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Rajeshwari S Durgad
Ph.D. Scholar, Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Horticulture, Bagalkot, Karnataka, India

Vishwanath YC
Assistant Professor, Department of Plantation, Spices, Medicinal and Aromatic crops, KRCCH, Arabhavi, Karnataka, India

Vijayakumar B Narayanapur
Associate Professor and Head, Department of Plantation, Spices, Medicinal and Aromatic crops, COH, Bagalkot, Karnataka, India

Rekha B Chittapur
Assistant Professor, Department of Genetics and Plant Breeding, COH, Bagalkot, Karnataka, India

Venkateshalu
Director of Extension, University of Horticultural Sciences, Bagalkot, Karnataka, India

Lokesh MS
Professor (Plant Pathology) and Special Officer (Seeds), University of Horticultural Sciences, Bagalkot, Karnataka, India

Corresponding Author:
Rajeshwari S Durgad
Ph.D. Scholar, Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Horticulture, Bagalkot, Karnataka, India

Quantitative determination of biochemical constituents in fenugreek seeds

Rajeshwari S Durgad, Vishwanath YC, Vijayakumar B Narayanapur, Rekha B Chittapur, Venkateshalu and Lokesh MS

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Abstract
Fenugreek (*Trigonella foenum-graecum* L.) is an important seed spice valued for its nutritional richness and medicinal properties, largely attributed to its bioactive phytochemical constituents. The present investigation was carried out to quantify major biochemical components in seeds of 55 fenugreek collections evaluated during two consecutive *rabi* seasons (2023-24 and 2024-25) at the College of Horticulture, Bagalkot, Karnataka. Quantitative estimation of total phenols, flavonoids, tannins, saponins and antioxidant activity was performed using standard biochemical methods and pooled mean data across both seasons were analyzed statistically. The results revealed highly significant variation among the collections for all the biochemical traits studied. Pusa Early Bunching recorded the highest levels of total phenols, flavonoids, tannins and antioxidant activity, followed by DFC-15 and Lam sel-2, whereas HUB-4 exhibited the lowest values for most of these parameters. In contrast, the highest saponin content was observed in HUB-4, while Afg-3 recorded the minimum. The biochemically superior collections identified in the present study possess enhanced antioxidant potential and nutraceutical value. These collections can be effectively utilized for the development of functional foods, nutraceutical products and for further improvement programmes in fenugreek.

Keywords: Fenugreek collections, seeds, biochemical constituents, nutraceutical potential

Introduction
Fenugreek (*Trigonella foenum-graecum* L.) is an annual dicotyledonous legume belonging to the family Fabaceae and is one of the important seed spice crops cultivated in many parts of the world. The crop is predominantly self-pollinated and well adapted to a wide range of agro-climatic conditions, particularly arid and semi-arid regions (Chaudhary *et al.*, 2018) [6]. It has been cultivated since ancient times and is extensively grown for its seeds and tender leaves, which are widely used in culinary preparations. Its ability to thrive under diverse environmental conditions, coupled with its multipurpose utility, has contributed to its economic importance and widespread cultivation, especially in South Asian and Middle Eastern regions.

The seeds of fenugreek possess a rich nutritional profile and are recognized for their high content of energy-giving and bioactive constituents. They contain carbohydrates (48%), proteins (approximately 25.5%), mucilage (around 20%), lipids (nearly 7.9%) and saponins (about 4.8%). They are a valuable source of several essential vitamins such as thiamine (B_1), riboflavin (B_2), niacin (B_3), pyridoxine (B_6), folic acid (B_9), along with vitamins A and C. In addition, fenugreek seeds supply important mineral nutrients including calcium, potassium, iron, copper, magnesium, zinc, manganese and selenium, which contribute significantly to their nutritional and therapeutic importance (Rao and Sharma, 1987) [15]. Anatomically, the endosperm of the seed develops into a gum-like matrix that surrounds the embryo and cotyledons. This mucilaginous layer is composed mainly of galactose, which is digestible, and mannan, a non-digestible polysaccharide, present in nearly equal proportions. The medicinal efficacy of fenugreek seeds is further enhanced by the presence of several secondary metabolites such as coumarin, diosgenin (0.4-1.26%), trigonelline (0.2-0.4%) and other steroidal compounds (Zandi *et al.*, 2015; Mahmooda and Yahyab, 2017) [19, 12].

