0.131	0.150	0.116	0.202	0.358	0.187	0.101	0.128	0.146	0.101	0.166	0.164	0.262	0.162	0.138	0.173	0.123
1	0.206	0.212	0.159	0.179	0.189	0.149	0.244	0.455	0.278	0.173	0.164	0.170	0.130	0.254	0.181	0.345	0.223	0.148	0.225	0.149

Figure 5. Mean Ferric reducing antioxidant power (FRAP) of Omani fenugreek accessions.

followed by the accession #49 (66.85 mg CAE/100g) and accession # 04 (66.53 mg CAE/100g), respectively (Fig. 4). The lowest tannin content was recorded in accession #212 (30.21 mg CAE/100g). The results showed significant differences in ferric reducing antioxidant power among Omani fenugreek accessions (Fig. 5). Accession #63 was superior among them (0.259 mM/L) followed by accession #49 (0.1926 mM/L) and accession #135 (0.1538 mM/L). The accession #209 showed the lowest values for ferric reducing antioxidant

Table 3. PCA results based on phytochemical contents for Omani fenugreek accessions.

Plant descriptors	PCA 1	PCA 2	PCA 3	PCA 4	PCA 5
Total phenolic contents	0.9037	-0.1114	-0.1075	-0.09585	-0.38750
Tannins	0.5716	-0.5426	0.6058	0.05654	0.09329
Flavonoids	0.8330	-0.0004	-0.2647	-0.41840	0.24700
Saponins	0.4366	0.7899	0.4296	-0.03016	-0.00274
Ferric reducing antioxidant Power	0.7947	0.0834	-0.2720	0.52340	0.11620
% variance	53.1900	18.7550	14.1410	9.24570	4.66790
Eigenvalue	2.6595	0.9377	0.7071	0.46228	0.23339

Table 4. Principle component analysis loadings of phytochemical composition based on Ward`s phenotypic distance of 20 fenugreek accessions obtained from six geographical regions in Oman.

Accessions	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5
2	0.83381	0.79021	-0.07492	-1.72820	1.43810
17	-0.07452	-0.16904	1.19520	-0.00764	-2.38190
31	0.03416	0.34124	0.85596	-0.95617	0.97736
35	-0.83058	0.39431	-0.60869	-0.69367	0.26189
49	2.82850	0.14539	0.04344	-0.97782	-1.96550
63	2.09060	-0.59296	-1.37730	2.19390	0.85910
97	0.29718	0.87127	0.10255	1.23190	0.05719
122	-0.37490	0.75985	0.24753	1.58910	0.49220
136	0.45311	-0.01967	1.25420	0.24523	0.82645
153	-0.77705	-0.93746	-0.14174	0.20148	-0.10596
160	0.26441	0.22897	-0.56596	-1.60700	0.07759
209	-0.33280	-1.36820	2.39470	-0.04788	0.26206
212	-1.47330	1.67010	-0.29944	0.55859	-1.17480
235	0.00650	0.92690	-0.62325	-0.28940	1.21520
240	-0.14918	0.88938	0.66645	0.44793	0.02305
246	-0.21125	0.07061	0.33834	1.03370	-0.38987
260	-0.60384	-1.36930	-1.36590	-0.26061	-0.77362
274	-0.70627	-1.11240	-1.90340	-0.26951	-0.48479
304	-0.40768	-2.31720	0.32419	-0.11546	0.87638

power among Omani fenugreek accessions (0.818 mM/L) (Fig. 5).

The quantitative data for all Omani fenugreek accessions for their phytochemical contents (total phenolic contents, tannins, flavonoids, saponins) and ferric reducing antioxidant power (FRAP) were evaluated statistically (Table 3). The three principle components analyses (PCA) of phytochemical contents of Omani fenugreek accessions were calculated according to their chemical analysis (Table 3). The PCA of the phytochemical contents showed high variability (86%) divided for PCA1, PCA2 and PCA3 (53%, 18% and 14%, respectively). PCA1 ranged from 0.43 to 0.90, while in PCA2, the characters contributions were between 0.08 to 0.78 except for total phenolic contents, flavonoids and tannins. Furthermore, PCA3 ranged between 0.42 and 0.60 except for total phenolic contents, flavonoids and ferric reducing antioxidant power (FRAP).

