Biochemical, Phytochemical and Antioxidant Analysis of Leaves and Seed of Fenugreek Plant

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Abstract:

In the present study leaves and seeds of two different varieties of Fenugreek plant viz. *Trigonella foenum gracium* and *Trigonella corniculata*, collected from Bahawalpur, Pakistan were subjected to the biochemical, phytochemical and antioxidant analysis. The content of total sugars, non reducing sugars and reducing sugars in selected parts of these varieties were found to be 5.007 ± 0.101 to 8.133 ± 0.223 , 4.255 ± 0.150 to 7.099 ± 0.316 and 0.751 ± 0.064 to 1.410 ± 0.071 g/100g of dry weight respectively. Water soluble, salt soluble and total salt soluble protein content range from 1.172 ± 0.001 to 6.015 ± 0.132 , 1.517 ± 0.038 to 1.174 ± 0.006 and 0.013 ± 0.001 to 0.018 ± 0.001 g/100 g of dry weight respectively and the total free amino acids ranged from 1.198 ± 0.014 to 4.554 ± 0.006 g/100 g of dry weight. The range of tannins and saponin contents in analyzed parts of these varieties was found to be 0.057 ± 0.009 to 0.117 ± 0.025 and 0.083 ± 0.012 to 0.0151 ± 0.045 g /100g of dry weight respectively. Similarly, flavonoids and hydrogen cyanide content ranged from 0.021 ± 0.000 to 0.055 ± 0.004 and 0.0234 ± 0.008 to 0.307 ± 0.009 g/100g of dry weight respectively; while ascorbic acid content was found to be in the range of 0.068 ± 0.08 to 0.267 ± 0.031 g/100g of dry weight. Total phenolic acid content of the selected parts of these varieties were estimated on the basis of difference in dipole moment and its range was observed from 0.0539 ± 0.024 to 0.856 ± 0.064 g/100g of dry weight.

The total antioxidants analysis showed that ascorbic acid and trolox contents (mg Eqv./100g of extract) were found to be lower in *Trigonella corniculata* seeds (0.066 ± 0.001 and 0.047 ± 0.001 mg Eqv./100g respectively) and higher in *Trigonella corniculata* leaves (0.069 ± 0.001 and 0.050 ± 0.001 mg Eqv./100g respectively). The DPPH radical scavenging activity and reducing power were found to be in the range of 31.736 ± 2.430 to 39.590 ± 2.432 and 0.974 ± 0.231 to 2.301 ± 0.068 respectively. The different parts of studied varieties of fenugreek plant, having higher values of %DPPH inhibition and reducing power showed least count of IC₅₀ and vice versa.

Keywords: *Trigonella foenum graecum*, *Trigonella corniculata*, Biochemical, Phytochemical, antioxidants, DPPH, Phenolic acids.

I. Introduction

Plants have been used for medicinal purpose long before recorded history (Barnes et al. 2002). Plants are the rich source of bioactive components that add to the

nutritional and medicinal importance of these plants. Fenugreek is also one of these plants. It is the best known member of *Trigonella*, which belong to family Fabaceae. The genus *Trigonella* having 37 known species. Its two most important species named *Trigonella foenum graecum* and *Trigonella corniculata* are known as sickle fruit fenugreek and cultivated fenugreek respectively (Braun, 2005). The leaves and seeds of these both species are used in medical and culinary purpose. In ancient time fenugreek is commonly used in Egypt for the treatment of burn and for the promotion of the child birth. It was taken with honey for the treatment of “Dyspepsia”. Much other disease like “Diabetes” and “ricket” can also be treated by the use of fenugreek with honey. It is also used in the medicine to cure the urine infection (Grieve, 2008). Later studies revealed that fenugreek plant also contain phytonutrients and phytoestrogens.

