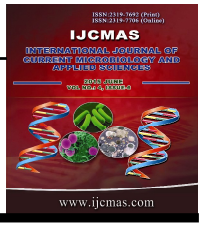
Int.J.Curr.Microbiol.App.Sci (2015) 4(6): 201-210

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 6 (2015) pp. 201-210**
http://www.ijcmas.com



Original Research Article

Biomolecular Characterization of Different Fenugreek Genotypes (*Trigonellafoenum-graecum* L.)

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Keywords

Trigonella foenum-graecum L., Methi, Greek hay, Antioxidant activity, secondary

A B S T R A C T

In the present investigation biomolecular characters in fenugreek seeds were estimated. Results revealed minimum moisture content in genotype JFG.261. Genotype PEB and JFG.261 recorded the higher ash and oil content, respectively. The lowest and the highest phenol content were recorded in the genotype JFG.266 and kasturi methi, respectively. Significantly higher total antioxidant activity was found in genotype kasturi methi. The maximum total soluble sugars and reducing sugars were observed in Guj. methi 2. Genotype PEB estimated the highest free amino acid content with regard to the protein content in the seeds. True protein and crude protein content was recorded maximum in genotype JFG.260 and kasturi methi respectively.

Introduction

Fenugreek (*Trigonellafoenum-graecum*L.) is a self-pollinating crop, which is the native plant of the Indian subcontinent and the eastern Mediterranean region. It is an old medicinal plant and has been commercially used as a traditional spice and medicine. Fenugreek is originally from southern Europe and western Asia, but today it is grown in many parts of world (Naidu *et al.*, 2011).

Fenugreek is known for its pleasantly bitter seeds. The seeds available in whole ground form are used to flavour in many foods including curry powders, spice blends and teas. The fenugreek seeds contain a central hard yellow embryo surrounded by a corneous and comparatively large layer of white semi-transparent endosperm (Betty, 2008).

India is the major producer and exporter of fenugreek. This crop is mainly grown in arid as well as semi-arid region of India. In India, it is grown in 93090 ha area with production of 112845 tonnes and productivity is 1.21 tonnes/ha (Balraj Singh, 2014). In India the major fenugreek growing states are Rajasthan, Gujarat, Madhya Pradesh, Tamilnadu, Uttar Pradesh and Punjab. More than 80% area and production is contributed by Rajasthan state alone. The major districts

growing fenugreek in Gujarat state are Patan and Dahod.

Fenugreek seeds were used as a spice in food preparation as well as an ingredient in traditional medicine. The seeds are vice source of calcium, iron, ß-carotene and other vitamins (Sharma and Raghuram, 1990). Fenugreek seeds and leaves should be included in normal diet of family, especially diet of growing kids, pregnant ladies, puberty regarding girls and elder members of family because they have haematinic value (Bukhari *et al.,* 2008). Fenugreek seed is widely used as galactagogue i.e. milk producing agent by nursing mother to increase inadequate breast supply. Many scientist have recorded that the seeds also contain lysine and L-tryptophan rich proteins, mucilaginous fibres and other biochemical such as saponin, coumarins, sapogenins, phytic acids etc. which are thought for many of its presumed therapeutic effects, many inhibit cholesterol absorption and thought to help lower sugar levels. Therefore fenugreek seeds are used as traditional remedy for the treatment of diabetes and hyper cholesteroleria in Indian medicines (Bhukri *et al.,* 2008, Sauvaire *et al.,* 1991 and Basch *et al.,* 2003).

The purpose of this study was to evaluate fenugreek as new potential source for its higher nutraceutical value. The mucilaginous seeds have medicinal values as a tonic, emollient, carminative, diuretic, restorative etc. (Duke, 2007). Now a days search for natural nutraceutical source is gaining much important. Thus it is important to identify new sources of safe and inexperience nutraceutical constituents of natural origin. Hence, in present investigation an attempt has been made to identify fenugreek genotypes for its higher nutraceutical value through bio molecular characterization.

