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**Nisha**  
Department of Biological  
Sciences C.B.S.H., G.B. Pant  
University of Agriculture &  
Technology, Pantnagar, India

**Pasaumarti Bhaskar Rao**  
Department of Biological  
Sciences C.B.S.H., G.B. Pant  
University of Agriculture &  
Technology, Pantnagar, India

## Gas Chromatography-Mass spectrometry analysis for identification of bioactive compounds in selected genotypes of *Trigonella foenum-graecum* L

**Nisha and Pasaumarti Bhaskar Rao**

### Abstract

Fenugreek is reported to have various medicinal properties and potent source of natural antioxidants, the medicinal properties attributed to this plant is due to the presence of different bioactive components which are identified by using GC-MS. In this study, the bioactive constituent present in methanolic seed extract of some selected genotypes of *Trigonella foenum-graecum* identified. GC-MS analysis of methanolic extract of fenugreek seed revealed the presence of various bioactive compounds such as vitamin E, retinol, lanseol sis, pregnane, reported for exhibiting various biological activities. Seeds of fenugreek genotypes can be used in pharmaceutical as natural source of medicine.

**Keywords:** Fenugreek, medicinal properties, bioactive compounds

### Introduction

Fenugreek or Methi is extensively cultivated in most regions of the world for its promising medicinal properties. Its leaves along with seeds are consumed in different countries around the world for different purposes. It is an annual crop belongs to the family fabaceae and the genus "*Trigonella*" comes from Latin means "little triangle" because of its yellowish-white triangular flowers<sup>[1]</sup>. It is native to South Europe and Asia and grown in many parts of India<sup>[2]</sup>. In order to meet the increasing demand for medicinal plants in indigenous systems of medicine as well as in pharmaceutical industries many medicinal plants are need to be cultivated commercially, but soil salinity and other forms of pollutants represent serious threats to plant production. In this context fenugreek is used as anti-diabetic, anti-fertility, antimicrobial, anti-parasitic, hypo-cholesterolaemic, antileptic, anti-bronchitis, carminative, aphrodisiac, analgesic, antipyretic anticancer antioxidant<sup>[3]</sup>.

India is the largest producer of fenugreek in the world, maximum comes from Rajasthan, Gujarat, Uttarakhand, Uttar Pradesh, Maharashtra, Madhya Pradesh Haryana and Punjab. In India fenugreek powder is also used for the lactation stimulating agent and protective against ethanol toxicity. Fenugreek seeds are aromatic, bitter and possess carminative galactogogue, antibacterial, rheological and antiviral properties. Galactomannans in seed endosperm are reported. Majority of phyto-constituents include saponins (fenugreekine, diosgenin), alkaloids (trigonelline, gentianine, carpaine), amino acids (4-Hydroxyisoleucin, argentine) proteins and flavanoids etc. Seeds have extensive use in Indian cuisine for flavouring many foods such as spice blends, curry powders and tea<sup>[4]</sup>. Seeds are also reported for various medicinal properties like anti-inflammatory, antitumor, cardio tonic, carminative, demulcent, diuretic, emollient, expectorant, febrifuge, galactogogue, hypoglycaemic, hypotensive and laxative attributed to this plant in the traditional system of Indian medicine<sup>[5]</sup>.

Fenugreek seeds also contain a substantial amount of fibre<sup>[6-7]</sup> phospholipids, glycolipids, oleic acid, linolenic acid and linoleic acid<sup>[9-4]</sup> choline, vitamin A, B1 and B2, C, nicotinic acid, niacin<sup>[4]</sup> and many other functional elements are present. Fenugreek seeds are rich source of fiber (50–65 g/100 g) mainly the non-starch polysaccharides<sup>[7]</sup>. Medicinally fibres present in fenugreek are capable of maintaining the glucose metabolism. More over tannins, mucilage, pectin, and hemi cellulose inhibits bile salt absorption in the colon and thus facilitate low density lipoprotein cholesterol (LDL), reduction in blood while binding food toxins, thus indirectly protects intestinal epithelial membrane from cancer onset. Moreover it also helps to lower the blood glucose absorption and thus control sugar level by facilitating the insulin action. Galactomannans on the other hand constitute the major portion of soluble fibre in seeds that lowers glucose absorption in body<sup>[6]</sup>.