This multipurpose crop has wide range of applications in food, fodder and traditional medicine. The fully matured seeds are extensively used as a spice and are an essential

component of many culinary preparations, particularly spice mixes like curry powders and masalas, where they enhance both flavor and aroma while also imparting health benefits. Fenugreek seeds are also a key ingredient of 'Panch Phoron', a traditional five-spice blend consisting of fenugreek, nigella, cumin, black mustard and fennel seeds, commonly used in the cuisines of eastern India. Apart from their culinary significance, the seeds have long been employed in indigenous medicine for the treatment of digestive disorders such as flatulence, colic, dysentery, diarrhea and dyspepsia, as well as for managing chronic cough, dropsy and diabetes (Shahrajabian *et al.*, 2021) [16]. Due to their high soluble fiber content, seeds are effective in reducing blood glucose and serum cholesterol levels. The medicinal efficacy of fenugreek seeds is largely attributed to their rich phytochemical composition, particularly secondary metabolites such as alkaloids, flavonoids, phenolic compounds, tannins, diosgenin and trigonelline, which are known to exhibit antioxidant, anti-inflammatory, antidiabetic, hypocholesterolemic and antimicrobial properties (Al-Asadi, 2014) [2]. Given the wide variability reported in the nutritional and phytochemical composition of fenugreek seeds, systematic quantitative estimation of key biochemical constituents is essential for identifying nutritionally superior and medicinally valuable genotypes, thereby facilitating their effective utilization in crop improvement and nutraceutical development.

Materials and Methods

The present investigation was carried out at the Department of Biochemistry, College of Horticulture and University of Horticultural Sciences, Bagalkot, Karnataka, which is situated in the Northern Dry Zone of Karnataka (Zone III, Region II) at 16°18′ N latitude and 75°69′ E longitude, with an average elevation of 542 m above mean sea level (MSL). During the *rabi* seasons of 2023-24 and 2024-25, a total of 55 fenugreek collections were evaluated for growth, yield and yield related traits, with the experimental material comprising accessions sourced from various research institutes across India as well as different agro-climatic regions of Karnataka. These collections were further evaluated for quantitative biochemical parameters during both seasons and the pooled results across the two seasons are presented in this paper.

Biochemical estimations

A) Total phenols (mg GAE/100 g)

The total phenol content of fenugreek seed samples was determined following the Folin-Ciocalteu method described by Singleton and Rossi (1965) [18], using gallic acid as the standard. For estimation, one gram of finely powdered seed sample was extracted with 10 mL of ethanol using a mortar and pestle. The homogenate was centrifuged at 3,000 rpm for 10 minutes, and the supernatant was collected. A 0.5 mL aliquot of the extract was diluted with 1 mL of distilled water, followed by the addition of 0.5 mL of Folin-Ciocalteu reagent (FCR). After incubating the mixture for 3 minutes, 2 mL of 10% sodium carbonate (Na_2CO_3) solution was added, and the reaction mixture was kept in a water bath at 60 °C for 10 minutes. A reagent blank was prepared by substituting the sample with distilled water, keeping other reagents constant. The absorbance of the reaction mixture was measured at 650 nm against the blank using a UV-VIS spectrophotometer. The total phenol content was computed using the formula given below and expressed as milligrams of gallic acid equivalent per 100 grams of dry weight (mg GAE/100 g).

$$\text{Total phenol content(mg GAE/100 g)} = \frac{\text{OD value at 650 nm x standard value x total volume of extract x100}}{\text{Assay volume x weight of sample (g) x 1000}}$$

B) Flavonoids (mg QE/100 g)

Total flavonoid content in fenugreek seed samples were estimated by aluminium chloride method using quercetin as standard (Kumar *et al.*, 2015) [11]. Sample of one gram was taken, 10 mL of ethanol was added and centrifuged at 4000 rpm for 10 minutes. From the resulting supernatant, one mL was pipetted out and to which 0.3mL of 5 per cent sodium nitrite ($NaNO_2$) was added. After 5 minutes 0.3 mL of 10 per cent aluminium chloride ($AlCl_3$) was added, followed by inclusion of 2 mL of 1N sodium hydroxide (NaOH) 6 minutes later. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm using spectrophotometer and expressed in mg quercetin equivalent per 100 g of dry weight

$$\text{Total flavonoid content (mg QE/100 g)} = \frac{\text{OD value at 510 nm x standard value x total volume of extract x100}}{\text{Assay volume x weight of sample (g) x 1000}}$$

C) Tannins (mg TAE/100 g)