Materials and Methods

The present experiment was conducted at the Biochemistry department, B. A. College of Agriculture, Anand Agricultural University, Anand (Gujarat) The materials for study consisted of thirteen genotypes/cultivars *viz.*, JFG.179, JFG.226, JFG.234, JFG.240, JFG250, JFG256, JFG.260, JFG.261, JFG263, JFG.266, GUJARATI-METHI.2, PEB, and KASTURI METHI were obtained from Main Vegetable Research Station, Anand Agricultural University, Anand.

The presence of flavonoids, cardiac glycoside, steroids, terpenoids and tannins in the fenugreek seed was determined by the methods described by Sofowara (1993) and Harborne (1973).The methods of A.O.A.C (1984) were used to determine the Moisture content and total ash. The oil content was determining using Petroleum ether in Soxhlet reflux extractor (Chaturvedi and Sanker, 2006). For the estimation of phenols the method as proposed by Malick and Singh (1980). The total antioxidant activity was determined by FRAP (Ferric reducing antioxidant power) assay (Varga *et al.,* 1998). The true protein content in the seed was estimated by Lowry *et al.* (1951) where as crude protein (N x 6.25) was determined using micro Kjeldahl method (A.A.A.C, 2000). The free amino acid was determined using the method suggested by Moore and Stein (1948). Reducing sugars were estimated by the method of Nelson (1944). Total soluble sugar was estimated by phenol sulphuric acid method (Sadasivam and Manickam, 1992). The content of non-reducing sugar was calculated from the difference between soluble sugar and reducing sugar.

The varieties of seed proteins was analysed by using SDS-PAGE. For extraction of

protein each genotypes had three replications and 25 seed for every replication was randomly selected and ground to fine powder with motor and pestle, and a total of 150µl sample buffer was added to a 0.05gm seed powder and mixed thoroughly by vortex in eppendorf tube (1.5ml) with a small glass rod. The extraction buffer contained 0.05 M Tris base 6.5 g/L; 0.007 M citric acid (monohydrate) 1.5 g/L; 0.1% cysteine hydrochloride 1 g/L; 0.1% ascorbic acid (Na salt or free acid) 1 g/L; 1.0% polyethylene glycol (H 3500) l0.0 g/L; 1 mM 2- mercaptoethanol 0.08 mL/L, the final pH 8.0 (Arulsekar and Parfitt, 1986), centrifuged at 18000rpm for 20min. to monitor the movement of the protein in gel, Bromophenol blue was used as a tracking dye. Seed protein was analysed through SDS-PAGE using 12% polyacrylamide gel. The molecular weight dissociated polypeptides were determined using protein standard (M.W SDS- 2 to 150 kDa) of Merck, India. The gels were stained with Coomassie brilliant blue and distained till the background became transparent.

Result and Discussion

Phytochemical screening

Phytochemicals means the chemicals which may have biological importance but are not established as important nutrients. In a narrower sense the terms phytochemical describe the number of secondary metabolic compounds found in plants. Scientists estimate that about 10,000 different phytochemicals having the capability to have an effect on diseases like cancer and metabolic syndrome etc.

The result of the preliminary phytochemical screening from fenugreek seeds (Table 1) shows the presences of different phytochemicals prepared indifferent solvent extracts. The phytochemical tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes. Quantitative assessments of the different phytochemicals detected during investigation was graded as –ve for 0, +ve for 1, ++ve for2 and +++ve for 3. The observations and inferences made in the phytochemical tests are presented as follows:

Flavonoids: Flavonoids are acts as anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity. A yellow coloration was observed in all the fenugreek genotypes except JFG-261 indicating thereby the presence of flavonoids in all fenugreek genotypes screened.

Cardiac glycosides: Cardiac glycoside is useful for heart failure and cardiac arrhythmia (Denwick, 2002). A brown ring obtained at the interface indicated the presence of a de-oxy sugar characteristic of cardenolide i.e. Cardiac glycosides was observed in seven genotypes of fenugreek out of thirteen genotypes.

Steroids: Steroids are important for cardio-tonic activity. Steroid possesses insecticidal and antimicrobial properties (Callow, 1936). A reddish brown ring at the interface was observed with the extract of all fenugreek genotypes/cultivars except JFG-261 plants indicating the presence of steroids.