### Correspondence

**Nisha**  
Department of Biological  
Sciences C.B.S.H., G.B. Pant  
University of Agriculture &  
Technology, Pantnagar, India

Gum obtained from fenugreek seeds consists of mannose and galactose that gives high viscosity to an aqueous solution [10]. It has higher water solubility due to more galactose in comparison to guar and many others members of the same family. However its gum has not been well exploited in the food industry. Purified gum contains 0.8% residual protein that is able to reduce the surface tension and form stable emulsions with oil droplets (2–3 ml) as compared to other hydrocolloids [6].

GC-MS is a technique used for screening, identification and quantification of many bioactive compounds present in plant extracts. Gas chromatography is now a day's used to separate drugs that might be present in the sample. The retention time identify characteristic of a drug; detector used for the GC is the mass spectrometry. Fragmentation pattern for a drug is always very unique and therefore, very helpful for identifying characteristic of a particular drug. The drug identification by its retention time and fragmentation patterns along with sample specific information makes GC-MS the foremost confirmation method for analyzing the constituent present in the herbal extract [11].

In recent years GC-MS analysis now days have increasingly applied for the analysis of bioactive compound presents in the medicinal plants. This technique is a proved valuable method for the analysis of non polar components and fatty acid, volatile essential oil, lipids and alkaloids [8].

### Material and Method

The healthy and mature seeds of *Trigonella foenum-graecum* L. genotypes was procured from different places in India AM-1 (Azad methi-1 from Chandra Shekhar Azad University Kanpur), FGK-75 and PR (Fenugreek-75 and Pant Ragini from Vegetable research centre GBPUAT Pantnagar), AFG-1 (Ajmer fenugreek from National research centre on seed spices Tabiji Ajmer) and GM-2 from (Gujarat methi-2 from Centre for Research on Seed Spices, S. D. Agricultural University, Jagudan Gujarat). The extraction procedure was carried out in the department of Biological Science GBPUAT Pantnagar. The glass and plastic wares used in this study were supplied by Borosil India, ASGL, India and Schott Duran Germany. All the chemicals and solvents used in the present study were of AR grade and HP grade and were purchased from Merck, Darmstadt, Germany, Hi Media Laboratories Pvt. Ltd, Mumbai, India.

Seeds were washed with fresh water to remove the traces of dust and soil and dried to make the fine powder. Powdered seed material 2 g was soaked in 15 mL of methanol for 48 hours; centrifuged at 10000 rpm for 10 minute and then filtered through Whatmann filter paper No.1 along with 2 g anhydrous sodium sulphate (Na<sub>2</sub>SO<sub>4</sub>) to remove the sediments and traces. The filtrate it is then further subjected for GC-MS analysis at Advance instrumentation research facility at Jawaharlal Nehru University, New Delhi.

### Gas Chromatography-Mass Spectrometry Analysis

The GC-MS analysis was performed in a GCMS-QP2010 Ultra Shimadzu system comprising a gas chromatograph interfaced to a mass spectrometer (GC-MS) at Javahar Lal Nehru University New Delhi. Instrument employing the following conditions: Plunger Speed(Suction):High, Viscosity Comp. Time:0.2 sec, Plunger Speed(Injection):High, Syringe Insertion Speed: High, Injection Mode: Normal, Pumping Times:5,Inj. Port Dwell Time:0.0 sec, Terminal Air Gap: No Plunger Washing Speed:High,Washing Volume:6uL,Syringe

Suction Position:0.0 mm Syringe Injection Position:0.0 mm,Solvent Selection: Column Oven Temp.:80.0 °C, Injection Temp: 260.00 °C Injection Mode: Split, Flow Control Mode: Linear Velocity,Pressure:81.9 kPa, Total Flow:16.3 mL/min, Column Flow:1.21 mL/min, Linear Velocity:40.5 cm/sec,Purge Flow:3.0 mL/min, Split Ratio:10.0,Ion Source Temp: 230.00°C Interface Temp.:270.00°C, Solvent Cut Time:3.50min, Detector Gain Mode Relative Detector Gain:+0.00kV,Threshold:1000,Start Time:4.00min End Time:50.24min ACQ Mode:Scan Event Time:0.20secScan Speed: 3333Start m/z:40.00End m/z: 650.00.

### Identification of Components

Interpretation of mass spectrum from GC-MS was conducted using the database of National Institute Standard and Technology (NIST) library having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

### Results and Discussion

In methanolic seed fraction of GM-2 revealed 50 bioactive compounds (%) out of which 2-Methylpyrrolidine (3.08), Mome inositol (12.22), Pentadecanoic acid (8), 9, 12-Octadecadienoic acid (Z, Z) - (32.8), (2,14-Dioxocyclotetradecyl) acetic acid methyl ester (6.10), dl- $\alpha$ -Tocopherol (0.45) and gamma-Sitosterol (1.70) are major compounds; and along with these, esters, amino acids, tocopherols are the compounds of keen interest as they are involved in various biological activities. For ex., tocopherols exhibit anti-inflammatory, antioxidant, antimicrobial properties [12]. The rest of the compounds identified in GM-2 are presented in Table 1.

