

PROXIMATE AND PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF INDIGENOUS LANDRACES OF OMANI FENUGREEK SEEDS

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## Abstract

**Background:** Fenugreek (*Trigonella foenum graecum* L) is receiving global attention as a functional food due to its unique nutritional and medicinal properties as anti-diabetic, hypocholesterolemic, antipyretic, anti-carcinogenic and seasoning and flavoring agent.

**Materials and Methods:** Seeds of indigenous fenugreek accessions were collected from three different ecological regions (Al-Dakhaliyah, Al-Dhahirah, and Al-Batinah) of Sultanate of Oman. The samples were analyzed for proximate chemical composition, phytochemical contents and antioxidant properties.

**Results:** Only significant ( $P < 0.05$ ) differences were observed in the crude fiber and gross energy values of fenugreek seeds collected from different regions of Oman. The highest crude fiber content (8.6 %) was observed in samples collected from Al-Dhahirah region whereas the lowest value (7.1%) was found in samples collected from Al-Dakhaliyah region. No significant ( $P < 0.05$ ) differences were however observed in the moisture, crude protein, crude fat, and ash contents of samples collected from the three regions of Oman. The regional variability significantly ( $P < 0.05$ ) affected the phytochemicals composition and the highest amount of total phenolics (139.2 mg GAE/100g) were recorded in samples collected from Al-Batinah, followed by Al-Dhakhliyah (130.0 mg GAE/100g) and Al-Dhahirah (127.8 mg GAE/100g) regions, respectively. A significant correlation was also observed between the total phenolic contents and the antioxidant properties of fenugreek seeds as determined by reducing power potential (FRAP).

**Conclusion:** Indigenous landraces of Omani fenugreek seeds are a rich source of protein, dietary fiber, and many important bioactive components, which were found to be significantly correlated with its antioxidant properties.

**Keywords:** Omani fenugreek, landraces, phytochemical composition, antioxidant properties.

## Introduction

Fenugreek (*Trigonella foenum graecum* L) is receiving global attention as a functional food because of its unique medicinal properties in human health. Fenugreek is an annual herb belonging to the family *Leguminaceae* or *Fabaceae* and widely grown in many parts of the world including North Africa, Mediterranean Europe, West and South Asia and some parts of Australia (Petropoulos, 2002). Fenugreek seeds are used as a whole or in the powdered form as seasoning and flavoring agents in the preparation of various meals and dishes. Although fenugreek belongs to the family *Fabaceae*, it is not classified as a legume from culinary perspectives as the other genera of this family. From culinary perspectives, it has basically 3 main uses: as herb (dried or fresh leaves); as spice (seeds); and as vegetable (fresh leaves and sprouts). Since fenugreek has long been used as a leafy vegetable, spice or condiment and medicinal herb, the Food and Drug Administration (FDA) has approved it as seasoning, spice, and flavoring agent and has classified it in the category of foods as "Generally Recognized as Safe (GRAS)" (FDA, 2008).

Fenugreek seeds are known to have a large number of pharmacological and therapeutic properties such as anti-diabetic in glycemic control (Puri et al., 2012; Haber and Keonavong, 2013; Marzouk et al., 2013; Neelakantan et al., 2014), hypocholesterolemic (Belguith-Hardriche et al., 2013; Chaturvedi et al., 2013), anti-leukemic, antipyretic, anti-carcinogenic, antimicrobial (Acharya et al., 2008; Alsemari et al., 2014; Yadav and Baquer, 2014; Zargar, 2014) and anti-lithogenic properties (Reddy and Srinivasan, 2009a and b). Recently it has also been shown to reduce the severity and systemic symptoms of dysmenorrhea (Younesy et al., 2014). The antioxidant properties of fenugreek have also been explored by many researchers (Genet et al., 2002; Thirunavukkarasu et al., 2003; Kaviarasan et al., 2004). The medicinal properties attributed to fenugreek have been reported to be associated with its phytochemicals and bioactive components such as complex carbohydrates (galactomannans), steroidal saponin (diosgenin, yamogenin, tigogenin, neotigogenin), alkaloids (trigonelline) and amino acids (4-hydroxyisoleucine) (Acharya et al., 2007; 2008). The non-starch polysaccharide (NSP) contents of fenugreek seeds include saponins, hemicelluloses, mucilage, tannins and pectin and help to decrease the level of low density lipoprotein-cholesterol (LDL-C) in blood by inhibiting the re-absorption of bile salts in the colon and help to reduce the risk of heart attack and colon cancer. These fibrous components bind to the toxins present in food and help to protect the colon mucus membrane from cancer causing toxins. The galactomannan or fenugreek gum is considered unique due to its ~1:1 ratio of galactose to mannose molecules. This high ratio of galactose substitution helps galactomannan to adsorb more water to form highly viscous solution at a relatively low concentration resulting in reduced glucose absorption within the digestive tract (Iskili et al., 2005; Prajapati et al., 2013).

