International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 612-618

Received: 15-01-2018
Accepted: 18-02-2018

Naveen Chandra Pant
Assistant Professor, Department of Agriculture, Dolphin (P.G.) Institute of Biomedical and Natural Sciences, Dehra Dun Uttarakhand, India

Rakesh Dhoundiyal
Assistant Professor, Department of Chemistry, Shri Guru Ram Rai P.G. College, Dehradun, Uttarakhand, India

Manoj Kumar
Department of Biochemistry. College of Basic Sciences and Humanties. GBPUAT. Pantnagar, U. S. Nagar. Uttarakhand, India

Upendra Dwivedi
Assistant Professor, Department of Agriculture, Dolphin (P.G.) Institute of Biomedical and Natural Sciences, Dehra Dun Uttarakhand, India

JP Singh
Professor, Department of Vegetable Sciences.
College of Agriculture. GBPUAT Pantnagar.US Nagar Uttarakhand, India

Sanjeev Agrawal
Professor (Head) Department of Biochemistry,
College of Basic Sciences and Humanties. GBPUAT Pantnagar, US Nagar Uttarakhand, India

Correspondence
Assistant Professor, Department of Agriculture, Dolphin (P.G.) Institute of Biomedical and Natural Sciences, Dehra Dun Uttarakhand, India

Fenugreek (*Trigonella foenum-graecum* L.) A potential source of dietary fibres and steroidal sapogenin (Diosgenin)

Naveen Chandra Pant, Rakesh Dhoundiyal, Manoj Kumar, Upendra Dwivedi, JP Singh and Sanjeev Agrawal

Abstract
Soluble, Insoluble and total dietary fiber content along with diosgenin (steroidal sapogenin) content of 25 fenugreek (*Trigonella foenum graecum*. L) genotypes from north India were evaluated, together with crude fiber and saponin content. The total dietary fiber content varied from 39.487±0.547 in UM-274 to 50.015±0.667 % in IC-143850. The genotype IC-143850 also showed, highest soluble dietary fiber content (i.e., 33.158±0.573 %), respectively. The diosgenin content within fenugreek genotypes varied from 7.153±0.388 in UM-265 to 17.822±0.602 mg g^{-1} dry wt. in RMt-303. The genotypes under investigation showed significant difference ($p \leq 0.05$) with respect to dietary fiber and diosgenin content. There was a significant ($p \leq 0.01$) positive correlation (0.960) between total dietary fiber and soluble dietary fiber, similarly a significant positive correlation (0.891) was observed between total and insoluble dietary fiber within the genotypes. There was also a significant positive correlation between diosgenin and different dietary fiber constituents i.e., soluble, insoluble and total viz., (0.298, 0.444 and 0.375, respectively). Saponin and diosgenin content among the genotypes under investigation were also positively correlated (0.646). The findings of the present investigation clearly show variation in dietary fiber and diosgenin content and significant among fenugreek genotypes. These variations within the genotypes may be attributed to the genetic differences among the fenugreek genotypes.

Keywords: Pullulanase, galactomannans, diosgenin, dietary fibers

Introduction
Fenugreek (*Trigonella foenum-graecum* L.) also known as 'methi' is among the oldest medicinal herbs used by human beings. It is extensively grown in winter season throughout tropical and subtropical regions of India during for its seeds, tender shoots and fresh leaves. The seeds are rich source of alkaloids proteins, free unnatural amino acids (4-hydroxyisoleucine), dietary fiber, steroidal saponins and individual spirostanols and furastanols like diosgenin, gitogenin, yamogenin etc. These components have been identified as the main phytochemicals responsible for varying biological effects shown by fenugreek seeds. Fenugreek soluble fibers are an indispensable component due to their potential applications in functional foods and nutraceuticals. The soluble fiber from fenugreek seeds has been identified chemically as galactomanns. These are the biopolymers having β-D-mannan backbone to which varying degrees of D-galactosyl substituents are attached via 1, 6-glycosidic linkages. Adequate intake of dietary fiber is associated with significant lower prevalence of coroner heart disease, stroke, and peripheral vascular disease (Merchant *et al.* 2003); [17] other risk factors i.e., obesity, hypertension and diabetes etc., are also less prevelant in individuals with the adequate intake of dietary fiber (Lairon *et al.* 2005) [14]. Fibers particularly soluble fibers usually delay gastric emptying due to slow transit of food materials through the small intestine, whereas insoluble fibers has inverse effect. In the small intestine, dietary fibers have profound effect on a wide variety of gastrointestinal hormones that serve as a stimulus effecting release of insulin (Anderson *et al.*, 2009) [4]. Dietary fibers are also known to bind bile acids and significantly increase excretion of bile acids and cholesterol from the body (Kirby *et al.* 1981) [12]. Fermentable fibers in the colon increase bacterial load thereby acting as prebiotics increasing growth health-promoting bacteria. The physiological effects of total dietary fiber i.e., soluble and insoluble derived from food have a significant effect on human.

Plant based foods are the only known sources of dietary fibers health and nutrition (Schweitzer & Edwards, 1992) [24] in human diet and they play a significant role in maintaining health and nutrition of different population groups.