The methanolic extract of AM-1 revealed the presence of 48 bioactive compounds (%) in which 2-Methylpyrrolidine (15), 6,6-Dideutero-Nonen-1-OL-3 (9.52), 2-Penten-1-OL, 5-(2,3-Dimethyltricyclo 2.2.1.0(2,6) (9.84), Pentadecanoic acid (5.69), Naphthalene, 1-(2-nitro-2-propenyl)- (0.82), Piperidin-1-yl-acetic acid (2,2-dichloro-3-phenyl-cyclopropylmethylene (0.11), cis-vaccenic acid (17.11) and vitamin E (0.24) are the major compounds. All these compounds are reported to have various biological activities. Apart from these, the essential oil, lanceol-cis (a major component of essential oil) is also identified [13]. The rest of the identified compounds are listed in Table 2.

The methanolic extract of genotype PR revealed presence of 57 bioactive compounds (%) out of which Pentadecanoic acid (7.28), 9-12- octadecanoic acid (Z,Z)- (16.40) it shows the properties of anti-inflammatory, anti cancer, hepatoprotective and anticoronary [12] in *Pleiospermium alatum* cis vaccenic acid (18.29) are the dominant comparison to other compounds. It is rich in essential oils, alcohols, esters and fatty acids. L- Menthol which is used as anaesthetic and Pregnane-3 is steroid are the compounds of keen interest and present only on this genotype, and reported first time in the present study. The rest of the compounds are presented in Table 3.

Seed extract of AFG-1 revealed 64 bioactive compounds (%), out of which fatty acids, esters of various compounds, naphthalene, vitamin A, vitamin E, silver salts and guaiene are the compounds which can be exploited by the pharmaceutical industries in the formulation of drugs,

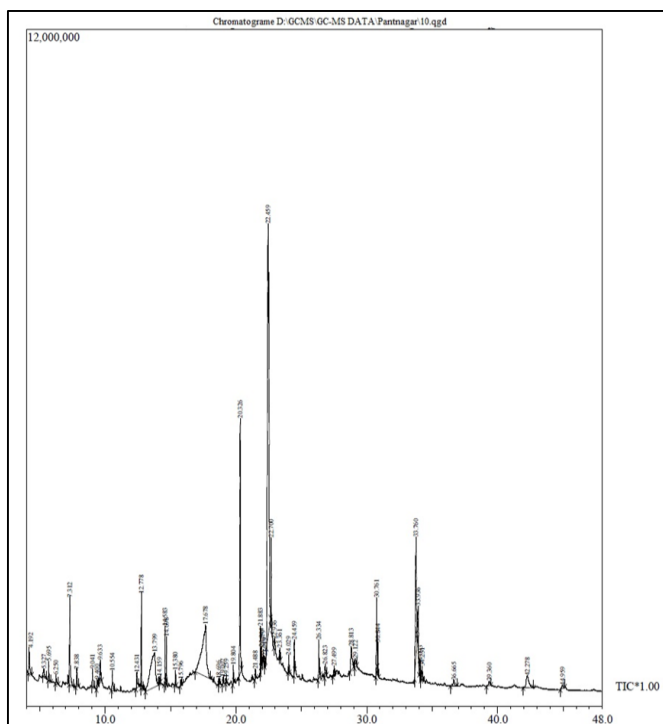
cosmetics, oils, soaps, and etc. Methylpyrrolidine (13.26), 6, 6-Dideutero-Nonen-1-OL-3 (10.41), pentadecanoic acid (5.64), cis-vaccenic acid (24.16), trilinolein (1.56), vitamin E (0.47) also reported in *Cassia angustifolia* [14] and retinol (0.59) are the major compounds. Alpha-guaiene is present only in this genotype, AFG-1. It is a sesquiterpenes used in flavouring, fragrance industries. Compounds major identified compound Table 4.

The methanolic seed extract of FGK-75 revealed the presence of 46 compounds (%). The main components are disogenin (0.73) and vitamin E (0.46). However, other compounds such as 2-Methylpyrrolidine (8.38%), 6, 6-dideutero-nonen-1-ol-3 (11.0), 9, 12-octadecadienoic acid (Z, Z) - (16.44) and cis-vaccenic acid (15.91) are the major compounds. The rest of the identified compounds are listed in Table 5.