In Ayurvedic and Unani medicine (the traditional medicine systems used in the Middle-East and South-Asian countries), fenugreek is used in the treatment of various ailments including diabetes, epilepsy, paralysis, gout, dropsy, chronic cough and piles (Bin-Hafez et al., 2003, Krishnaswamy, 2008). In Oman the fenugreek seeds are traditionally used to cure baldness, alleviate certain kidney problems, to maintain blood sugar level and in cosmetic preparations. Fenugreek seeds are added to the porridge given to new mother to help her sweat out infections, impurities and poisons from the body. Fenugreek seeds are also given to the nursing mothers as they act as galactagogue in augmentation of milk production.

Fenugreek is cultivated in Oman since centuries. It is a useful legume crop that is incorporated into short-term crop rotation systems as it helps in nitrogen fixation in the soil through symbiosis with nodule bacteria, and enhances the soil fertility (Acharya et al., 2008; Sadeghzadeh-Ahari et al.,

2009). Although fenugreek is appreciated for its versatility as all the components leaves, seeds and sprouts are used for different culinary and medicinal purposes. However, the Omani people mainly use the seeds for human consumption whereas the leaves are exclusively used for hay and silage making for livestock feeding. The indigenous landraces of Omani fenugreek are well adapted to local agro-Climatic conditions and farming system. However, their genetic diversity and nutritional and medicinal properties have not been fully explored. In a previous study we reported the nutritional quality, phytochemical composition and antioxidant properties of indigenous Omani legumes (Ali et al., 2013).

The present study was conducted to evaluate the proximate chemical composition, phytochemicals contents and antioxidant properties of indigenous landraces of Omani fenugreek seeds.

## Materials and Methods

The representative samples of indigenous Omani fenugreek (*Trigonella foenum-graecum* L) seeds were collected from 3 different regions of Oman; Al-Dakhaliyah (N 22 57.078, E 57 31.670), Al-Dhahirah (N 23 14 33.0, E 57 02 28.4), and Al-Batinah (N 23 12 47.205 E 57 27 33.622). The collection sites varied in their soil characteristics, environmental conditions and altitude. The fenugreek accessions were selected based on their significance in socio-cultural and farming system as well as on their frequency of consumption in human nutrition. The samples were manually cleaned to remove any foreign materials such as dust, dirt, weeds, immature and broken seeds etc. The samples were evaluated for their proximate chemical composition and phytochemical contents as well as for their reducing potential (FRAP). The analysis for all the chemical parameters was carried out in triplicate and the data has been reported on dry matter basis. The samples were analyzed for their proximate chemical composition according to the methods as described by AOAC (1990). The gross energy value (GE, KJ/100 g) was calculated by multiplying the % g of protein, lipids and carbohydrates with 16.7, 37.7 and 16.7 respectively.

The extraction of phenolic contents was carried out according the procedures described by Biglari et al. (2008). The total phenol contents (TPC) were determined using the Folin-Ciocalteu assay as described by Singleton and Rossi (1965). The results on total phenolic content (TPC) are expressed as mg of Gallic Acid Equivalents (mg GAE/100 g sample). The condensed tannin content (CTC) was determined according to Broadhurst and Jones (1978). Total flavonoids were determined calorimetrically as described by Heimler et al. (2005). The results for CTC and total flavonoids are expressed as mg of (+)-catechin equivalents (mg CAE/100 g sample). The saponins were determined according to AOAC (1990) and the results are expressed as g/100g. The amount of oxalates was determined according to the method described by Abaza et al. (1968). The results are expressed as mg/100g of sample. The reducing power (described as the ability of the sample extracts to reduce the ferric chloride (FeCl<sub>3</sub>) solution, FRAP) of the sample extracts was measured according to Pulido et al. (2002). The details on the extraction procedures and sample preparation for the analysis of various phytochemicals components are given in our earlier publication (Ali et al., 2013).