Fenugreek seeds are also a rich source of diosgenin, a steroidal sapogenin belonging to triterpene group. It has great significance for pharmaceutical industry due to its oestrogenic effect on the mammary gland. Diosgenin is generally used as starting material for partial synthesis of oral contraceptives, sex hormones and other steroids. It also plays an important role in the control of cholesterol metabolism, variation in the lipoxygenase activity of human ethyroleukemia cells. Diosgenin is known to ameliorated diabetes by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues (Uemura *et al.*, 2010). Fenugreek containing diosgenin may be useful for ameliorating the glucose metabolic disorder associated with obesity. Administration of diosgenin to HFD-STZ diabetic rats caused a decrease in body weight gain, blood glucose, insulin, insulin resistance and also it modulated lipid profile in plasma and tissues (Naidu *et al.*, 2015). [18] Diosgenin is known to improve insulin resistance through an estrogen receptor-mediated activation pathway and may serve as a new strategy for type 2 diabetes mellitus, especially for women in an estrogen-deficient condition (Fang *et al.*, 2016) [8]. Diosgenin has potential effects on cardiovascular risk, insulin secretion and beta cell regeneration in STZ induced diabetic rats (Kalailingam *et al.*, 2014) [14]. Fenugreek is an important source of both Soluble fiber (galactomannan) and diosgenin There are various studies on presence of diosgenin and dietary fibers in fenugreek seeds, leaves and other plant parts but few studies have been made on genotypic difference among the genotypes w.r.t dietary fiber and diosgenin content within fenugreek genotypes. So the present investigation was undertaken to investigate different fenugreek genotypes w.r.t dietary fiber and diosgenin content, so that they may be commercialized as potential functional food providing desired health benefits.

Materials & Methods

The present study was undertaken to investigate fenugreek genotypes w.r.t dietary fibers and diosgenin content in seeds of different genotypes. The seeds of the 25 fenugreek genotypes viz., AM-316, UM-271, UM-222, UM-258, UM-265, UM-273, UM-274, UM-279, UM-325, IC-143843, IC-143850, IC-143816, IC-066843, UM-126, RMt-303, RMt-305, Pusa early bunching, RMt-1, GM-2, RMt-2, RMt-361, RMt-143, Pant ragini, HM-355 and UM-366 (under varietal trials of All India Coordinated Research Project (AICRP) on Spices) were collected from Vegetable Research Centre (VRC), GBPUA&T, Pantnagar, district Udham Singh Nagar (Uttarakhand, India).

Total dietary fiber determination

Total dietary fiber as total non-starch polysaccharides (NSP) was determined using method of Englyst and Hudson, 1987. 100 mg powder seed samples were mixed with 40 ml of acetone for 30 min. The contents were centrifuged at 5000 rpm and the supernatant was removed by aspiration without disturbing the residue. 2 ml of DMSO was added to the samples and mixed for about 2 min at room temperature. Samples were placed in boiling water bath for 1 h. Sample were removed and 8 ml of 0.1 M sodium acetate buffer (pH 5.2) pre-equilibrated at 50^0 C was added immediately and again placed in a water bath at 40° C. The reaction mixtures were removed after 3 min and 0.5 ml of α-amylase solution (approx 18,000 units), followed by 0.1 ml of pullulanase solution (1:100) was added and incubated at 42° C mixed and kept for about 16 h at 42^0 C. 40 ml of ethanol was added, mixed well by inversion and left for 1 h at room temperature and centrifuged at 1500 g for 10 min. The residue was washed twice using 50 ml of 85 % ethanol. Acetone (40 ml) was added to the washed residue and centrifuged at 1500 g for 10 min. Supernatant was removed by aspiration and dried at 65^0 C on a hot plate. 2ml 12 M sulphuric acid was added to the dried residue and the reaction mixture was left at 35^0 C for 1 h. 22 ml of distilled water was added and the reaction mixture was placed in boiling water bath for 2 h.

1 ml of the hydrolysates was used for further analysis by DNS method. Absorbance was measured at 530 nm and calculated the concentration of total dietary fiber by reference to the standards. A mixture of arabinose/xylose/glucose in the proportions 3:4:3 (by weight) was used for standard curve.

Estimation of soluble and insoluble NSP

The procedure for the measurement of insoluble NSP was identical with that described above, for total NSP omitting the precipitation with ethanol, 40 ml of 0.2M phosphate buffer (pH 7) was added and the tubes placed in a boiling water bath for 1 h. The reaction mixture was cooled and centrifuged at 1500 g,, the supernatant was remove and discarded. The residue washed once with 50 ml of the phosphate buffer, and once with 85 % (v/v) ethanol. The residue was dried and treated as described for total NSP.

Soluble NSP = Total NSP - Insoluble NSP.

Crude fibre determination

The crude fibre content (%) in fenugreek seeds was estimated using modified AOAC method (Ahuja and Bajaj, 1999). 100 g powdered seeds from different genotypes was mixed with 25 ml petroleum ether, and kept overnight. The petroleum ether was decanted and the powdered material was dried. The powder was treated with 5 ml of 1.25 % H_2SO_4 and kept in boiling water bath for half an hour, and the remaining material was washed thrice with distilled water. The residue left was treated with 5 ml of 1.25 % NaOH and again kept for 30 min. in boiling water bath and the remaining material was again washed twice with distiller water. Finally the remaining residue was oven dried. The residue was oxidized with 5 ml of 0.06 M potassium dichromate reagent and kept in boiling water bath for 30 min and cooled to room temperature. The volume was made 50 ml with distilled water, and the absorbance measured at 590 nm. The crude fibre content (mg/100 g) of the seed samples was expressed as percent. Pure cellulose (5-25 mg) was used as a standard.

