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Nutritional and antioxidant components of fenugreek (*Trigonella foenum-graecum* L.) seedlings

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Abstract
Fenugreek (*Trigonella foenum-graecum* L.) is regarded as a useful medicinal plant for the treatment of various dysfunctions and diseases in recorded history and in Ayurveda also. Fresh leaves are also used as vegetables in the diets. Fenugreeks leaves is known for its medicinal qualities such as antidiabetic, anticarcinogenic, hypocholesterolemic and antioxidant. In present investigation, promising genotype of fenugreek were evaluated for antioxidant activity and its related component from leaves at different growth stages. The result showed that the genotype JFG-204 and JFG-205 exhibits statistically at par as well as higher mean value compared to other genotypes, for ascorbic acid content, Flavonoid content as well as for antioxidant activity. Highest mean total phenol content recorded for genotype JFG-80, whereas lowest value was recorded for GM-2 (0.600 $mg.g^{-1}$ %). A strong correlation between total phenolic content and antioxidant activity was found, high total phenol content increases antioxidant activity.

Keywords: *Trigonella foenum graceum* L. phenol, antioxidant activity, DPPH, carotenoid, flavonoid

Introduction
Fenugreek (*Trigonella foenum-graecum* L.) is one of the well known spices in human food. Its seeds and green leaves are used in food as well as in medicinal application for the treatment of various dysfunctions and diseases in recorded history and in Ayurveda also. Fresh leaves are also used as vegetables in the diets. It is used in functional food, traditional food, nutraceuticals as well as in physiological utilization such as antibacterial, anticancer, antiulcer, anthelmintic, hypocholesterolemic, hypoglycemic, antioxidant, and antidiabetic agent. It has beneficial influence on digestion and also has the ability to modify food texture. Fenugreeks leaves rich in bitterness are loaded with lots of medicinal properties (Agarwal *et al.*, 2013) [1]. It is known for its medicinal qualities such as antidiabetic, anticarcinogenic, hypocholesterolemic, antioxidant, and immunological activities. In general, plants equipped with various compound having a phenolics, ascorbic acid etc., for their defense system also possessed higher antioxidant property. Thus the present experiment was planned to evaluate the antioxidant property and its contributing components in leaves of promising genotypes of fenugreek.

Materials and Methods
The present investigation was conducted in green house condition at Department of Biochemistry, College of Agriculture, Junagadh Agricultural University, Junagadh (Gujarat). The seeds of following 10 genotypes of fenugreek used for the present study were obtained from the Centre of Research for seed spices, SDAU, Jagudan. Seeds were grown in pots. The leaves were collected from different time intervals and used for the analysis for different parameters.
The phenol content in was determined by method of Malik and Singh (1980) [15] using methanolic extract. Standard graph was prepared for quantification using gallic acid as a standard. Total Ascorbic acid was quantified according to the method described by Omaye *et al.*, (1979) [18]. Total ascorbic acid was ex-pressed in mg per 100g leaves sample. Total flavonoid was estimated using 1 ml of methanolic extract in which 0.5 ml of 2% w/v $AlCl_3$ in methanol and 0.5 ml potassium acetate (120 mM) were added and incubated at room temperature for 30 minutes. Absorbance was read at 415 nm (Chanda and Dave, 2009) [4]. Total chlorophyll in seedling was determined by DMSO method (Hiscox and Israelstam, 1979) [7]. Total carotenoid was measured from leaves of fenugreek at three different growth stages as per Mahadevan and Shridhar, (1986) [14].

Qualitative test for alkaloids was carried out using Mayer's reagent. A cream colour precipitation was obtained showed the presence of alkaloid (Hossain *et al.,* 2013) [8]. The percentage of DPPH scavenging activity for determining antioxidant property was measured as per (Gyamfi *et al.,* 1999) [6].