The data was analyzed using one way analysis of variance (ANOVA) and the results are expressed as means  $\pm$  standard deviation (SD). The statistical software package SPSS v.16 was used for the statistical analysis of data. The means were compared with least significant difference (LSD) as described by Snedecor and Cochran (1989). The value of P < 0.05 was considered as statistically significant.

## Results and Discussion

The data on the proximate chemical composition of indigenous landraces of Omani fenugreek seeds collected from different regions of Oman is presented in Table 1. Regional variability only significantly (P<0.05) affected the crude fiber and gross energy values of fenugreek. The highest crude fibre content (8.6 %) was observed in samples collected from Al-Dhahirah region whereas the lowest values (7.1%) were found in samples collected from Al-Dakhaliyah region. No significant (P<0.05) differences were however observed in the moisture, crude protein, crude fat, and ash contents of samples collected from the three regions of Oman. The crude protein values ranged from 24.9 to 25.9%, the highest (25.9%) was in samples collected from Al-Dhahirah region. The crude fat contents ranged from 5.1 to 5.6%. Variable results have been reported in the literature on the proximate chemical composition in fenugreek seeds by various researchers from different parts of the globe (Isikli et al., 2005; Acharya et al., 2006; Erum et al., 2011). Naidu et al. (2011) reported slightly higher amounts of crude protein (27.57%) and crude fat (6.71%) in fenugreek seed samples from India as compared to our results. The protein content of legume has been reported to depend on variety, climatic condition, agricultural

**Table 1:** Proximate composition of fenugreek seeds from different regions of Oman

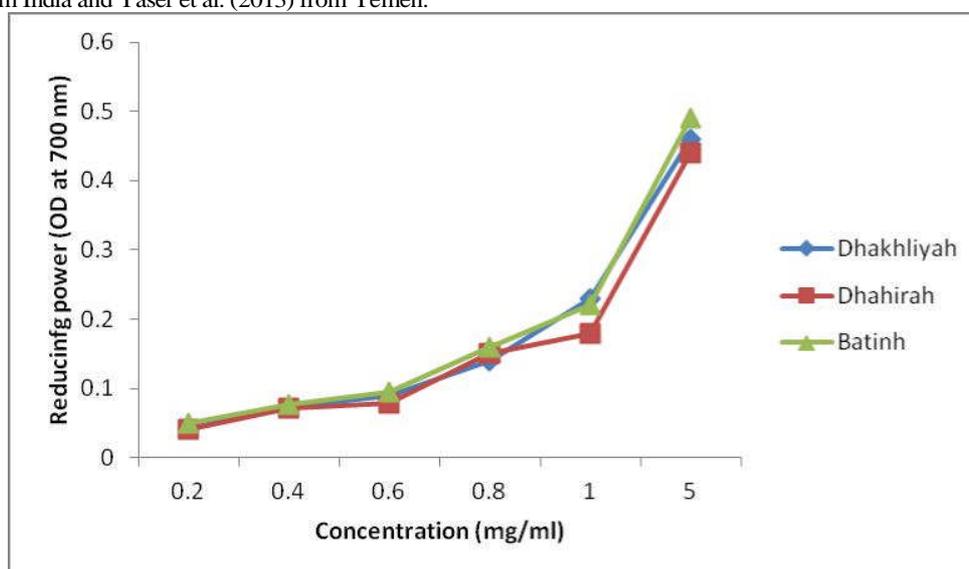
Region of Oman	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	NFE (%)	Gross energy kJ/ 100g
Al-Dakhaliyah	9.3 $\pm$ 1.224	9 $\pm$ 1.2	5.2 $\pm$ 0.7	7.1 $\pm$ 1.1 <sup>b</sup>	3.1 $\pm$ 0.7	50.4	$\pm$ 2.31457 $\pm$ 15 <sup>a</sup>
Al-Dhahira	9.5 $\pm$ 1.125	9 $\pm$ 1.7	5.1 $\pm$ 0.9	8.6 $\pm$ 1.2 <sup>a</sup>	3.2 $\pm$ 0.2	47.6	$\pm$ 1.91427 $\pm$ 8 <sup>b</sup>
Al-Batinah	9.4 $\pm$ 1.325	5 $\pm$ 1.4	5.6 $\pm$ 0.8	7.9 $\pm$ 1.4 <sup>ab</sup>	3.8 $\pm$ 0.5	47.7	$\pm$ 1.31440 $\pm$ 17 <sup>a</sup>
Mean values	9.4 $\pm$ 1.125	5 $\pm$ 1.3	5.3 $\pm$ 0.8	7.8 $\pm$ 1.1	3.4 $\pm$ 0.8	48.5	$\pm$ 3.61441 $\pm$ 13