India, Pakistan, Bangladesh, Egypt, France, Spain, Turkey, Morocco, and China are the main cultivated countries of fenugreek (Parthasarathy, 2008). *Trigonella foenum graecum* is an aromatic plant and can grow up to 60cm annually (Grieve, 2008; Parthasarathy, 2008; Chevallier, 2001). The fenugreek is rich in carbohydrates, proteins, free amino acid, steroidal saponin and flavonoids and because of this reason, its crop management depends on the climate conditions. According to climate fenugreek is sown in mostly autumn or spring. Fenugreek seed are the rich source of the polysaccharides galactomannan. Its seed also shows the diuretic activity because of the presence of alkaloid, terpenoids, flavonoids, phytochemical terpenoids and steroidal glycosides (Parthasarathy, 2008; Chevallier, 2001). Antioxidants play a vital role in the betterment of the health. If the diet contains more antioxidant than it is helpful for the prevention of the chronic disease. According to research, cancer development is inhibited by antioxidants as the antioxidants remove the oxidation of the given substrate by free radicals (Gall, 2011). In case of oxidative stress reactive oxygen species are generated. These reactive oxygen species are superoxide, hydroxyl and peroxyl radicals. These are very important for various diseases like aging, tumor, cardiac infarction, senile psychosis etc. (Thirunavukkarasu, 2003). The study is based on comparison of biochemical, phytochemical and antioxidant analysis of different parts of two varieties i.e. *Trigonella foenum graecum* and *trigonella corniculata*.

II. Material and Methods

2.1 Plant material

Leaves and nuts of *Trigonella foenum graecum* and *Trigonella corniculata* were purchased from local market of District Bahawalpur, Pakistan. Leaves and seed were cleaned from dust, washed with running water and dried under shade. These plant parts were ground properly using electric grinder and stored.

2.2 Extraction

1 g of each sample was extracted twice using ethanol over night. The extracts were filtered through muslin cloth and then through percolater and finally dried using rotary evaporator. The estimation of the sugars and free amino acids was performed after the reconstitution of these extracts.

Biochemical Parameters

Sugars and free amino acids were extracted in 75% aqueous methanol for 24 hr at solid to solvent ratio 1:25 w/v by following the method of Shad et al. 2009 (Shad et al. 2009). Total sugars and reducing sugar contents were estimated by the method developed by Travekyan and Harrison (Travekyan and Harrison, 1952) and Hulme and Naraian (Hulme and Naraian, 1934) respectively. Non reducing sugars content was calculated by taking

the difference of total sugars and reducing sugars. The free amino acid content was determined by the method described by Hamilton and Slyke (Hamilton and Slyke, 1931). Water soluble and salt soluble protein contents of *E. debile* stem were obtained by successive extraction in distilled water and 0.5 M ammonium sulfate solution respectively for 4 hours. Biuret's method was followed to estimate the protein content of each fraction (Plummer, 1979).

2.3 Phytochemical analysis

Tannins contents

The tannins content in each sample were calculated as g equivalent of tannic acid by following the method Fagbemi et al. 2005. The reaction mixture was allowed to stand for 30 minutes at room temperature and the contents were centrifuged. The absorbance of the filtrate was recorded at 725nm by using UV-Visible spectrophotometer (Fagbemi et al. 2005).

Saponins contents

Saponins were determined by method of Obadoni and Ochuko (Obadoni and Ochuko, 2001). The sample (20 g) was extracted twice with equal volumes of 20% ethanol (100 mL) at 55°C with continuous stirring. The extracts were combined, and volume was reduced to 40 mL by evaporation in boiling water bath. The concentrate was extracted twice with diethyl ether (20 mL) and the aqueous layer was further extracted twice with n-butanol (60 mL). The butanolic extract was washed twice with 5% aqueous NaCl solution (10 mL) and evaporated to dryness in boiling water bath. The residue was weighed and saponins contents were calculated as.

Saponins content (g /100g dry weight) = $Wr \div Ws \times 100$

where *Wr* is the weight of the residue and *Ws* is the weight of the sample.

Flavonoids contents

Flavonoids were extracted in 70% ethanol for 30 min at $25 \pm 5^\circ\text{C}$ and the flavonoids content were calculated as g catechin equivalent/100g dry weight by following the method of Michlaska (Michlaska et al, 2007). Absorbance was recorded at 510nm and the catechin was used as standard solution.

Hydrogen cyanide content

HCN content were extracted in phosphoric acid and water (1:20 v/v) for 12 hrs (Gilchrist, 1967). The cyanide contents were estimated as;

$1\text{MOI CN} = 2\text{AgNO}_3$.

Ascorbic acid content

Vitamin C was determined by using the procedure as outlined by Nielsen (1998). Samples were extracted with 20 ml of metaphosphoric acid-acetic acid mixture. The extracts were titrated separately with the indophenol dye solution until a light rose pink persisted for 5 s. The amount of dye used in the titration were determined and used in the calculation of vitamin C content. In each sample the amount of ascorbic acid was then calculated as g/100 g dry weight (Nielsen, 1998).