Tannin content in fenugreek seed samples was determined using the modified Folin-Ciocalteu method as described by Paramesha *et al.* (2023) [13], with tannic acid serving as the standard. One gram of powdered seed sample was extracted with 10 mL of 80% ethanol, and the mixture was centrifuged at 3,000 rpm for 10 minutes. The resulting supernatant was collected and filtered. From this extract, 0.5 mL was mixed with 3.75 mL of distilled water, followed by the addition of 0.25 mL of Folin-Ciocalteu reagent and 0.5 mL of 35% sodium carbonate solution. The reaction mixture was thoroughly mixed and incubated at room temperature for 10 minutes. A blank was prepared by replacing the sample with distilled water while maintaining the same reagent composition. Absorbance was read at 725 nm using a UV-VIS spectrophotometer. The tannin content was calculated using the following formula and expressed as milligrams of tannic acid equivalent per 100 grams of dry weight (mg TAE/100 g).

$$\text{Tannin content (mg TAE/100 g)} = \frac{\text{OD value at 725 nm x standard value x total volume of extract x100}}{\text{Assay volume x weight of sample (g) x 1000}}$$

D) Saponins (mg OAE/100 g)

Saponin content in fenugreek seed sample was estimated using vanillin-perchloric acid colorimetric method (Abdouli *et al.*, 2012) [1]. Powdered seed sample (0.5g) was extracted with 40 mL of acetone and methanol using a Soxhlet apparatus for 3 hours to obtain crude saponins. 0.05 mL of crude extract was evaporated to dryness in a water bath at 70 °C for 2 hours. To the dried residue, 0.1 mL of 5% vanillin in glacial acetic acid and 0.4 mL of perchloric acid were added and the mixture was vortexed thoroughly. The tubes were then incubated in a water bath at 70 °C for 15 minutes, followed by immediate cooling in an ice-water bath. After cooling, 2.5 mL of glacial acetic acid was added to each tube and the contents were mixed well. The absorbance of the resulting solution was measured at 540 nm against a freshly prepared reagent blank. Quantification was performed using a standard calibration curve generated with known concentrations of oleic acid and expressed as mg oleic acid equivalent per 100 gram dry weight of sample (mg OAE/100 g).

$$\text{Saponin content (mg OAE/100 g)} = \frac{\text{OD value at 540 nm x standard value x total volume of extract x100}}{\text{Weight of sample (g) x 1000}}$$

E) Antioxidant activity (mg AAE/100 g)

Antioxidant activity in fenugreek seed sample was determined by FRAP (Ferric Reducing Antioxidant Power) assay (Elkadousy *et al.*, 2020) [7]. The following reagents were used for preparation of FRAP reagent.

Reagents

- Acetate buffer-4.08 g of sodium acetate trihydrate in 1.6 mL of glacial acetic acid and make up to 100 mL
- TPTZ-31.2 mg in 10 mL of distilled water and add few drops of HCL
- $FeCl_3$-32.4 mg in 10 mL of distilled water
- FRAP = 50 mL of acetate buffer + 5 mL of TPTZ + 5 mL of $FeCl_3$

The antioxidant potential of fenugreek seed extract was determined using the Ferric Reducing Antioxidant Power (FRAP) assay. One gram of finely powdered seed sample was extracted with methanol, and a 10 µL aliquot of the extract was subsequently diluted with 990 µL of distilled water to obtain a final volume of 1 mL. Thereafter, 2 mL of freshly prepared FRAP reagent was added, and the reaction mixture was incubated at room temperature for 30 minutes. The absorbance of the samples was measured at 593 nm using a UV-VIS spectrophotometer. Ascorbic acid served as the standard, and the antioxidant activity was expressed as milligrams of ascorbic acid equivalent per 100 grams of dry weight (mg AAE/100 g).

$$\text{Antioxidant activity (mg AAE/100 g)} = \frac{\text{Sample OD x volume made up x 100}}{\text{Aliquot taken x sample weight (g) x 1000}}$$

Results and Discussion

The biochemical analysis of fenugreek collections revealed highly significant variation in total phenols, flavonoids, tannins, saponins and antioxidant activity (Table 1.)

Phenolic compounds constitute one of the most important groups of bioactive constituents in plants and play a vital role in regulating growth and developmental processes through their involvement in lignification, enzyme regulation, auxin metabolism, cell wall synthesis and defense responses against microbial invasion via phytoalexin production. In the present investigation, the pooled mean analysis over both seasons revealed significantly higher differentiation among collections, with Pusa Early Bunching recording the highest phenol content (242.34 mg GAE/100 g), followed by DFC-15 (229.31 mg GAE/100 g), while HUB-4 exhibited the lowest phenolic content (99.27 mg GAE/100 g). Such variation reflects underlying genetic diversity among the collections, influencing phenylpropanoid pathway efficiency. The present results are well supported by the earlier works of Kenny *et al.* (2013) [10], Al-Maamari *et al.* (2016) [3], Jashwitha, (2019) [9] and Bouhenni *et al.* (2021) [5].