The distribution of measured phytochemical contents is shown in a scatter diagram of PCA presented by the first two principal components. The total PCA's were scattered in all four quadrants (Fig. 6). PCA1 was the most important in the separation of accessions as it contributed 53% of the total variation. All the accessions were grouped into one main group except for accession #49, which was far away from the main group. The weighted clustering for plant performance using the five key Phytochemical parameters viz., total phenolic compounds, Tannins, flavonoids, saponins and ferric reducing antioxidant power (FRAP) were carried out among all the 20 Omani fenugreek accessions (Fig. 7). The cluster analysis showed two clusters (Fig. 7). The dendrogram showed two main clusters of accessions, groups A and B. The group-B was the largest group and it was divided into two sub-clusters, B1 and B2. Accession # 49 and accession #63 were included together in sub-cluster A1 according to their performance in the total phenolic contents and FRAP. Out of these two sub-clusters, the accessions 49 and 63 were grouped together in one cluster as they showed close affinity to each other according to their phytochemical compositions. The PCA analyses of phytochemical composition of Omani fenugreek accessions showed two main closely clusters, first cluster containing the

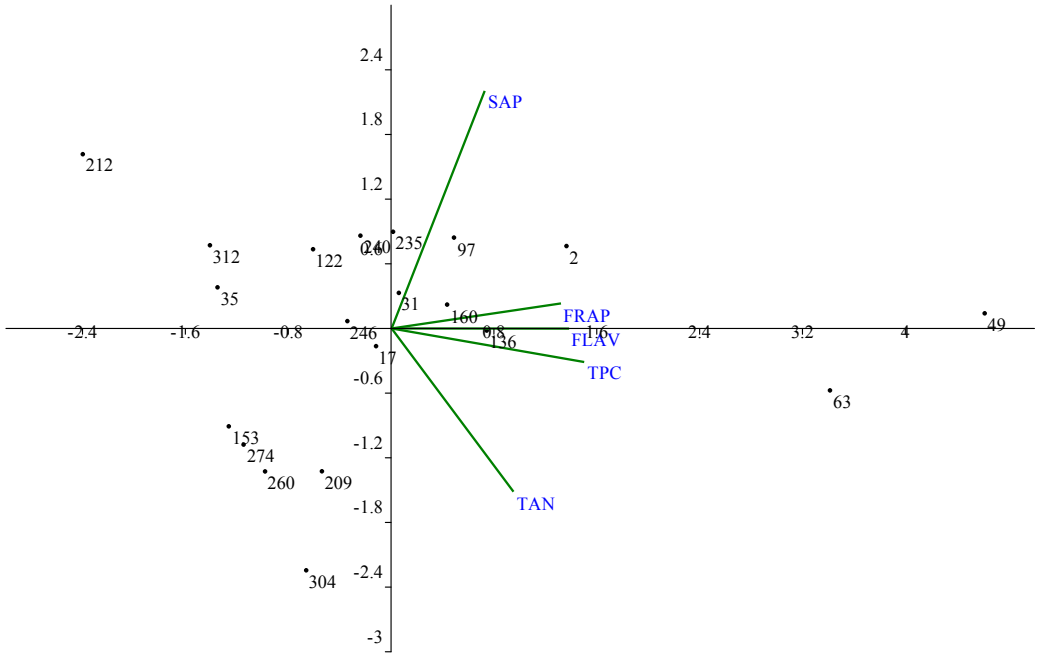


Figure 6. Eigenvalue scatter diagram for Omani fenugreek accessions based on five phytochemical compositions.

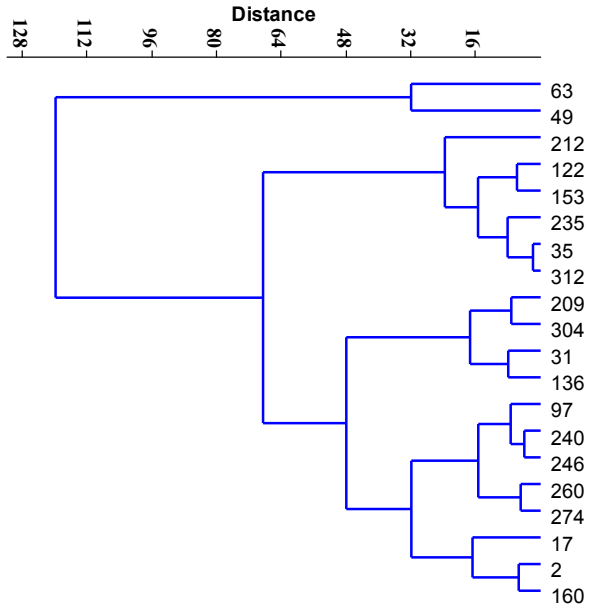


Figure 7. UPGMA dendrogram illustrating Nei (1978) genetic similarity of 20 Omani fenugreek accessions from different regions in Oman based on their phytochemical composition.

accessions 63 and 49 and the second cluster containing accessions 122, 97, 17, 35 and 31 (Fig. 7).