Antioxidant analysis

Total phenolic acid contents

TPC in crude methanolic extract was determined using the method of Taga *et al.* (Taga et al. 1884). The sample (5 g) was extracted twice in 70% methanol (50 mL) at room temperature for 24 h. The methanolic extract (1 mL) was treated with Folin-Ciocalteu's reagent (0.1 mL) followed by the addition 2% Na₂CO₃ solution (2 mL). The mixture was

allowed to stand for 30 min and absorbance was measured at 750 nm. TPC was calculated as g/100 g dry weight from the standard curve of gallic acid ($R^2=0.996$).

Total antioxidant content

The Trolox and ascorbic acid equivalent total antioxidant activity of methanolic extracts was determined by 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical and phosphomolybdenum assays using the methods described by Shad et al. (Shad et al. 2012) and Prieto et al. (Prieto et al. 1999) respectively. TAO was expressed as mg of Trolox and mg of ascorbic acid equivalent/100g (Travelyan and Harrison, 1952).

DPPH activity

The methanolic extract (1 mL) of each sample was mixed with 40 μ M methanolic DPPH solution (3 mL) and allowed to stand in dark (30 min). The absorbance of reaction mixture was recorded at 517 nm and the total antioxidant content was calculated as g/100g of extract from standard curve of Trolox ($R^2=0.993$) as well as ascorbic acid ($R^2=0.990$).

The percent DPPH inhibition deliberated:

$$[\text{DPPH}] \text{ inhibition (\%)} = 100 \times \frac{\text{Abs}_0 - \text{Abs}_{30}}{\text{Abs}_0}$$

Statistical Analysis

All the values are result of three concordant readings and are expressed in Mean $\pm$ SD. The data were statistically analyzed by one-way variance analysis (ANOVA) and the means with significant difference at 95% confidence level ($p<0.05$) were separated in to subsets using Tukey's multiple range test. All the statistical tests were performed on the statistical software (SPSS version 12.0).

III. Results and Discussion

The current study deals with biochemical, phytochemical and antioxidant analysis of different parts of *Trigonella corniculata* and *Trigonella foenum graecum*. Reducing sugars, non-reducing sugar and total sugar content were present in higher quantity in leaves of the *Trigonella corniculata* (5.263 ± 0.212 , 0.789 ± 0.631 , 4.474 ± 0.426) g/100g dry weight respectively as compared to those in the leaves of the *Trigonella foenum graecum* (5.007 ± 0.101 , 0.751 ± 0.064 , 4.255 ± 0.15) g/100g dry weight respectively. The total sugar, and non-reducing sugars content were higher in the seeds of the *Trigonella corniculata* (8.133 ± 0.223 , 7.099 ± 0.316) g/100 g dry weight respectively and lower in the seeds of *Trigonella foenum graecum* (5.900 ± 0.265 , 4.490 ± 0.297) g/100 g dry weight respectively. While the reducing sugars content were found to be higher in the seed of *Trigonella foenum graecum* (1.410 ± 0.071 g/100 g dry weight) as compared to seeds of *Trigonella corniculata* (1.034 ± 0.260 g/100 g dry weight). Water soluble, salt soluble and total salt soluble proteins content were found to be higher in the leaves of *T. corniculata* (4.419 ± 0.003 , 1.596 ± 0.019 , 6.015 ± 0.132) g/100 g dry weight as compare to those in the leaves of *T. foenum graecum* (3.332 ± 0.001 , 1.517 ± 0.038 , 4.849 ± 0.003) g/100 g dry weight respectively. While the seeds of *T. corniculata* were found to have higher water soluble, salt soluble and total salt soluble proteins content (1.37 ± 0.102 , 1.174 ± 0.001 , 3.11 ± 0.102 g/100 g dry weight respectively as compared to those in the seeds of *T. foenum graecum* (1.172 ± 0.001 , 0.531 ± 0.001 , 1.703 ± 0.003) g/100 g dry weight. While the amino acids content are found to be higher in the leaves of *T. corniculata* (4.554 ± 0.006 g/100 g dry weight) as compared to those in leaves *T. foenum graecum* (1.198 ± 0.014 g/100 g dry weight) while the seeds of the *T. foenum graecum* has greater amount of amino acids content (2.375 ± 0.057 g/100 g dry weight) as compared to those in the *T. corniculata* (2.059 ± 0.085 g/100 g dry weight).