Results and Discussion

The assessment of antioxidant potential and its contributing components like total phenol, ascorbic acid, carotenoids etc., might be a fruitful approach for advocating fenugreek leaves for their nutraceutical importance. The results of individual are depicted as under.

Total phenol content

Total phenol content from seedlings (10, 20 and 30 DAG (Days after germination)) of different fenugreek genotypes were found statistically significant for different stage, genotypes as well as for the interaction (Table 1).

Table 1: Total phenol and ascorbic acid content (mg.g^{-1}%) of fenugreek genotypes at different growth stages.

Sr	Genotype (G)	Total phenol (mg.g^{-1}%)				Ascorbic Acid t (mg.g^{-1}%)			
		Stages			Mean (G)	Stages			Mean (G)
		S1 (10 DAG)	S2 (20DAG)	S3 (30 DAG)		S1 (10 DAG)	S2 (20DAG)	S3 (30 DAG)	
1	JFG-80	0.442	0.510	0.759	0.570	1.91	3.92	5.61	3.81
2	JFG-194	0.372	0.410	0.597	0.460	1.60	4.42	5.23	3.74
3	JFG-204	0.333	0.447	0.610	0.463	2.10	4.39	5.89	4.12
4	JFG-205	0.335	0.407	0.610	0.451	2.06	3.68	6.50	4.08
5	JFG-208	0.334	0.450	0.607	0.464	1.46	4.50	5.46	3.80
6	JFG-211	0.378	0.411	0.615	0.468	2.41	3.74	5.88	4.00
7	JFG-220	0.384	0.457	0.587	0.476	1.67	4.59	6.15	4.13
8	JFG-225	0.371	0.403	0.617	0.464	2.62	3.86	5.76	4.08
9	JFG-241	0.388	0.410	0.587	0.462	1.62	4.51	5.35	3.82
10	GM2	0.297	0.447	0.527	0.424	2.01	3.59	5.95	3.85
Mean (S)		0.363	0.435	0.611		1.946	4.120	5.778	
		S	G	S x G		S	G	S x G	
S.Em+		0.008	0.012	0.024		0.039	0.072	0.126	
C.D. at 5%		0.022	0.034	0.069		0.113	0.206	0.357	
C.V.%		7.46				5.54			

The lowest mean value of phenol was found at 10 DAG (0.363 mg.g^{-1}%) and highest value recorded at 30 DAG (0.871 mg.g^{-1}%). Genotypic differences for total phenol content were also found significant. Among the genotypes, mean total phenol content varied from 0.424 to 0.570 mg.g^{-1}%. The highest value recorded for genotype JFG-80, whereas lowest value was recorded for GM-2 (0.600 mg.g^{-1}%). While In case of 10 to 30 days after germination, there was increased in the total phenol content in a leaves of all fenugreek genotypes. At 30 days, among the 10 genotypes the total phenol was remain significantly higher in JFG-80 (0.759 mg.g^{-1}%) and the lowest was found in GM-2 (0.527 mg.g^{-1}%). Researcher also reported higher phenol content was the characteristic of resistant variety (Kandoliya and Vakhariya, 2013; Mori *et al.*, 2017; Patel *et al.,* 2015; Joshi *et al.,* 2018) [10, 17, 19, 9]. It was also revealed gradual accumulation of proline and total phenols in seedling leaves. Phenols in plants may perform useful effects through free radicals scavenging (Chun *et al.,* 2003) [5]. This also reflects by increase in antioxidant activity (Table 4).