DISCUSSION

Fenugreek (*Trigonella foenum graecum* L.) is considered as a promising medicinal plant crop having triangular shape of small yellowish-white flowers. The fenugreek seed is the most useful part of the plant as it contains significant quantities of many important phytochemicals, such as saponins flavonoids, tannins, and alkaloids, which are regarded as bioactive components with various disease

preventing potential, immuno-modulatory functions and antioxidant properties (Goswami, 2012; Khole *et al*., 2014). This study evaluated the genetic diversity of certain phytochemicals (total phenolic contents, flavonoids, saponins and tannins,) in seeds of twenty Omani fenugreek accessions. The seeds of Omani fenugreek accessions exhibited significant ($P<0.05$) variability in the mean values for total phenolic contents (TPC), saponins, flavonoids and tannins. The mean values in various accessions ranged for TPC from 107.88 to 216.47 mg GAE/100g; for saponins from 7.27 to 17.03 g/100g; for flavonoids 8.46 to 32.81 mg CAE/100g and for tannins from 30.21 to 74.54 mg CAE/100g, respectively. Our results showed relatively high contents of total polyphenol components in Omani fenugreek accessions compared to the previously reported values by Ali *et al*. (2015) for various land races of indigenous Omani fenugreek seeds. The accession 49 showed the highest TPC (216.47mg GAE/100g) whereas the accession 212 produced the lowest values (107.88mg GAE/100g). Omezzine and Haouala (2013) reported that the aerial parts of fenugreek were rich in phenols, alkaloids, flavonoids and tannins and possess high allelopathic activities. Our results on the TPC values are also in line with the findings reported by Naidu *et al*. (2011) from India, and Yaser *et al*. (2013) from Yemen.

The presence of saponins in the fenugreek seeds is well documented (Lim, 2012). Fenugreek seeds mainly contain steroidal saponins, which contribute to bitter taste and foaming characteristics of seeds. Variable results have however been reported about the saponins contents of fenugreek seeds (Naidu *et al*., 2011; Yaser *et al*., 2013; Ali *et al*., 2015). Thediosgenin levels in 10 different accessions of mature fenugreek seeds from Western Canada were reported to range from 0.28 to 0.92% (Taylor *et al*., 2002). Erum (2011) reported that fenugreek seed contained 0.6 - 1.7% of saponins. Our results are in line with these findings. Our results, however, showed that the amount of saponins (7.27 to 17.03 g/100g) in these Omani fenugreek accessions is higher compared to the values reported from other parts of the world (Erum *et al*., 2011; Naidu *et al*., 2011; Yaser *et al*., 2013; Ali *et al*., 2015).

The flavonoids in fenugreek give a peculiar taste to meals/dishes and act as potential antioxidants to prevent the oxidative cell damage (Valaquez *et al* 2010; Singh *et al*., 2014). The mean values for flavonoids in these Omani fenugreek accessions ranged from 8.46 to 32.81mg CAE/100g. Our results are in line with the results reported by various authors from various parts of the world (Erum *et al*., 2011; Naidu *et al*., 2011; Yaser *et al*., 2013, Ali *et al*., 2015). Considerable variability has been reported in the phytochemical composition among the fenugreek genotypes (Acharya *et al*., 2006b). However, the variability in the reported results may be attributed to different methodologies used in determining the amount of these phytochemicals and flavonoids in plant samples. Yilmaz and Toledo (2006) reported that the methanolic extracts were better for flavonoids extraction such as catechin, epicatechin and epigallocatechin. Belguith-Hadriche *et al*. (2010) determined the total flavonoids in the extract using a catechin standard (19.01 mg Catechin/g) whereas Khole *et al.* (2014) found that flavonoid content was 27.8±0.025 mg quercetin equivalent/g in germinated fenugreek seeds powder. Our results using catechin standard protocol are in line with the earlier reports related to flavonoids.