Extraction & Estimation of Diosgenin

Extraction

500 mg dried seed powder from different fenugreek genotypes was used for the extraction of diosgenin. The seed powder was hydrolyzed with 30 ml of 1 M H_2SO_4 (prepared in ethanol) for 30 minutes at 100^0C the solution was further diluted with distilled water (20 ml). Diosgenin was extracted (thrice) from the hydrolysate using 20 ml hexane as solvent. The pooled hexane extract (60 ml) was washed with 20 ml of 0.1 M NaOH to remove free fatty acids. Hydrophilic contaminants were removed by washing the extract with 10 ml of distilled water the resulting hexane extract was used for the determination of diosgenin. The estimation was performed employing the method of Rishi *et al.*, 1976. To 1 ml of the

hexane extract was taken in a test tube and kept in a water bath at 75^0C. 5 ml of concentrated $HClO_4$ followed by 0.1 ml $SbCl_5$ solution (24 % in 70 % $HClO_4$) were added to each tubes, the tubes were incubated for 30 min at room temperature and the absorbance measured at 486 nm against blank. The diosgenin content (µg/g dry.wt) was calculated using a standard curve (10-100 µg) prepared by using diosgenin as standard.

Saponins

500 mg of powdered seeds were homogenized in acetone (5ml) and kept for 24 hrs and then solvent was removed. The procedure was repeated by using methanol (5 ml) as solvent. The methanolic extract was allowed to cool and volume was made to 15 ml with methanol. 1 ml of extracted sample was taken in a test tube and kept in boiling water bath in order to remove alcohol. After cooling 2 ml of ethyl acetate was added, to this 1 ml of p-anisaldehyde reagent and 1 ml of concentrated H_2SO_4 were added. After stirring, these test tubes were kept for 10 min at room temperature. The intensity of red color developed was measured at 430nm (Fenwick and Oakenfull, 1983). A standard curve of diosgenin was used for calculation of saponin (10-80 µg).

Statistical Analysis

Analysis of variance (ANOVA) and Duncan LSD post hoc test were carried out on the values obtained in the experiment. Correlation analysis (bivariate) was also carried out to determine the relationship between soluble dietary fiber, insoluble dietary fiber, total dietary fiber, crude fiber, saponin and diosgenin content present in different fenugreek genotypes. The software IBM SPSS Statistics 20 (IBM Corporation) and SigmaPlot for Windows 11.0 (Systat Software, Inc.) were used to perform statistical analysis and graphing respectively. Results are expressed as mean ± SEm (n = 3). A statistical difference at $p \leq 0.05$ was considered to be significant.

Results

Total Dietary Fiber

The total dietary fiber content expressed as percent among the fenugreek genotypes (Table. 1) under investigation varied from 39.487±0.547 in UM-274 to 50.015±0.667 % in IC-143850. No significant ($p \leq 0.05$) difference in total dietary fiber content was observed in UM-273 & pant ragini (i.e., 14.316±0.620 & 45.000±2.125 %, respectively), UM-265 & UM-271 (i.e., 44.457±0.697 & 44.126±0.676 %, respectively), IC-066843 & IC-143816 (i.e., 42.816±0.558 & 42.756±0.596 %, respectively), GM-2, UM-222, UM-325 & HM-355 (i.e., 41.777±0.563, 41.551±0.564, 41.506±0.600 & 41.475±0.562 %, respectively), RMt-305 & UM-258 (i.e., 41.219±0.737 & 41.129±0.674 %, respectively), IC-143843 & RMt-1 (i.e., 40.466±0.891 & 40.436±0.598 %, respectively). Thus according to Duncan LSD posthoc analysis (SPSS 20), the 25 fenugreek genotypes can be grouped into 17 distinct groups on the basis of their total dietary fiber content (Table. 1). The genotypes differed significantly ($p \leq 0.05$) between different groups, whereas within individual groups no significant difference in total dietary fiber content was observed between the genotypes.

Soluble Dietary Fiber

The soluble dietary fiber content expressed as percent among the fenugreek genotypes (Table. 1) under investigation varied from 26.175±0.390 % in UM-274 to 33.158±0.573 % in IC-143850. No significant ($p \leq 0.05$) difference in soluble dietary fiber content was observed in IC-143816 & RMt-361 (i.e., 28.389±0.560 & 28.208±0.388 %, respectively), UM-325 & HM-355 (i.e., 27.511±0.390 & 27.494±0.428 %, respectively), RMt-305, UM-258 & RMt-143 (i.e., 27.315±0.366, 27.258±0.364 & 27.217±0.362, respectively), IC-143843 & RMt-1 (i.e., 26.811±0.404 & 26.801±0.373 %, respectively). Thus according to Duncan LSD posthoc analysis (SPSS 20), the 25 fenugreek genotypes can be grouped into 20 distinct groups on the basis of their soluble dietary fiber content (Table. 1). The genotypes differed significantly ($p \leq 0.05$) between different groups, whereas within individual groups no significant difference in soluble dietary fiber content was observed between the genotypes.

Insoluble Dietary Fiber

The insoluble dietary fiber content expressed as percent among the fenugreek genotypes (Table. 1) under investigation varied from 13.312±0.370 % in UM-274 to 17.962±0.813 % in pant ragini. No significant ($p \leq 0.05$) difference in insoluble dietary fiber content was observed in IC-143850 & UM-366 (i.e., 16.856±0.407 & 16.559±0.426 %, respectively), IC-066843, IC-143816 & RMt-361 (i.e., 14.427±0.315, 14.406±0.306 & 14.351±0.441 %, respectively), UM-222, UM-325, HM-355, RMt-305, UM-258, RMt-143, IC-143843 & RMt-1 (i.e., 14.006±0.375, 13.994±0.412, 13.981±0.372, 13.904±0.489, 13.871±0.457, 13.852±0.472, 13.655±0.551 & 13.634±0.411, respectively). Thus according to Duncan LSD posthoc analysis (SPSS 20), the 25 fenugreek genotypes can be grouped into 15 distinct groups on the basis of their insoluble dietary fiber content (Table. 1). The genotypes differed significantly ($p \leq 0.05$) between different groups, whereas within individual groups no significant difference in insoluble dietary fiber content was observed between the genotypes.