Table 1: Biochemical composition of (g/100g dry weight) of leaves and seeds of selected species of fenugreek plant

Parameter studied	Trigonella corniculata		Trigonella foenum graecum	
	Leaves	Seeds	Leaves	Seeds
Sugars				
Total sugars	5.263±0.212b	5.007±0.101a	5.007±0.101a	5.900±0.265c*
Reducing sugars	0.789±0.631b	1.034±0.260a	0.751±0.064a	1.410±0.071b*
Non-Reducing sugars	4.474±0.426b	3.973±0.316a	4.256±0.150a	4.490±0.297b*
Proteins				
Water soluble	6.015±0.132a	3.110±0.102b	4.849±0.003b	1.172±0.001b
Salt soluble	1.596±0.019a	1.174±0.006a	1.517±0.038a	1.703±0.003b
Total salt soluble	0.018±0.003a	0.013±0.001a	0.018±0.001a	0.013±0.001a
Free amino acid	4.554±0.006c	2.059±0.085b	1.198±0.014a	2.375±0.057b

In current study tannins content were found to be higher in leaves of *T. corniculata* (0.074±0.013 g/100 g dry weight) as compared to that in the leaves of *T. foenum graecum* (0.057±0.009 g/100 g dry weight). While the tannins content were observed greater in the seeds of *T. foenum graecum* (0.117±0.025 g/100 g dry weight) as compared to that seeds of *T. corniculata* (0.092±0.004 g/100 g dry weight). The saponin content were present in higher quantity in the leaves of *T. foenum graecum* (0.151±0.45 g/100 g dry weight) as compared to the leaves of the *T. corniculata* (0.083±0.012 g/100 g dry weight). While seeds of *T. corniculata* (0.118±0.017 g/100 g dry weight) have higher amount of saponin as compared to that in seeds of *T. foenum graecum* (0.093±0.003 g/100 g dry weight). Flavonoids content were found to be higher in the leaves of *T. corniculata* (0.055±0.004 g/100 g dry weight) as compared to that in the leaves of *T. foenum graecum* (0.042±0.015 g/100 g dry weight). While flavonoids content were observed greater in the seeds of *T. foenum graecum* (0.040±0.001 g/100 g dry weight) as compared to that in the seeds of the *T. corniculata* (0.021±0.001 g/100 g dry weight). The HCN content were found to be higher in *T. foenum graecum* (0.307±0.009 g/100 g dry weight) as compared to that in the leaves of *T. corniculata* (0.234±0.008 g/100 g dry weight). The ascorbic acid was present in higher amount in the leaves of *T. corniculata* (0.267±0.031 g/100 g dry weight) as compared to that in the leaves of *T. foenum graecum* (0.153±0.006 g/100 g dry weight). While the seeds of *T. foenum graecum* (0.085±0.009 g/100 g dry weight) has greater amount of ascorbic acid as compared to the seeds of *T. corniculata* (0.068±0.008 g/100 g dry weight).

Diabetes and cancer are some of the most widespread diseases seriously affecting its victims. *Trigonella foenum graecum* and *Trigonella corniculata*, both herbal plant have been usually used for the treatment of diabetes all over the world [22]. Various phytochemicals have widespread activities that help in defense against chronic infections. Numerous physiological effects like an irritant, antiseoretolytic, antiphlogistic, antimicrobial have been shown by the tannins. They are very helpful in protecting the plants from bacteria and insects' attacks. The fruits which have pits such as cherries,

apricots, apples and bitter almonds are the rich source of HCN. Several pits have small quantity of cyanohydrins such as mandelonitrile which discharges hydrogen cyanide.