Ascorbic acid

Ascorbic acid is a part of defense system of plant against pathogen along with other antioxidant component (Kandoliya and Vakhariya, 2015) [11]. In present experiment, ascorbic acid content in fenugreek tissues of 10 genotypes at three different growth stage viz., 10 DAG, 20 DAG and 30 DAG is depicted in Table 1. Among the different growth stages, mean value of ascorbic acid content varied between 1.94 to 5.77 mg.g^{-1}%. Among the genotypes, mean Ascorbic acid content varied from 3.74 to 4.13 mg.g^{-1}%. The highest value recorded for genotype JFG-220, whereas lowest value was recorded for JFG-194 (3.74 mg.g^{-1}%). At 10 DAG, the ascorbic acid was significantly higher in JFG-225 (2.62 mg.g^{-1}%) which was followed by JFG-211, JFG-204, JFG-205. The lowest content was found in JFG-208 (1.46 mg.g^{-1}%). While in case of 20 days after germination, there was continuous increase in the ascorbic acid content of the seedlings of all the fenugreek genotypes. The highest ascorbic acid value were found in JFG-220 (4.59 mg.g^{-1}%). The lowest ascorbic acid was found in GM-2 (3.59 mg.g^{-1}%). At 30 days, the ascorbic acid was remain significantly higher in JFG-205 (6.50 mg.g^{-1}%) which was followed by JFG-220, GM-2, JFG-204. The lowest ascorbic acid content was found in JFG-194 (5.23 mg.g^{-1} %). Our data were in agreement with Singh *et al*. (2010) [21]. They also found that ascorbic acid content increased with maturity in methi leaf, stem and fruit.

Total carotene

Among the different growth stages, mean value of total carotene content varied between 0.564 to 2.294 mg.g^{-1} % (Table 2). The highest value was found at 30 DAG (2.564 mg.g^{-1}%). Among the genotypes, mean total carotene content varied from 1.103 to 1.554 mg.g^{-1}%. The highest value recorded for genotype GM-2, whereas lowest value was recorded for JFG-204 (1.103 mg.g^{-1}%). At 10 day among the 10 genotypes the total carotene content was significantly higher in JFG-241 (0.634 mg.g^{-1}%) which was followed by JFG-204, JFG-220, JFG-208. The lowest total carotene content was found in JFG-194 (0.494 mg.g^{-1}%). Total carotene continuously increased with the growth of plants. The lowest total carotene content was found in JFG-80 (0.87 mg.g^{-1}%). At 30 days, among the 10 genotypes, the carotenoid content was significantly higher in JFG-208 (2.71 mg.g^{-1}%) which was followed by JFG-194, GM-2, JFG-205. The lowest total carotene content was found in JFG-204 (1.79 mg.g^{-1}%). During growth of seedlings, carotenoid follows the trend of chlorophyll (Aggrwal *et al.,* 2013) [1].This was also reflects in results of present experiment.

Table 2: Total carotene (mg.g^{-1}%) and Total chlorophyll (mg.g^{-1}) content of fenugreek genotypes at different growth stages.

Sr	Genotype (G)	Total carotene (mg.g^{-1}%)				Total Chlorophyll (mg.g^{-1})			
		Stages (S)			Mean (G)	Stages (S)			Mean (G)
		S1 (10 DAG)	S2 (20DAG)	S3 (30 DAG)		S1 (10 DAG)	S2 (20DAG)	S3 (30 DAG)	
1	JFG-80	0.575	0.877	2.067	1.173	1.040	2.067	2.653	1.920
2	JFG-194	0.494	1.068	2.557	1.373	1.077	2.033	2.687	1.932
3	JFG-204	0.599	0.912	1.799	1.103	0.997	2.037	2.790	1.941
4	JFG-205	0.553	0.954	2.487	1.331	1.093	2.243	2.370	1.902
5	JFG-208	0.586	1.164	2.712	1.487	1.110	1.963	2.740	1.938
6	JFG-211	0.512	0.970	1.994	1.159	0.947	2.177	2.437	1.853
7	JFG-220	0.597	1.246	2.317	1.387	1.263	2.200	2.760	2.074
8	JFG-225	0.526	1.503	2.482	1.504	1.020	1.910	2.800	1.910
9	JFG-241	0.634	0.927	1.958	1.173	1.187	2.110	2.780	2.026
10	GM2	0.564	1.534	2.564	1.554	1.103	2.033	2.763	1.967
Mean (S)		0.564	1.116	2.294		1.084	2.077	2.678	
		S	G	S x G		S	G	S x G	
S.Em+		0.038	0.069	0.120		0.024	0.044	0.977	
C.D. at 5%		0.107	0.197	0.341		0.079	NS	0.218	
C.V.%		15.76				6.84			