Crude Fiber

The crude fiber content expressed as percent among the fenugreek genotypes (Figure. 1) under investigation varied from 6.717±0.049 % in AM-316 to 10.884±0.032 % in RMt-305. No significant ($p \leq 0.05$) difference in crude fiber content was observed in UM-325 & UM-274 (i.e., 9.126±0.065 & 9.098±0.016 %, respectively), IC-143816 & IC-143843 (i.e., 8.616±0.762 & 8.588±0.049 %, respectively), UM-258, RMt-303 & UM-279 (i.e., 8.503±0.032, 8.446±0.032 & 8.418±0.016, respectively), Pusa early bunching, UM-265 & RMt-1 (i.e., 8.021±0.016, 7.993±0.333 & 7.936±0.232 %, respectively), RMt-143, UM-222 & HM-355 (i.e., 7.341±0.081, 7.256±0.032 & 7.227±0.049 %, respectively). Thus according to Duncan LSD posthoc analysis (SPSS 20), the 25 fenugreek genotypes can be grouped into 17 distinct groups on the basis of their crude fiber content. The genotypes differed significantly ($p \leq 0.05$) between different groups, whereas within individual groups no significant difference in crude fiber content was observed between the genotypes.

Diosgenin Content

The diosgenin content expressed as mg g^{-1} dry wt. within fenugreek genotypes (Table. 1) under investigation varied from 7.153±0.388 in UM-265 to 17.822±0.602 mg g^{-1} dry wt. in RMt-303. No significant ($p \leq 0.05$) difference in diosgenin content was observed in RMt-303 & UM-126 (i.e., 17.822±0.602 & 17.742±0.787 mg g^{-1} dry wt., respectively), pant ragini & pusa early bunching (i.e., 17.443±0.661 &

17.304±0.010 mg g^{-1} dry wt., respectively), HM-355, RMt-305 & AM-316 (i.e., 16.695±0.060, 16.690±0.611 & 16.685±0.440 mg g^{-1} dry wt., respectively), RMt-361, UM-271, IC-143816, RMt-1, UM-366 & IC-143850 (i.e., 14.528±0.030, 14.328±0.264, 14.208±0.271, 14.079±0.756, 13.887±0.205 & 13.874±0.588 mg g^{-1} dry wt., respectively), GM-2 & RMt-2 (i.e., 12.837±0.185 & 12.754±0.438 mg g^{-1} dry wt., respectively), UM-258, UM-222, IC-143843 & UM-279 (i.e., 11.869±0.078, 11.813±0.276, 11.796±0.785 & 11.619±0.768 mg g^{-1} dry wt., respectively). Thus according to Duncan LSD posthoc analysis (SPSS 20), the 25 fenugreek genotypes can be grouped into 12 distinct groups on the basis of their diosgenin content (Table. 1). The genotypes differed significantly ($p \leq 0.05$) between different groups, whereas within individual groups no significant difference in diosgenin content was observed between the genotypes.

Saponin Content

The saponin content expressed as mg g^{-1} dry wt. within fenugreek genotypes (Figure. 2) under investigation varied from 11.406±0.544 in UM-265 to 19.847±0.186 mg g^{-1} dry wt. in UM-325. No significant ($p \leq 0.05$) difference in saponin content was observed in RMt-303 & AM-316 (i.e., 19.353±0.546 & 19.345±0.610 mg g^{-1} dry wt., respectively), HM-355, pant ragini & UM-273 (i.e., 18.314±0.046, 18.244±0.111 & 18.096±0.737 mg g^{-1} dry wt., respectively), IC-143850 & UM-271 (i.e., 16.232±0.217 & 16.132±0.739 mg g^{-1} dry wt., respectively), UM-366 & RMt-1 (i.e., 15.407±0.298 & 15.289±0.850 mg g^{-1} dry wt., respectively), UM-258 & RMt-2 (i.e., 13.613±0.059 & 13.480±0.611 mg g^{-1} dry wt., respectively). Thus according to Duncan LSD posthoc analysis (SPSS 20), the 25 fenugreek genotypes can be grouped into 19 distinct groups on the basis of their saponin content. The genotypes differed significantly ($p \leq 0.05$) between different groups, whereas within individual groups no significant difference in saponin content was observed between the genotypes.

Discussion

Total Dietary Fiber

Dietary fibers have an important role in human nutrition, they are known to exhibited health promoting effects particularly in individuals with diabetes plant based foods with high dietary fiber are known to increase insulin sensitivity and consequently lower insulin production & thus effectively controlling glucose concentration within diabetic individuals. The genotypes under investigation differed significantly ($p \leq 0.05$) w.r.t total dietary fiber content. The genotypes with high dietary fibers i.e., IC-143850, UM-366, UM-126 & Pusa early bunching can well be Promoted as suitable functional foods on the basis of their total dietary fiber content and their possible utility for individuals with diabetes. Similar results have also been reported Kochhar *et al.*, 2006 & Roberts, 2011 in fenugreek. The Total dietary Fiber content among the genotypes under investigation showed a significant ($p \leq 0.01$) positive correlation (Figure. 3A) with soluble Fiber, in soluble fiber & diosgenin content (i.e., 0.960, 0.891 & 0.375, respectively). Total dietary Fiber content among the genotypes also showed positive correlation with crude fiber & saponin content i.e., 0.106 & 0.118, respectively. Fenugreek genotypes with high total dietary fiber content have high nutritional value and can be promoted as novel functional &/or nutritional foods.