Parameter studied (g/100g dry weight)	<i>Trigonella corniculata</i>		<i>Trigonella foenum-gracium</i>	
	Leaves	Seeds	Leaves	Seeds
Tannin	0.074±0.013a	0.092±0.004a	0.057±0.009a	0.0117±0.025b
Saponins	0.083±0.012a	0.118±0.017b	0.151±0.045b	0.093±0.003a
HCN	0.234±0.008a	0.268±0.065a	0.307±0.009a	0.294±0.061a

Table 2: Anti-nutritional composition of (g/100g dry weight) of leaves and seeds of selected species of Fenugreek plant

Taking ascorbic acid as standard the total antioxidants contents were found to be comparable in all samples and are arranged in decreasing order as under *T. corniculata* leaves (0.069±0.001 g/100 g dry weight), *T. foenum-gracium* leaves (0.068±0.001 g/100 g dry weight), *T. foenum* seeds (0.067±0.001 g/100 g dry weight) and *T. corniculata* seeds (0.006±0.001 g/100 g dry weight). The highest amount of total phenolic content have been found to be present in *T. foenum-gracium* leaves (0.856±0.064) followed by *T. corniculata* leaves (0.741±0.050 g/100 g dry weight), *T. corniculata* seed (0.559±0.003 g/100 g dry weight) and *T. foenum-gracium* seed (0.539±0.024 g/100 g dry weight). The IC₅₀ values for the DPPH assay were arranged in the following order; *T. corniculata* leaves (0.298±0.015 g/100 g dry weight), *T. foenum-gracium* seed (0.295±0.011 g/100 g dry weight), *T. corniculata* seed (1.193±0.096 g/100 g dry weight) and *T. foenum-gracium* leaves (0.153±0.001 g/100 g dry weight).

Ascorbic acid capable of reducing molecular oxygen, nitrate, and cytochromes a and it is water soluble chain breaking antioxidant known as a reducing agent. Antioxidants are important in the prohibition of human diseases. Compounds with antioxidants activity may function as free radical scavengers, reducing agents, and ROS, protecting the body from degenerative diseases such as Cancer (Parthasarathy et al. 2008). The effect of antioxidants on DPPH is thought to be due to their reducing property (Molham, 2015). Both species of Fenugreek are potent antioxidants and show great DPPH radical scavenging capacity, however the leaves and seeds of *Trigonella foenum-gracium* showed relatively more antioxidant capacity as compared to *Trigonella corniculata*.

Table 3: Phytochemical composition of (g/100g dry weight) of leaves and seeds of selected species of Fenugreek Plant

Parameter studied	<i>Trigonella corniculata</i>		<i>Trigonella foenum-gracium</i>	
	Leaves	Seeds	Leaves	Seeds
TPA	0.741±0.050a	0.599±0.003a	0.856±0.064a	0.539±0.024a
Flavonoids	0.055±0.004a	0.021±0.000a	0.042±0.015a	0.040±0.001a
Ascorbic Acid	0.267±0.031a	0.068±0.008b	0.153±0.006a	0.085±0.009b
IC ₅₀	0.298±0.015a	1.193±0.096b	0.295±0.011a	0.153±0.001a

The DPPH radical scavenging capacity (DPPH RSC) with reference to ascorbic acid and trolox was calculated and represented in Figure 1. The DPPH RSC value in *T. foenum graecum* leaves (39.59%) was found to be more as compared to *T. corniculata* leaves (33.283%). The seeds of the *T. foenum graecum* (31.736%) showed greater DPPH RSC value as compared to *T. corniculata* seeds (36.04%).

T.c = *Trigonella corniculata*; T.f.g = *Trigonella foenum graecum*

Figure 1: DPPH radical scavenging capacity in parts of studied varieties of Fenugreek Plant

The ascorbic acid equivalent total antioxidant activity (AAE-TAOA) and trolox equivalent antioxidant activity (TE-AOA) of the leaves and seeds of studied plants was calculated and is shown in Figure 2. The ascorbic acid and trolox equivalent antioxidant activity of different parts of studied varieties showed that the leaves and seeds of *Trigonella foenum graecum* showed relatively high AAE-TAOA and TE-AOA as compared to leaves and seeds of *Trigonella corniculata*.

Figure 2: Ascorbic acid equivalent total antioxidant activity (AAE-TAOA) and Trolox equivalent total antioxidant activity (TE-AOA) in parts of studied varieties of Fenugreek Plant

IV. Conclusions

Our results showed that the seeds of *T. foenum gracium* are found to possess higher amount of reducing sugars, non-reducing sugars, tannins and saponin as compared to other parts of fenugreek plant. The amino acids, proteins, HCN, ascorbic acid and trolox content were found to be higher in the leaves of *Trigonella corniculata*. Due to their valuable biochemical, phytochemical and antioxidants composition seeds of *T. foenum gracium* and leaves of *Trigonella corniculata* are considered to be important source for nutritional and medicinal purposes.

Conflict of interest

The authors have no conflict of interest.

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