Chlorophyll content

The data on total chlorophyll content from seedlings of different fenugreek genotypes recorded at 10 day, 20 day and 30 day after germination is presented in Table 2. Among the different growth stages, mean value of chlorophyll content varied between 1.084 to 2.678 mg.g^{-1}. The highest value was found at 30 DAG (2.678 mg.g^{-1}). Genotypic difference for chlorophyll content were found to be nonsignificant. The chlorophyll content at 10 days, the mean value varied from 0.947 to 1.263 mg.g^{-1}. In this day the highest chlorophyll content was observed in JFG-220 which followed by JFG-241, JFG-208, GM-2. The content gradually increased at 20 DAG and 30 DAG for all the genotypes. At 30 days after germination, the mean of chlorophyll content was ranged from 2.370 to 2.800 mg.g^{-1}. In this day the maximum chlorophyll content was observed in JFG-225(2.800 mg.g^{-1}). Brinda *et al.* (2015) [3] also reported significant increased in chlorophyll content during germination. Increase in total chlorophyll content cope with increased carbohydrate content requirement of plants (Aggrwal *et al.*, 2013) [1]. These results was in agreement with our results showing accumulation of chlorophyll content during seedling growth.

Alkaloid

Qualitative test of alkaloid based on intensity of colour from fenugreek tissues of 10 genotypes at various growth stage viz., 10 DAG, 20 DAG and 30 DAG were described in Table. 4. It showed that the initial stages, alkaloid remains very high in the imbibed seed of fenugreek. However, JFG-80, JFG-220 and JFG-241 contain moderate alkaloids compared to other genotypes of fenugreek. The contains continuously decreased in all the genotypes during germinations, however no clear trend found for the genotypes during growth stages. Meghwal and Goswami (2012) [16] considered the alkaloid of fenugreek seed have pharmacological effect.

Table 3: Presence of alkaloid in fenugreek genotypes at different growth stages

Stages	Genotypes									
	JFG-80	JFG-194	JFG-204	JFG-205	JFG-208	JFG-211	JFG-220	JFG-225	JFG-241	GM-2
10 DAG	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+
20 DAG	+	+	+ +	+ +	+ +	+ +	+	+	+	+
30 DAG	+	+	+ +	+	+	+	+	+	+	+

+=Low, ++= Moderate and +++=High, based on turbidity of color appeared

Flavonoid

The data on Flavonoid content of seeds and seedlings of different fenugreek genotypes recorded at 10, 20 and 30 days after germination is presented in Table. 4.

Table 4: Flavonoid content (mg.g^{-1}%) and Antioxidant activity (%) of fenugreek genotypes at different growth stages.