Soluble Dietary Fiber

The soluble dietary fiber derived from fenugreek seeds are similar to other soluble fiber reported in guar seeds i.e., galactomannans (Song *et al.*, 1989). The soluble fibers (galactomannans) from fenugreek has galactose to mannose ratio of 1:1 as they are uniformly linked, thus providing greater hydration and thereby their solubility. These characteristics make fenugreek soluble fibers an excellent ingredient for various food applications (Brummer *et al.*, 2003; Kumar & Maliakel, 2007). Fenugreek genotypes with high soluble fibers viz., IC-143850, UM-366, UM-126 & Pusa early bunching are excellent source of soluble dietary fibers and may find potential use in food industry. Presence of soluble dietary fibers in fenugreek has also been reported by Roberts, 2011 and Ramulu *et al.*, 2011. The soluble dietary fiber content among the genotypes under investigation showed a significant ($p \leq 0.01$) positive correlation with Insoluble dietary fiber, Total dietary fiber & diosgenin content (i.e., 0.737, 0.960 & 0.298, respectively). There was also a positive correlation between soluble dietary fiber and crude fiber, saponin content within the genotypes i.e., 0.177 & 0.080, respectively (Figure. 3A). Thus fenugreek genotypes with high soluble dietary fiber content could well be promoted as suitable functional food apart from having significant application in food industry as soluble dietary fiber content within fenugreek seeds can be directly correlated to their galactomannans content (Shakuntala *et al.*, 2011).

Insoluble Dietary Fiber

Insoluble dietary fiber together with other fractions of dietary fiber forms an indispensable part of human diet with pronounced health benefits. The most obvious benefits of insoluble dietary fiber consumption is increased laxation. Insoluble dietary fiber have low solubility due to lower water binding capacity & has been reported to exert beneficial effects in divirticular disease, varicose veins, appendicitis, phlebitis. Rimm *et al.*, (1996) [21] reported increased consumption of IDF correlates with reduced coronary heart disease. The genotypes showed a significant difference in their insoluble dietary fiber content, genotypes with high insoluble dietary fibers viz., Pant ragini, IC-143850, UM-366, UM-126 & Pusa early bunching are excellent source of insoluble dietary fiber. Presences of insoluble dietary fiber have also been reported by Roberts, 2011 & Ramulu *et al.*, 2011 [23] in fenugreek. IDF content within fenugreek genotypes showed a significant ($p \leq 0.01$) positive correlation (Figure. 3A) with soluble dietary fiber, total dietary fiber & diosgenin content (i.e., 0.737, 0.891 & 0.444, respectively). Thus fenugreek genotypes with high IDF content have higher possibility of providing desired nutritional benefits & can be promoted as suitable functional and/or nutritional foods with well known therapeutic effects.

Crude Fiber

Crude fiber is the non-digestible residues within plant based foods. They constitute of cellulose & non-cellulose polysaccharides (Mehta & Kaur, 1992). The results of the present investigation clearly show that fenugreek genotypes under evaluation differ significantly w.r.t crude fiber content. The genotypes with high crude fiber content i.e., RMt-305, UM-126 and IC-143850 could be utilized and promoted as important functional food with myriad physiological properties associated with them. The crude fiber content among the genotypes showed a positive correlation (Figure. 3A) with total dietary fiber, diosgenin and saponin content

(i.e., 0.106, 0.054 and 0.234, respectively). The health promoting effect of crude fiber in fenugreek may be attributed to their association with total dietary fiber content among the genotypes.

Diosgenin

Diosgenin is an important steroidal sapogenin present in fenugreek and other plants viz., *Dioscorea* and *Costus* etc. It is known to enhance insulin dependent glucose uptake (Uemura *et al.*, 2010). Diosgenin is also known to exert estrogenic effect on the mammary glands (Aradhana, 1992) [5]and plays an important role in cholesterol metabolism (Cayen & Dvornik, 1979.)[8] The results clearly show that fenugreek genotypes under investigation differed significantly w.r.t diosgenin content. Presence of significant amount of diosgenin in fenugreek seeds have also been reported by Ortuno *et al.*, (1999). [19] Thus the results are in prefect correlation with previously reported studies on presence of significant amount of steroidal sapogenin i.e., diosgenin in fenugreek seeds. Diosgenin content within fenugreek genotypes under investigation showed a significant ($p \leq 0.01$) positive correlation (Figure. 3B) with saponins, soluble, insoluble and total dietary fiber (i.e., 0.646, 0.298, 0.444 & 0.375, respectively). The genotypes with high diosgenin content i.e., RMt-303, UM-126 & Pant ragini can wall be promoted and commercialized for diosgenin production. Fenugreek has the selective advantage over other potential sources (*Dioscorea*, *Costus*) in being an annual leguminous plant with short crop cycle (Leung & Foster, 1996).

Saponins

Saponins are important phytoconstituents of leguminous plants and are known to have potent pharmacological properties viz., hypocholesterolemic, antimicrobial and anticarcinogenic activities (Francis *et al.*, 2002). [10] Numerous studies have shown that saponins have well known effect on cell proliferation, transcription and gene expression. The results of the present investigation clearly show the presence on significant amount of saponins in fenugreek genotypes. The genotypes differed significantly w.r.t saponin content. Similar results have also been reported by Kochhar *et al.*, (2006) [13] in fenugreek seeds. The saponin content among the genotypes showed a significant ($p \leq 0.01$) positive correlation (0.646) with diosgenin content among the genotypes (Figure. 3B). The difference in genotypes w.r.t saponin content can well be utilized in promoting genotypes with high saponins i.e., UM-325, UM-126, RMt-303 and AM-316 as potential functional foods.

Conclusion

The present investigation clearly reveals that fenugreek is a potential source of dietary fibers and diosgenin. Fenugreek genotypes viz., IC-143850, UM-366, UM-126, RMt-303, Pant ragini and pusa early bunching are potential sources of both dietary fibers and diosgenin. The investigation also revealed the presence of significant positive correlation between different dietary fiber constituents (viz., soluble, insoluble and total dietary fibers) and diosgenin. Thus these genotypes with high dietary fibers and diosgenin may find potential use in different food and pharma industry. These may therefore be commercialized and used as potential functional foods.