Flavonoids have been reported to modulate the body's response to allergens and to exhibit a wide range of biological activities, including antiallergic, anti-inflammatory and anthelmintic effects (Siddhartha *et al.*, 2009) [17]. In the present study, pooled mean data across both seasons revealed significant variation in flavonoid content among fenugreek collections. Pusa Early Bunching recorded the highest flavonoid content (132.91 mg QE/100 g), followed by Lam sel-2 (121.18 mg QE/100 g) and DFC-15 (120.75 mg QE/100 g), whereas Lam sel-3 exhibited the lowest flavonoid content (61.34 mg QE/100 g). The observed variability suggests differential genetic control over flavonoid biosynthesis and accumulation among the collections. This pattern of variation is consistent with earlier findings reported by Bouhenni *et al.* (2021) [5] and Paramesha *et al.* (2023) [13].

Tannins constitute a diverse group of polyphenolic compounds widely distributed across plant species, particularly in external tissues such as bark, roots and stems, where they function as protective agents against biotic stress. Their high polyphenolic nature imparts an astringent property, enabling them to form stable complexes with proteins, carbohydrates and other macromolecules (Hattenschwiler *et al.*, 2000) [8]. In the present investigation, pooled analysis revealed pronounced variation in tannin content among fenugreek collections. Pusa Early Bunching exhibited the highest tannin content (441.41 mg TAE/100 g), which was statistically on par with Afg-3 (440.45 mg TAE/100 g), while HUB-4 recorded the lowest tannin content (97.74 mg TAE/100 g). This variability may be attributed to genotype-specific regulation of polyphenol biosynthetic pathways. Similar findings have been reported by Mahmooda and Yahya (2017) [2] and Paramesha *et al.* (2023) [13].

Saponins are naturally occurring glycosides characterized by an amphiphilic structure consisting of a hydrophobic aglycone linked to one or more sugar moieties. They are widely distributed in seeds, roots and leaves, where they play an important role in plant defense against pathogens and herbivores. In addition, saponins exhibit several biological activities, including antimicrobial, hypocholesterolemic and antidiabetic effects. In the present

study, pooled mean analysis across both seasons revealed that HUB-4 registered the highest saponin content (7555.24 mg OAE/100 g), which was statistically on par with Lam sel-3 (7477.99 mg OAE/100 g). In contrast, the lowest saponin content was recorded in Afg-3 (1124.49 mg OAE/100 g). The wide variation observed highlights the strong genetic influence on saponin biosynthesis among fenugreek collections. Similar trends were noted by Pavithra and Anuradha (2021) [14], Al-Maamari *et al.* (2016) [3] and Abdouli *et al.* (2012) [1].

Table 1: Biochemical estimation of total phenols, flavonoids, tannins, saponins and antioxidant activity in fenugreek collections