Tannins have been reported to be present in foods of plant origin (fruits, legume seeds, cereal grains) in and in beverages like wine, tea and cocoa cider (Santos-Buelga and Scalbert, 2000). The amount of tannins may vary according to types of plants, plant parts as well as plant varieties (De Mejia *et al*., 2005; Ali *et al*., 2013). Nikolopoulou *et al.* (2006) found significant differences in the contents of tannins while comparing two varieties of chickpea. They argued it as higher surface to volume ratio in smaller seeds and reported that tannins are located on the surface of the seed. It was also reported that high amount of tannins is related to more pigmented seeds and also with antioxidant and anti-mutagenic properties (Cardador-Martinez *et al*., 2002). Kenny *et al*. (2013) reported that fenugreek seeds are not enriched with the complex tannins and carotenoids . Our results showed significant ($P<0.05$) differences in the tannin contents of different fenugreek accessions that ranged from (30.21 to 74.54 mg CAE/100g). The highest tannin content (74.54 mg CAE/100g) was recorded in accession 209. The amount of tannins in legume crops is controlled by different metabolic regulations and it can be different in different environmental conditions and genetic background. It has been suggested that the cultivation area, time of cultivation and plant variety, as well as the interactions of these factors may affect the seed composition such as the sugar contents, the total tannins and phytic acid in chickpea (Nikolopoulou *et al*., 2006).

It has been reported that fenugreek seeds have ferric reducing antioxidant power (Khole *et al*., 2014). Kenny *et al*. (2013) reported high antioxidant activity of phenolic contents in the ethyl acetate extract of fenugreek seed samples. Omani fenugreek accessions showed significant differences in their potential for ferric reducing antioxidant power. The highest values for ferric reducing antioxidant power was observed in accession 63 followed by accession 49. Although there were apparent differences in the phytochemical contents in the genetically diverse verities of fenugreek seeds the differences were however, non-significant (Table 2). These non-significant differences might have been responsible in identifying the significant

differences in FRAP values, which is an indicator of antioxidant activities of fenugreek seeds. Our findings are in line with the result reported by Dixit *et al*. (2005) and Khole *et al*. (2014). Dixit *et al*. (2005) reported that an aqueous extract of germinated fenugreek seeds exhibited the highest antioxidant activity as measured by ferric reducing antioxidant power. Khole *et al*. (2014) reported that the ethyl acetate extract of germinated fenugreek seeds showed the highest radical scavenging, ferric reducing ability and prevented the oxidative damage induced through lipid peroxidation in rat liver mitochondria. Malky and Gouda (2007) reported that germination of fenugreek seeds increases the total flavonoids and phenolic contents and they are considered as the most important compounds with antioxidant properties (Chandrasekara and Shahidi, 2010; Chandrasekara and Shahidi, 2011). Khole *et al*. (2014) showed higher phenolics (39.2±1.25 mg gallic acid equivalent/g) in germinated fenugreek seeds. Bukhari *et al*.(2008) showed higher quantities of phenolic compounds in ethanol extract of fenugreek seeds. Comparing the amount of ferric reducing antioxidants power (FRAP) contents in fenugreek with other legume crops, it was reported that fenugreek contain highest ferrous ion chelating activity (38.14–76.95%) at concentrations of 10–50mg (Gupta and Prakash, 2009). Our studies revealed differences among Omani fenugreek accessions for their diverse phytochemical composition. The diversity in seed chemistry in fenugreek may be largely attributed to the type of cultivar, agro-climatic conditions, other agricultural practices (Hoeck *et al*., 2000; Bell *et al*., 2012).

***Conclusions*:** The phytochemical composition of Omani fenugreek accessions exhibited genetic diversity in seed reserves and this variation may be attributed to variety, agro-climatic conditions and other agricultural practices. The results showed high values for total phenolic contents; saponins, flavonoids, tannins and ferric reducing antioxidants power (FRAP) in the Omani fenugreek accessions. The accession #49 was found to be superior in terms of its total phenolic contents, saponins, flavonoids and tannins among all other Omani fenugreek accessions while accession #63 was better in terms of its antioxidant potential. The genetic diversity in the phytochemical composition in the seeds of fenugreek accessions showed the presence of a wide range of phytochemicals in Omani fenugreek accessions. It is suggested that the selection of potential fenugreek accessions for future propagation should therefore be based on their phytochemical composition and antioxidant potential properties to harvest the maximum health benefits.

***Acknowledgement*:** We greatly acknowledge the financial and administrative support of Sultan Qaboos University (SQU) in conducting this study.

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