Terpenoids: Terpenoidshave been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, anti-hyperglycaemic, anti-inflammatory and immunomodulatory properties (Rabi *et al.*, 2009; Wagner and Elmadfa, 2003). A deep

red colour was observed in ten genotypes of fenugreek out of thirteen genotypes.

Tannins: Plant tannins are a large, diverse group of polyphenolic compounds found throughout several species in the plant kingdom. Tannins have a protective function in the bark of the roots and stems, or any outer layers of plants. They are astringent in nature due to their high polyphenol content. This attribute confers the ability to form strong complexes with proteins, starches and other macromolecules (Catherine Clinton, 2009). Tannins are able to inhibit HIV replication selectivity and are used as diuretic. Tannin has recognized for pharmacological properties and make trees and shrubs a difficult meal for many caterpillars (Heslem, 1989). A green precipitate was observed in only Kasturi Methi indicating thereby the presence of tannins in only this genotype analysed.

Moisture

The mean value for genotypes (Table 2) showed significantly the highest and the lowest moisture content was found in JFG-226 and JFG.260 (5.92%), respectively. The genotypes JFG.261, Kasturi methi and JFG.250 were recorded at par, in case of genotype JFG.234 and JFG.240also recorded more or less same. Singh and his colleagues have studied the contribution of fenugreek (*Trigonellafoenum-graecum*L.) seeds towards the nutritional characterization. They have reported that the mean moisture per cent in seeds were recorded to be 8.86% and it was ranged from 7.57 to 11.51%.

Ash

The present investigation revealed that the ash content was found to be significant ($p<0.05$) among all genotypes of fenugreek. The lowest ash content found in genotype JFG.263 (1.25%) and the highest found in genotype PEB (5.23%). The non-significant differences were recorded among genotype JFG.226, JFG.250, JFG.179 and JFG.266. The presence of ash in fenugreek plant in such quantities are satisfying, because of the high importance of mineral for health maintenance and development, where the minerals are essential for human body, they are basic content of many of body tissues, such as calcium and phosphorous for bone and iron for blood and muscles. Ash consider basic element of biomolecules in addition to their roles in connectivity process and in all of biochemical reaction (Mann Jim and Truswell Stewart, 2002).

Oil

The data on fenugreek seed oil is presented in Table 2. The oil content was varied from 2.56% to 4.74%. Significantly the highest oil percentage was recorded in JFG.261 (4.74%) and lowest oil percentage was recorded in Gujarat methi-2 (2.56%). The genotype JFG.266, Kasturi methi, JFG.179, JFG.263, JFG.266, PEB and JFG.240 were recorded at par.

Phenol

The total phenol of different fenugreek seeds cultivars were duplicated in table 2. The content was varied from 1.69 to 3.22%. The higher percentage of total phenol was recorded in kasturi methi (3.22%) and lower was observed in JFG.266 (1.69%).The results indicate that vegetables containing high phenolic may provide a source of dietary anti-oxidants. The phenolic compounds may contribute directly to the antioxidant action; therefore, it is necessary to investigate total phenolic content (Bukhari *et al.*, 2008).

Total antioxidant activity

Total antioxidant activity of Fenugreek seeds was calculated and data are presented in Table- 2. The content was varied from 8.5 to 13.85 mg/g. significantly the highest percentage of total antioxidant activity was recorded kasturi methi (13.85 mg/g) and the lowest was observed in JFG.261 (8.5 mg/g). The genotype JFG.256 and JFG.226 recorded were at par. The reducing power of bioactive compounds is associated with antioxidant activity. So it is necessary to determine the reducing power of phenolic constituents elucidate the relationship between the antioxidant effects and there reducing power (Yildirim *et al.,* 2001; Siddhuraju *et al.,* 2002). Our results are agreement with results of Bukhari *et al.* (2008). They have reported that all extracts of the fenugreek exhibit antioxidant activity. These findings suggest that the fenugreek extracts could act as potent source of antioxidants.