Variety/Genotype	Total phenols (mg GAE/100 g)	Flavonoids (mg QE/100 g)	Tannins (mg TAE/100 g)	Saponins (mg OAE/100 g)	Antioxidants (mg AAE/100 g)
Lam sel-1	165.63	75.50	126.07	4464.62	32.74
Lam sel-2	218.19	121.18	412.80	1532.41	50.56
Lam sel-3	103.60	61.34	129.56	7477.99	22.86
GM-1	113.20	90.77	331.00	4712.26	40.48
GM-2	170.02	81.90	180.14	3679.96	45.77
GM-3	141.97	122.45	162.72	3535.34	38.37
CO-1	101.99	68.83	105.57	5755.75	23.36
CO-2	129.49	118.65	238.79	4366.46	47.40
Rmt-1	189.15	107.21	314.39	5121.21	28.19
Rmt-143	200.31	94.73	259.88	2416.54	27.71
Rmt-303	200.55	90.03	216.58	5297.43	31.34
Rmt-305	141.88	111.24	268.17	2061.68	32.95
Rmt-351	197.04	77.73	146.42	2702.71	42.05
Rmt-354	166.94	104.91	281.27	4196.36	32.75
Rmt-361	148.22	112.50	270.71	3562.65	34.45
Afg-1	114.95	89.62	254.45	4485.74	22.96
Afg-2	187.03	93.70	198.69	4019.56	43.45
Afg-3	225.32	117.55	440.45	1124.49	47.82
Afg-4	120.19	106.38	159.96	2591.24	30.74
Afg-5	131.31	84.22	241.13	4486.24	38.98
Pusa Early Bunching	242.34	132.91	441.41	1508.72	53.64
HM-57	104.56	79.45	113.47	5354.58	23.62
DFC-21	147.72	76.69	290.29	4351.60	40.26
HUB-1	165.76	78.00	291.44	2943.52	29.20
HUB-2	189.99	65.02	233.45	3099.25	34.05
HUB-3	174.92	88.30	237.30	5055.82	38.60
HUB-4	99.27	64.80	97.74	7555.24	22.11
HUB-5	209.16	84.99	148.04	1866.29	42.75
HUB-6	122.02	74.05	211.89	4295.17	25.32
HUB-7	159.74	64.53	283.64	3127.14	28.13
HUB-8	209.01	118.00	336.61	5549.63	47.38
HUB-9	140.93	67.57	181.85	3652.69	28.91
DFC-1	145.62	69.30	166.54	3147.68	29.51
DFC-2	110.57	74.15	152.36	4200.97	39.34
DFC-3	116.02	81.21	275.92	2964.81	41.09
DFC-4	121.40	74.12	138.00	3265.67	40.94
DFC-5	191.59	89.18	283.18	2665.14	44.81
DFC-6	133.54	90.82	147.46	4944.45	25.75
DFC-7	161.88	98.86	218.33	3703.24	44.07
DFC-8	123.21	93.58	164.09	3316.82	32.72
DFC-9	186.55	109.97	305.28	3052.56	42.77
DFC-10	199.21	103.44	266.81	1700.89	46.73
DFC-11	128.79	69.86	216.62	2723.97	29.60
DFC-12	146.58	108.31	207.08	3539.77	30.25
DFC-13	130.27	98.29	177.57	4178.56	33.62
DFC-14	104.13	80.32	288.82	1987.30	39.08
DFC-15	229.31	120.75	327.30	1201.20	51.43
DFC-16	151.16	115.81	146.27	2388.42	38.86
DFC-17	158.42	106.67	153.19	3280.91	24.02
DFC-18	210.90	103.61	236.21	4952.65	40.54
DFC-19	167.00	78.13	166.87	4111.48	40.53
DFC-20	153.47	99.35	129.04	3907.82	33.14
DFC-22	197.65	92.88	252.09	4582.17	40.93
DFC-23	166.67	106.94	306.65	4727.14	39.11
DFC-24	159.56	97.24	218.44	4691.68	28.20
S. Em ±	2.36	1.02	3.21	30.66	0.49
C.D. (5%)	6.70	2.89	9.11	86.93	1.40

Antioxidant activity in plants is primarily attributed to the presence of phenolic compounds, flavonoids and other secondary metabolites capable of scavenging free radicals and neutralizing reactive oxygen species. These compounds protect plant tissues from oxidative stress and contribute significantly to human health by reducing oxidative damage associated with aging and chronic diseases. In the pooled mean across both seasons, significant variation in antioxidant activity was observed among fenugreek collections. Pusa Early Bunching exhibited the highest antioxidant activity (53.64 mg AAE/100 g), followed by DFC-15 (51.43 mg AAE/100 g) and Lam sel-2 (50.56 mg AAE/100 g), whereas HUB-4 recorded the lowest antioxidant activity (22.11 mg AAE/100 g). The results indicate a strong association between antioxidant activity and phenolic-based metabolites. The variation in these biochemical constituents under normal growing conditions can be attributed primarily to genetic variability among the collections, which regulates the efficiency of phenylpropanoid and terpenoid biosynthetic pathways. Additionally, genotype × environment interactions, including soil type, microclimate and nutrient uptake efficiency, influence their differential accumulation. These results are in agreement with the findings of Babita (2013) [4].

Conclusion

The present study revealed that fenugreek collections namely, Pusa Early Bunching, DFC-15 and Lam sel-2 exhibited comparatively higher concentrations of total phenols, flavonoids, tannins and antioxidant activity, whereas HUB-4 recorded superior saponin content, collectively indicating their enhanced biochemical potential. The richness of these bioactive constituents contributes significantly to antioxidant properties and enhances the nutraceutical value of fenugreek seeds. Owing to their edible nature and widespread consumption, these biochemically superior collections can be directly exploited for the development of functional foods and health-oriented products. Further isolation and characterization of individual phytochemical constituents from these promising collections may facilitate their effective utilization in nutraceutical improvement and future clinical research.

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