\*Different superscripts in same column indicate significant regional differences (P<0.05)

**Table 2:** Phytochemical composition of fenugreek seeds from different regions of Oman

Region of Oman	Total Phenolics mg GAE/100g	Tannins mg CAE/100g	Flavonoids mg CAE/100g	Oxalates mg/100g	Saponins mg/100g
Al-Dakhliyah	130.0 ± 4.2 <sup>ab</sup>	61.7 ± 8.5 <sup>b</sup>	11.6 ± 1.2 <sup>b</sup>	82.0 ± 6.011171.4 ± 125.6 <sup>b</sup>	
Al-Dhahirah	127.8 ± 8.8 <sup>b</sup>	59.5 ± 5.3 <sup>b</sup>	11.1 ± 2.1 <sup>b</sup>	81.1 ± 7.012661.4 ± 48.6 <sup>a</sup>	
Al-Batinah	139.2 ± 9.8 <sup>a</sup>	94.3 ± 9.9 <sup>a</sup>	14.0 ± 0.8 <sup>a</sup>	77.4 ± 8.09711.5 ± 134.2 <sup>c</sup>	
Mean values	132.3 ± 7.5	71.8 ± 9.7	12.3 ± 1.5	80.1 ± 5.411181.4 ± 147.4	

\*Different superscripts in same column indicate significant regional differences ( $P < 0.05$ ) GAE = Gallic acid equivalent; CAE = Catechin equivalent practices as well as nutrient and water status of soil (Acharya et al., (2006). Fenugreek protein is believed to contain high amount of lysine and tryptophan, which have been shown to have high potential for insulin-stimulating activity. It has been suggested that the composition of fenugreek seed may not be similar in all environments and all genotypes may not produce the high quality seeds every year (Acharya et al., 2006; Taylor et al., 2002). Our results on the proximate chemical composition of indigenous landraces of Omani fenugreek seeds are in line with those reported by Naidu et al. (2011) from India and Yaser et al. (2013) from Yemen.

**Figure 1:** The reducing power of methanolic extracts of fenugreek seeds from different regions of Oman

The data on the phytochemicals composition of indigenous landraces of Omani fenugreek seeds collected from different regions of Oman is presented in Table 2. Significant ( $P < 0.05$ ) differences were observed in the phytochemicals composition of indigenous landraces of fenugreek accessions collected from various regions of Oman. The highest amount of total phenolics (139.2 mg GAE/100g), tannins (94.3 mg CAE/100g), and flavonoids (14.0 mg CAE/100g), were found in samples collected from Al-Batinah region, whereas the lowest values were observed in samples from Al-Dhahirah region (127.8 mg GAE/100g, 59.5 mg CAE/100g and 11.1 mg CAE/100g respectively). On the other hand the highest amount of saponins (12.6 g/100g) were found in samples collected from Al-Dhahirah region, whereas the lowest values (9.7g/100g) were found in samples from Al-Batinah region. Yaser et al. (2013) reported higher TPC values (505.29 mg/100g), but lower amount of tannins (2.03 g/100 g) and flavonoids (4.99 g/100g) in different varieties of fenugreek seeds from Yemen as compared to the values observed in Omani fenugreek seeds. Naidu et al. (2011) also reported higher TPC values but lower contents of saponins in fenugreek seeds from India as compared to the values observed in Omani fenugreek seeds. Saponins in fenugreek contribute to the bitter taste and foaming characteristic of seeds. Omani fenugreek seed showed higher concentrations of saponins (9.7 to 12.7 g/100g) as compared to the values (4.8% and 5.12%) reported by Rao et al. (1996) and Naidu et al. (2011) respectively. Fenugreek in particular its steroidal saponin fractions have been shown to have hypocholesterolemic and hypoglycemic effects. Fenugreek is therefore used as an anti-diabetic herb in both normal and diabetic subjects (Sharma 1986; Gupta et al., 2001; Taylor et al., 2002; Chaturvedi et al., 2013; Marzouk et al., 2013; Neelakantan et al., 2014). Although under experimental conditions, the saponins produced some toxic effects, the acute poisoning in humans and animals is relatively rare under normal dietary consumption patterns (Cortes-Giraldo et al., 2012).