Total soluble sugars, reducing sugars and non-reducing sugars

Total soluble sugars, reducing sugars and non-reducing sugars of different fenugreek seeds cultivars are depicted in Table- 3. The content of total soluble sugars was varied from 4.15% to 11.01%. Significantly the highest percentage of total soluble sugar was recorded in Gujarat methi-2 (11.01%) and the lowest were recorded in JFG.226 (4.15%). The content of reducing sugars was varied from 1.99% to 3.47%. Significantly the highest percentage of reducing sugars was recorded in Gujarat methi-2 (3.47%) and the lower reducing sugars found in genotype JFG.260 (1.99%) which was at par with genotype JFG.226 and Kasturi methi. The content of non-reducing sugars was varied from 2.12% to 7.94%. Significantly the highest percentage of non-reducing sugars was recorded in JFG.250 (7.94%) and the lowest non-reducing sugars found in genotype JFG.226 (2.12%).

The principle function of carbohydrates is to serve as a major source of energy for the body. Each gram of carbohydrate yields 4 Kcal of energy regardless of its source. In Indian diets 60 to 80% of energy is derived from carbohydrates. The findings are in consonance with Gopalan *et al.,* (1992); Kochhar *et al.,* (2006); Saibaba and Raghuram, (1977) and Sumayya *et al.* (2012). Reducing sugars and non-reducing sugar of plants varied from 0.78 to 4.43, 1.03 to 8.0. Lower total, reducing and non-reducing sugar and almost similar starch content of fenugreek seed has been reported by E1-mandy and Sebaiy (1983).

Free amino acid

The free amino acid in different genotype of fenugreek seeds were depicted in Table- 3. Significantly the highest and lowest percentage of free amino acid was recorded in PEB (13.85%) and JFG.240 (10.63%); respectively. Sauvaire *et al.* (1984) have observed that 4-hydroxy isoleucine represent up to 80% of free amino acid in fenugreek seeds. The concentration does not decrease in later stage of maturation of the seeds, but it absent from the seed reserve protein.

True protein

The true protein in different genotype of fenugreek seeds were recorded in Table- 3. The content was varied from 7.26% to 11.26%. Significantly the highest and lowest percentage of true protein was recorded in JFG.260 (11.26%) and JFG.263 (7.26%); respectively. The non-significant differences were recorded among JFG.250, JFG.179, JFG.234 and JFG.261.

Table.1 Phytochemical screening of 13 different genotypes

Name of Genotype	Flavonoid	Cardiac glycoside	Steroid	Terpenoid	Tanin
JFG.179	1	0	3	1	0
JFG.226	1	0	2	1	0
JFG.234	1	0	1	0	0
JFG.240	1	1	1	0	0
JFG.250	3	3	1	3	0
JFG.256	1	1	1	1	0
JFG.260	1	0	2	3	0
JFG.261	0	0	0	0	0
JFG.263	1	2	1	2	0
JFG.266	3	3	1	3	0
Guj.methi 2	1	0	2	2	0
PEB	2	2	1	3	0
KM	2	3	3	2	1

(3) High presence; (2) Moderate; (1) Low; (0) absent

Table.2 The moisture, ash, oil, phenol and total antioxidant activity in different fenugreek seeds

Genotype	Moisture (%)	Ash (%)	Oil (%)	Phenol (%)	Total Antioxidant Activity(mg/g)
JFG.179	9.58	2.22	3.16	2.56	11
JFG.226	13.63	2.16	2.56	1.98	9.25
JFG.234	10.75	2.56	3.62	2.38	10.5
JFG.240	10.57	3.30	3.43	2.52	10.9
JFG.250	7.8	2.20	4.47	2.07	10
JFG.256	8.74	2.51	3.59	2.36	9.21
JFG.260	5.92	2.69	4.26	1.90	10.62
JFG.261	7.18	2.90	4.74	1.98	8.5
JFG.263	6.86	1.25	3.25	1.78	9.71
JFG.266	8.71	2.23	3.34	1.69	9.9
Guj.methi-2	8.33	5.05	2.56	1.71	11.7
PEB	6.73	5.22	3.35	1.72	11.5
Kasturi methi	7.47	3.80	3.05	3.22	13.8
S.Em±	0.129	0.109	0.317	0.047	0.034
C.D. at 5%	0.376	0.318	0.922	0.137	0.100
CV%	2.592	6.456	15.715	3.804	0.566