Table 1: Total, soluble, insoluble dietary fiber and diosgenin content in different fenugreek genotypes

S. No	Genotypes	Total Dietary Fibres (%)	Soluble Dietary Fibres (%)	Insoluble Dietary Fibres (%)	Diosgenin (mg/g)
1	AM-316	42.168±0.591^{ghi}	27.960±0.558^{hij}	14.208±0.299^{fghi}	16.685±0.44^{b}
2	UM-271	44.126±0.676^{df}	29.246±0.399^{g}	14.880±0.464^{efg}	14.328±0.264^{c}
3	UM-222	41.551±0.564^{ghij}	27.544±0.426^{hijkl}	14.006±0.375^{hij}	11.813±0.276^{fg}
4	UM-258	41.129±0.674^{hijk}	27.258±0.364^{jklm}	13.871±0.457^{hij}	11.869±0.078^{fg}
5	UM-265	44.457±0.697^{f}	29.465±0.398^{fg}	14.992±0.477^{def}	7.153±0.388^{j}
6	UM-273	45.316±0.620^{ef}	30.045±0.573^{ef}	15.270±0.336^{de}	11.348±0.874^{gh}
7	UM-274	39.487±0.547^{l}	26.175±0.390^{n}	13.312±0.370^{j}	10.53±0.186^{h}
8	UM-279	39.969±0.569^{kl}	26.493±0.382^{mn}	13.476±0.389^{ij}	11.619±0.768^{fg}
9	UM-325	41.506±0.600^{ghij}	27.511±0.390^{ijkl}	13.994±0.412^{hij}	12.446±0.814^{ef}
10	IC-143843	40.466±0.891^{jkl}	26.811±0.404l^{mn}	13.655±0.551^{hij}	11.796±0.785^{fg}
11	IC-143850	50.015±0.667^{a}	33.158±0.573^{a}	16.856±0.407^{b}	13.874±0.588^{c}
12	IC-143816	42.756±0.596^{g}	28.349±0.560^{hi}	14.406±0.306^{fgh}	14.208±0.271^{c}
13	IC-066843	42.816±0.588^{g}	28.388±0.545^{h}	14.427±0.315^{fgh}	13.599±0.097^{cd}
14	UM-126	48.132±0.695^{b}	31.903±0.453^{bc}	16.228±0.477^{bc}	17.742±0.787^{a}
15	RMt-303	46.746±0.634^{cd}	30.988±0.481^{d}	15.758±0.421^{cd}	17.822±0.602^{a}
16	RMt-305	41.219±0.737^{hijk}	27.315±0.366^{jklm}	13.904±0.489^{hij}	16.69±0.611^{b}
17	Pusa early bunching	47.906±0.654^{bc}	31.763±0.601^{c}	16.143±0.357^{bc}	17.304±0.01^{ab}
18	RMt-1	40.436±0.598^{jkl}	26.801±0.373l^{mn}	13.634±0.411^{hij}	14.079±0.756^{c}
19	GM-2	41.777±0.563^{ghij}	27.698±0.506^{hijk}	14.078±0.323^{ghij}	12.837±0.185^{de}
20	RMt-2	45.963±0.644^{de}	30.467±0.447^{de}	15.496±0.439^{cde}	12.754±0.438^{de}
21	RMt-361	42.560±0.642^{gh}	28.208±0.388^{hi}	14.351±0.441^{fgh}	14.528±0.030^{c}
22	RMt-143	41.069±0.703^{ijk}	27.217±0.362^{jklm}	13.852±0.472^{hij}	8.128±0.019^{i}
23	Pant ragini	45.000±2.125^{ef}	27.037±0.463^{klm}	17.962±0.813^{a}	17.443±0.661^{ab}
24	HM-355	41.475±0.562^{ghij}	27.494±0.428^{ijkl}	13.981±0.372^{hij}	16.695±0.06^{b}
25	UM-366	49.126±0.658^{ab}	32.567±0.526^{ab}	16.559±0.426^{b}	13.887±0.205^{c}

The genotypes with same superscript are not significantly different ($P \leq 0.05$) according to Duncan LSD posthoc analysis

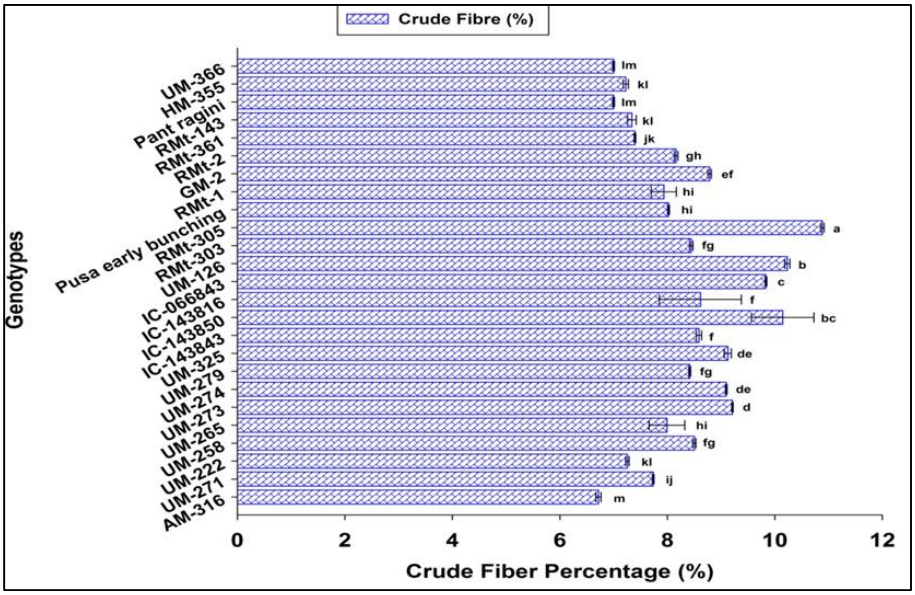

Fig 1: Crude fiber content (%) within fenugreek genotypes. Values shown are mean values of three replicates. The genotypes with same superscript are not significantly different (P≤0.05) according to Duncan LSD posthoc analysis