Significantly ( $P < 0.05$ ) higher amounts of tannins were found in Al-Batinah region, followed by Al-Dhakhliyah and Al-Dhahirah region. Tannic acid has been found to be the most effective phenolic compound in reducing the enzyme activity as compared to other phenolic acids as it can reduce the enzyme activity up to 78% whereas ferulic acid up to 30%, gallic acid up to 26% and caffeic acid by only 18% reduction (Welsch et al., 1989). Ferulic acid has been reported to act as a powerful natural antioxidant, particularly in photo-protection of skin. Tannic acid has also been reported to reduce the starch digestibility by 16% whereas phytic acid can reduce up to 60% of starch digestibility (Thompson and Yoon, 1984). Regional variability did not significantly ( $P > 0.05$ ) affect the oxalate contents in Omani fenugreek seeds. Oxalates are generally considered as undesirable components in foods as they can lead to the formation of non-absorbable insoluble compounds with certain minerals such as  $Ca^{+2}$ ,  $Fe^{+2}$  and  $Mg^{+2}$  rendering these minerals unavailable and can lead to kidney stone formation (Almeida et al., 2008; Akhtar et al., 2011).

However, the oxalate content in Omani fenugreek was found less as compared to many other foods as reported by Akhtar et al. (2011). The flavonoids in fenugreek contribute to maintain the peculiar taste of prepared meals/dishes. They act as potent antioxidants by scavenging the free radicals and preventing the oxidative cell damage. Therefore, flavonoids are capable of treating certain physiological disorders including the protection against all stages of carcinogenesis (Ross and Kasum, 2002; Velazquez et al., 2010).

The data on the reducing power of methanolic extracts of fenugreek is presented in Figure 1. The reducing power was measured over a concentration of 0.2 to 5 mg/ml of FeCl<sub>3</sub> (FRAP). A linear increase in the reducing power was obtained in a dose-dependent assay of fenugreek samples and it was higher in sample from Al-Batinah region. A significant linear correlation was observed between the total phenolic contents and the antioxidant properties of fenugreek seeds as determined by reducing power potential (FRAP). Al-Batinah region is considered as one of the most important geographical and economic region of Oman, which includes country's largest agricultural plain (MOI, 2010). The fenugreek samples from Al-Dhahira region showed the lowest amount of total phenolics and consequently the lower reducing power potential. The reducing power potential is considered as an indicator of antioxidant activity and it directly relates to the amount of phenolic compounds present in different foods including fenugreek (Campos-Vega et al., 2010; Doss et al., 2011; Siger et al., 2012).

## Conclusion

Phytochemicals are receiving much attention for their distinctive therapeutic properties and wide use in human and animal health (Chaturvedi et al., 2013; Neelakantan et al., 2014; Yadav and Baquer, 2014). Phenolic compounds present in fenugreek can reduce the glycemic index of the carbohydrate rich foods by inhibiting the action of digestive enzymes such as salivary amylase, pancreatic amylase and glucosidase (Puri et al., 2012; Haber and Keonavong, 2013; Marzouk et al., 2013). In addition to this, the polyphenols contained in fenugreek also interact with hydroxyl (OH) groups of starch molecules, thus reducing the re-association of starch polymers during the retrogradation process (Baquer et al., 2011; Roberts et al., 2011; Prajapati et al., 2013). Only significant (P<0.05) differences were observed in the crude fiber and gross energy values of fenugreek seeds collected from different regions of Oman. The regional variability significantly affected the phytochemicals composition and the highest amount of total phenolics (139.2 mg GAE/100g) was recorded in samples collected from Al-Batinah region. The total phenolics were found to be significantly correlated with the antioxidant properties of fenugreek seeds. Omani fenugreek is a rich source of protein, dietary fiber, and contains many important phenolics and bioactive components with distinctive therapeutic properties. In addition to its nutritional value, it can be used as a good source of natural antioxidants. However, its use is limited because of its bitter taste. Based on the results obtained in this study, it is difficult to conclude that the one particular geographical region is better in the production of high quality fenugreek seeds than the other. It is therefore suggested that the genetic diversity of these landraces of Omani fenugreek should be explored further to improve their nutritional and bioactive components for improved human health benefits.

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