Table.3 Total soluble sugar, reducing sugar, total protein, crude protein, free amino acid in different fenugreek seeds

Genotype	Total Soluble Sugar (%)	Reducing Sugar (%)	Non reducing sugar (%)	Free Amino Acid (%)	True Protein (%)	Crude Protein (%)
JFG.179	6.33	1.99	4.34	12.04	9.62	14.23
JFG.226	4.15	2.03	2.12	11.58	10.17	14.73
JFG.234	5.87	2.51	3.35	12.18	10.01	17.50
JFG.240	8.36	2.36	5.99	10.63	8.63	18.67
JFG.250	10.22	2.28	7.94	11.93	9.14	21.56
JFG.256	7.40	2.40	5.00	13.37	8.56	17.65
JFG.260	7.45	2.51	4.98	12.55	11.26	23.34
JFG.261	8.66	2.49	6.17	12.20	9.86	22.90
JFG.263	9.12	2.54	6.66	12.58	7.26	21.15
JFG.266	8.82	2.29	6.53	12.35	10.91	22.47
Guj.methi-2	11.01	3.47	7.54	11.02	10.45	27.57
PEB	7.90	3.07	4.82	13.85	8.02	26.40
Kasturi methi	9.26	2.04	7.22	11.16	9.17	32.24
S.Em±	0.01	0.02	0.154	0.05	0.03	0.25
C.D. at 5%	0.28	0.07	0.78	0.15	0.1	0.73
CV%	2.1	1.62	2.20	0.72	0.57	2.00

Fig.1 Dandogram of 13 different fenugreek seeds genotype based on Jaccard's similarity coefficients

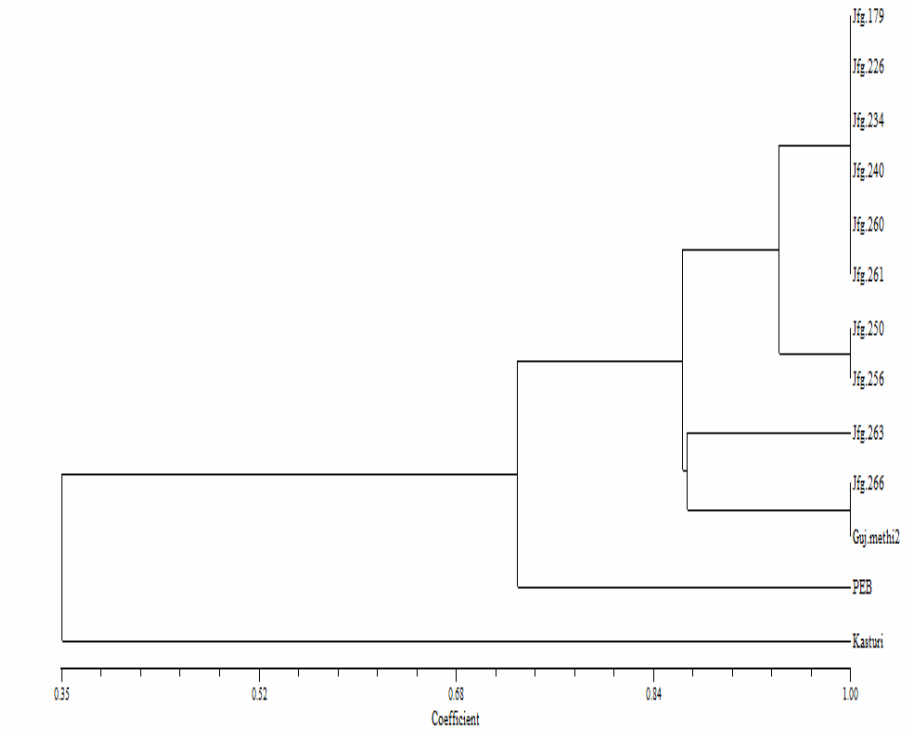
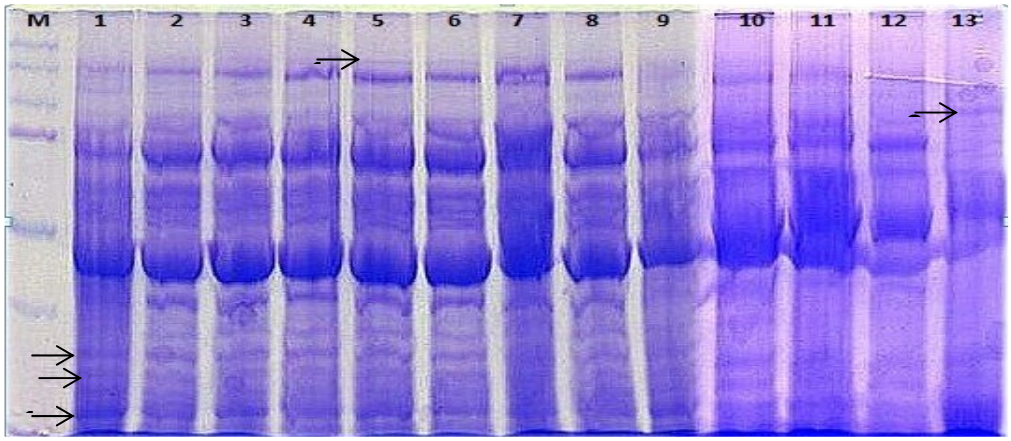