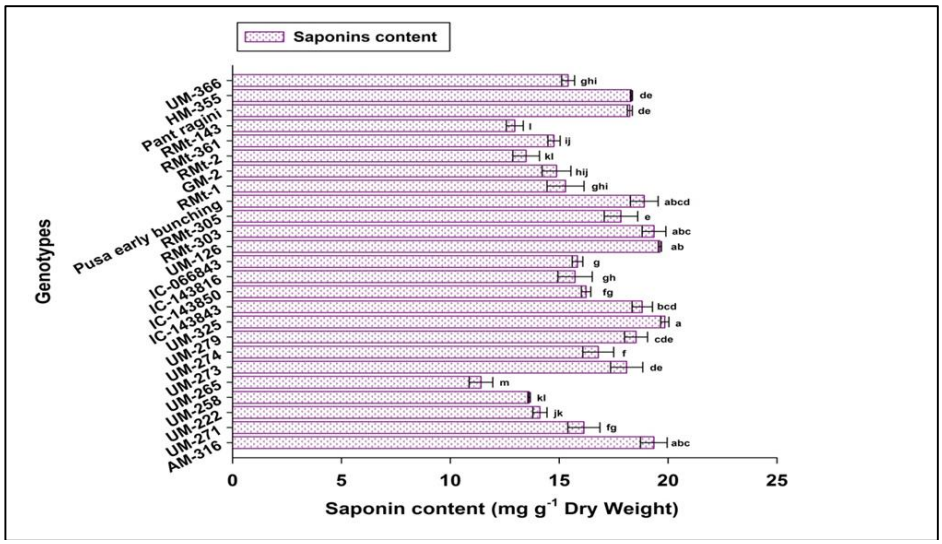

Fig 2: Saponin content (mg g^{-1} Dry Wt.) within fenugreek genotypes. Values shown are mean values of three replicates. The genotypes with same superscript are not significantly different (P≤0.05) according to Duncan LSD posthoc analysis

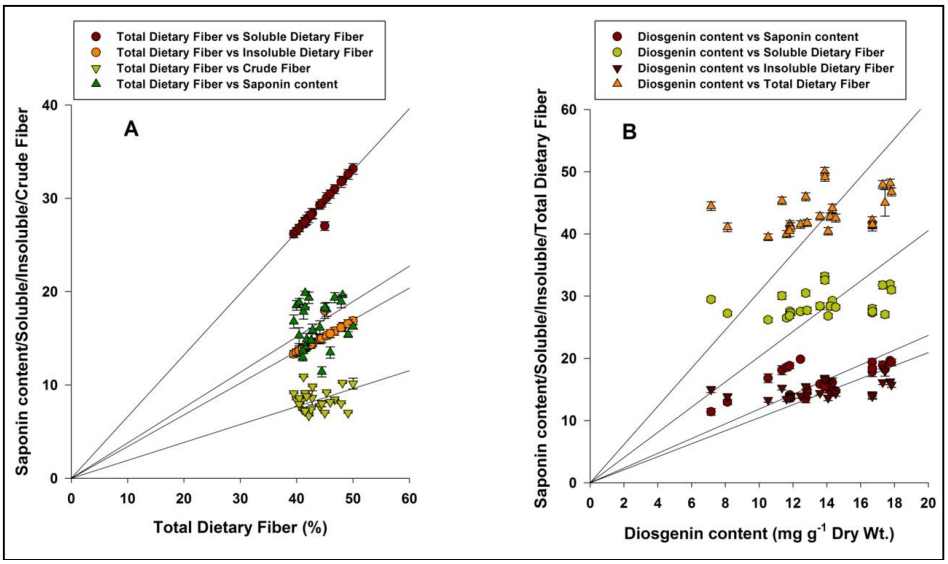

Fig 3: Correlation analysis of Total Dietary fiber and Diosgenin content with saponin content and soluble/insoluble/crude fiber within different fenugreek genotypes. (A) Correlation between total dietary fiber content with soluble/insoluble/crude fiber and saponin content. (B) Correlation of Diosgenin content with saponins, soluble, insoluble and total dietary fiber

Conflict of Interest
We declare that we have no conflict of interest.

Acknowledgements
The financial support provided by Council of Scientific and Industrial Research (CSIR) and the infrastructural grant provided by DST (Department of Science and Technology, Govt. of India) is duly acknowledged.