Sr	Genotype (G)	Flavonoid (mg.g^{-1}%)				Percent radical scavenging activity (DPPH)			
		Stages (S)			Mean (G)	Stages (S)			Mean (G)
		S1 (10 DAG)	S2 (20DAG)	S3 (30 DAG)		S1 (10 DAG)	S2 (20DAG)	S3 (30 DAG)	
1	JFG-80	6.890	4.900	2.190	4.660	35.417	44.297	52.802	44.172
2	JFG-194	7.420	4.870	2.767	5.019	39.127	51.123	61.000	50.417
3	JFG-204	6.837	4.960	3.463	5.087	40.897	51.547	63.230	51.891
4	JFG-205	7.047	4.993	2.983	5.008	40.240	52.003	62.876	51.706
5	JFG-208	7.123	4.853	2.903	4.960	40.320	50.743	62.735	51.266
6	JFG-211	7.483	4.887	2.407	4.926	39.673	51.750	61.659	51.027
7	JFG-220	7.053	5.813	2.590	5.152	40.220	47.927	57.867	48.671
8	JFG-225	6.543	5.657	2.267	4.822	38.993	49.147	58.534	48.891
9	JFG-241	6.897	4.563	2.357	4.606	41.460	50.903	61.216	51.193
10	GM2	6.620	5.167	2.143	4.643	40.380	50.933	60.952	50.755
Mean (S)		6.991	5.066	2.607		39.673	50.037	60.287	
		S	G	S x G		S	G	S x G	
S.Em+		0.7	0.11	0.22		0.243	0.383	0.766	
C.D. at 5%		0.19	0.31	0.62		0.682	1.078	2.157	
C.V.%		6.53				3.14			

Among the genotypes, mean Flavonoid content varied from 5.354 to 6.283 mg.g^{-1}%. The highest value recorded for genotype JFG-204 (6.283 mg.g^{-1} %) followed by JFG-205(6.251 mg.g^{-1} %), whereas lowest value was recorded for JFG-80 (5.354 mg.g^{-1}%). The mean of Flavonoid content was varied from 7.437 to 9.980 mg.g^{-1}% among 10 fenugreek genotypes. The maximum Flavonoid content was observed in JFG-205 (9.980 mg.g^{-1}%) which followed by JFG-204. The lowest Flavonoid content was found in JFG-80 (7.437 mg.g^{-1}%). It was also noted that high content of Flavonoid contributed to antioxidant activity in other crops also (Kandoliya *et al.,* 2015 and 2016) [12, 13].

Antioxidant Activity

The assessment of antioxidant potential might be a fruitful approach for advocating them as nutraceutical importance. Antioxidant activities of 10 fenugreek genotypes were measured as DPPH free radical scavenging capacity. Methanol extract of seed and seedling showed significant variation in antioxidant activity at different growth stages (Table 4). Among the different growth stages, mean value of antioxidant activity varied between 39.673 to 60.267 % and found statistically significant. Genotypic difference for antioxidant activity was also found statistically significant. Among the genotypes, mean activity was varied from 44.172 to 51.891 %. The highest value was recorded for genotype JFG-204 followed by JFG-205, whereas lowest value was recorded for JFG-80 (44.172 %). The activity increased with the increase in seedling growth. At 30 days after germination, the mean of antioxidant activity was increased in all genotypes varied from 52.802 to 63.230%. This was coinciding with the higher ascorbic acid and phenol content at this stage in present study. Behairy *et al.,* (2012) [2] also reported that the application of ascorbic acid to fenugreek seed apparently increased antioxidant activity. Phenols also perform useful effects through free radicals scavenging activity (Chun *et al.,* 2003) [5]. In this day the maximum antioxidant activity was observed in JFG-204 which followed by JFG-205. The lowest antioxidant activity was found in JFG-80 (37.260%). The results reveal that at all the stages, fenugreek exhibit antioxidant activity. These findings suggest that the fenugreek could act as potent source of antioxidants (Seasotiya *et al.,* 2014) [20].

Conclusion

The assessment of antioxidant potential and its contributing componants like total phenol, ascorbic acid, carotenoids etc., might be a fruitful approach for advocating fenugreek leaves for their nutraceutical importance. The result showed that the genotype JFG-204 and JFG-205 exhibits statistically at par as well as higher mean value compared to other genotypes, for ascorbic acid content, Flavonoid content as well as for antioxidant activity. Highest mean total phenol content recorded for genotype JFG-80, whereas lowest value was recorded for GM-2. A strong correlation between total phenol content and antioxidant activity was found, high total phenol content increases antioxidant activity. These findings suggest that the fenugreek could act as potent source of antioxidants.

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