Plate.1 SDS PAGE banding pattern of different genotype of fenugreek seeds



Srinivasan (2006) reported that cooking does not affect the quality of fenugreek seed proteins. It is evidenced in the animal study that the replacement of casein diet up to 10% by fenugreek seeds did not produce any harmful effect in protein quality of casein as studies on animal subjects has been evidenced for protein efficiency ratio, protein digestibility and net protein utilization, debitterized fenugreek seeds are rich in protein and lysine.

Crude protein

The percentage of crude protein present in different genotype of fenugreek seeds were recorded in Table- 3.The content was varied from 14.23% to 32.24%. Significantly the highest percentage of total protein was recorded in kasturi methi (32.24%) and the lower percentage of crude protein was recorded in JFG.179 (14.23%), which was at par with JFG.226. The genotype JFG.263 and JFG.250 recorded were at par. The estimation of nitrogen was done by Kjeldahl method wherein the protein content was obtained by multiplying the nitrogen value with 6.25, (A.O.A.C., 1990). Gopalan *et al.* (1992) reported that fenugreek seeds richest source of crude protein (25.8%).

Protein electrophoresis

The soluble protein of fenugreek seeds were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method. The banding pattern was present in plate-1. The band no.1 was present in genotype JFG.250 and JFG.256 with Rm value 0.130cm. In all genotypes total eighteen bands having Rm value ranged from 0.130 to 0.937 were recorded. The band no.2 was present in all genotypes except genotype JFG.263 and Kasturi methi. The band no.3 having Rm value 0.247 was present only in genotype Kasturi methi. In case of band no.4, 5 and 6 was absent in genotype Kasturi methi. Band no.9, 13, 15 and 18 were absent in genotype PEB. The protein band no.7, 11 and 17 were present in all fenugreek genotypes. Sanjeevrajan *et al.* (2012) evaluated protein from various legumes seeds using different protein extraction methods. They have compared seven different protein extraction methods from the seeds of four different leguminous plants.

Jaccard's similarity coefficient on the basis of presence and absence of bands was calculated for all possible pairs of 13

genotypes of fenugreek. The highest similarity Index value 1.0 was found among genotype JFG.179, JFG.226 and JFG.250. The highest similarity index was also found between genotype JFG.240 and JFG.263, while the minimum similarity index value 0.40 was found between JFG.266 and guj.methi-2.

Over all it can be concluded that from present experiment that seeds of kasturi methi contain higher amount of cardiac glycoside and steroid whereas moderate amount of flavonoids and terpenoids. Flavonoids and steroids were present in all genotypes whereas tannin was only present in kasturi methi. The genotypes JFG.250 and JFG.266 contain higher amount of flavonoids, cardiac glycoside and terpenoids. JFG.261 genotype doesn't contain any of phytochemicals. Kasturi methi registered with higher relative water content, phenol, crude protein and Total antioxidant activity. Seeds of PEB have maximum ash and free amino acids. Thus this genotype may be used for breeding purpose.

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