References

1. Abo Omar J, Hejazi A, Badran R. Performance of Broilers Supplemented with Natural Herb Extract. Open Journal of Animal Sciences 2016; 5:68-74.
2. Ahuja KL, Bajaj KL. Colorimetric determination of crude fibre in cruciferous oilseeds. Cruciferae News. 1999; 21:61-62.
3. Englyst HN, Hudson GJ. Colorimetric method for routine measurement of dietary fibre as non-starch polysaccharides. A comparison with gas-liquid chromatography. Food Chem. 1987; 24:63-76.
4. Anderson JW, Baird P, Davis RH, Ferreri S, Knudtson M, Koraym A. *et al.*, Health benefits of dietary fiber. Nutr. Rev. 2009; 67(4):188-205.
5. Aradhana M, Rao AC, Kale RK. Diosgenin a growth stimulator of mammary gland of ovariectomized mouse. Indian J. Exp. Biol. 1992; 30:367-370.
6. Brummer Y, Cui W, Wang Q. Extraction, purification and physicochemical characterization of fenugreek gum. Food hydrocolloids. 2003; 17:229-236.
7. Cayen MN, Dvornik D. Effect of diosgenin on lipid metabolism in rats. J Lipid Res. 1979; 20:162-174.
8. Fang K, Dong H, Jiang SLiF, Wang D, Yang D, Gong J, *et al*, Diosgenin and 5-Methoxypsoralen Ameliorate Insulin Resistance through ER-α/PI3K/Akt-Signaling Pathways in HepG2 Cells. Evid Based Complement Alternat Med 2016; doi: 10.1155/2016/7493694.
9. Fenwick DE, Oakenfull D. Saponin content of food plants and some prepared foods. J Sci Food Agric. 1983; 34:186-191.
10. Francis G, Levavi Sivan B, Avitan A, Becker K. Effects of long term feeding of Quillaja saponins on sex ratio, muscle and serum cholesterol and LH levels in Nile tilapia (Oreochromis niloticus L.) Comparative Biochemistry and Physiology Part C. 2002; 133:593-603.
11. Kalailingam P, Kannaian B, Tamilmani E, Kaliaperumal R. Efficacy of natural diosgenin on cardiovascular risk, insulin secretion, and beta cells in streptozotocin (STZ)-induced diabetic rats. Phytomedicine 2014; 21(10):1154-61.
12. Kirby RW, Anderson JW, Sieling B. Oat-bran intake selectively lowers serum low-density lipoprotein cholesterol concentrations of hypercholesterolemic men. Am. J Clin. Nutri. Rev. 1981; 34:824-829.
13. Kochhar A, Nagi M, Sachdeva R. Proximate composition, available carbohydrates, dietary fibre and anti nutritional factors of selected traditional medicinal plants. J Hum. Ecol. 2006; 19(3):195-199.
14. Lairon D, Arnault N, Bertrais S. Dietary fiber intake and risk factors for cardiovascular disease in French adults. Am. J Clin. Nutri. Rev. 2005; 82:1185-1194.
15. Leung AY, Foster S. Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics. 2nd Edition, John Wiley and Sons, Inc., New York. 1996
16. Mehta K, and Kaur A. Reviews: dietary fiber. Int J Diabetes Dev Ctries 1992; 12:12-8.
17. Merchant AT, HU FB, Spiegelman D, Willett WC, Rimm EB, Ascherio A. Dietary fiber reduces peripheral arterial disease risk in men. J. Nutri. Rev. 2003; 133:3658-3663.
18. Naidu PB, Ponmurugan P, Begum MS, Mohan K, Meriga B, Ravindar Naik R, *et al.*, Diosgenin reorganises hyperglycaemia and distorted tissue lipid profile in high-fat diet-streptozotocin-induced diabetic rats. J Sci Food Agric. 2015; 95(15):3177-82.
19. Ortuno A, Oncina R, Botia JM, Del Rio JA. Regulation of the diosgenin expression in Trigonella foenum-graecum plants by different plant growth regulators. Food Chem. 1999; 65:227-232.
20. Ramulu P, Giridharan NV, Udayasekhararao P. Hypolipidemic effect of soluble dietary fiber (galactomannan) isolated from fenugreek seeds in WNIN (GR-Ob) obese rats. J Med Plants Res. 2011; 5:4804-13.
21. Rimm EB, Klatsky A, Grobbee D, Stampfer MJ. Review of moderate alcohol consumption and reduced risk of coronary heart disease: Is the effect due to beer, wine, or spirits? British Medical Journal. 1996; 312:731-736.
22. Rishi AK, Kapoor R, Vakhlu VK. A rapid spectrophotometric method for the quantitative determination of Diosgenin in Discorea tubers. Curr. Sci. 1976; 45:326-328.
23. Roberts KT. The potential of fenugreek (*Trigonella foenum graecum*) as a functional food and nutraceutical and its effects on glycemia and lipidemia. J. Med. Food. 2011; 14(12):1485-1489.
24. Schweitzer TF, Edwards CA. Dietary fiber, nutritional function, health and disease-a component of food Springer. London, UK. 1992; 295-332.
25. Shakuntala S, Jarpala PN, Thangraj J, Madineni MN, Pullabhatla S. Characterization of germinated fenugreek (*Trigonella foenum graecum* L.) seed fractions. Int J Food Sci. 2011; 46:2337-2343.
26. Singh KP, Beena Nair B, Prem Chand P, Naidu AK. Contribution of fenugreek (*Trigonella foenum graecum* L.) seeds towards the nutritional characterization. Journal of Medicinal Plant Research 2013; 7(41):3052-3058.
27. Snehlata HS, Payal DR. Fenugreek (*Trigonella foenum graecum* L.): an overview. Int. J. Curr. Pharm. Rev. Res. 2012; 2(4):169-187.
28. Song BK, Winter WT, Taravel FR. Crystallography of highly substituted galactomannans: fenugreek and lucerne gums. Macromolecules 1989; 22:2641-2644.
29. Sowmya P, Rajyalakshmi P. Hypocholesterolemic effect of germinated fenugreek seeds in human subjects. Plant Food Hum. Nutr. 1999; 53:359-365.
30. Uemura T, Hirai S, Mizoguchi N, Goto T, Lee JY, Taketani K. Diosgenin present in fenugreek improves glucose metabolism by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues. Mol Nutr Food Res. 2010; 54(11):1596-1608