The major bioactive compounds identified in five different genotypes which have potent biological effects and presented in Table 6. There are many compounds which are specific in a particular genotype. The compound, dihydro-nor-dicyclo-pentadienyl acetate used as perspirant and dl-alpha-

tochopherol an anti-inflammatory drug are identified only in GM-2. Cytidine an anti-depressant, spirost-5-en-3-ol, (3.beta. 25r) anti-cancerous, and Pregnane -3, 11, 20-triol steroid, identified only in genotype PR. Trilinolein - a cardio protective drug and retinol - an anti-aging found only in AFG-1. Diosgenin - an anti-cancerous drug and diallyl maletate - a polyester resin present only in FGK-75.

The major bioactive compounds identified in five different genotypes have potent biological effects. There are many compounds specific in a particular genotype. These compounds are dihydro-nor-dicyclo-pentadienyl acetate used as perspirant and dl-alpha-tochopherol an anti-inflammatory drug identified in GM-2 [11]. Cytosine an anti-depressant, spirost-5-en-3-ol, (3.beta. 25r) anti-cancerous, Pregnane -3, 11, 20-triol steroid, identified in genotype PR. Trilinolein - a cardio protective drug and retinol - an anti-aging present only in AFG-1. Diosgenin - an anti-cancerous drug [16] and diallyl malate - a polyester resin present only in FGK-75.



**Fig 1:** GC-MS chromatogram of methanolic seed fraction in genotype GM-2 of *Trigonella foenum-graecum*.

**Table 1:** Bioactive compounds present in methanolic extract in genotype GM-2 of *Trigonella foenum-graecum*

Peak	RT	Area	Area (%)	Name of compounds
1	4.192	2169528	1.19	2-Cyclopenten-1-one, 2-hydroxy-
2	5.327	737581	0.40	Oxirane, 2-butyl-3-methyl-, cis-
3	5.695	967972	0.53	1-hexanol, 2-ethyl-
4	6.250	675581	0.37	2,4-dihydroxy-3,3-dimethylbutanoic acid. ga
5	7.312	5621348	3.08	2-methylpyrrolidine
6	7.838	988441	0.54	2-Butenoic acid, 2,3-dimethyl-
7	9.041	1089786	0.60	2-oxonanone
8	9.400	621772	0.34	2,3-dihydro-benzofuran
9	9.633	1243254	0.68	3-Amino-4,5-dimethyl-2(5H)-furanone
10	10.554	1390464	0.76	2-oxonanone
11	12.431	1139453	0.63	1-isopropyl-2,2-dimethyl-propylideneamine
12	12.778	4048839	2.22	Dihydro-nor-dicyclo-pentadienyl acetate
13	13.799	17165438	9.42	Cyclopentanol
14	14.159	154631	0.08	7-hexadecenal, (z)-
15	14.583	1809015	0.99	2-(4-methyl-3-cyclohexen-1-yl)-1-propanol
16	14.698	1591286	0.87	2h-pyran-2-on, tetrahydro-4-(2-methyl-1-pro

17	15.380	700074	0.38	9-methyl-6-oxo-9-azabicyclo[3.3.1]non-2-yl a
18	15.796	226272	0.12	2-hydroxy-2,4,4-trimethyl-3-(3-methyl-buta-1
19	17.678	22276262	12.22	Mome inositol
20	18.696	179275	0.10	2,6,10-trimethyl,14-ethylene-14-pentadecne
21	19.007	213693	0.12	Pentadecanoic acid
22	19.259	182560	0.10	11-octadecenal (spectrum disagrees)
23	19.804	583000	0.32	Hexadecanoic acid, methyl ester
24	20.326	14577058	8.00	Pentadecanoic acid
25	21.488	374702	0.21	Heptadecanoic acid
26	21.883	1298188	0.71	9,12-octadecadienoic acid (z,z)-, methyl este
27	21.944	742137	0.41	9-octadecenoic acid (z)-, methyl ester
28	22.079	407734	0.22	2-cyclopenten-1-one, 2-methyl-3-(2-pentenyl)
29	22.219	295563	0.16	Methyl stearate
30	22.459	59575701	32.68	9,12-Octadecadienoic acid (Z,Z)-
31	22.700	2965473	1.63	Octadecanoic acid
32	22.936	265716	0.15	6-Nonen-1-ol, (E)-
33	23.361	590076	0.32	10,12-hexadecadien-1-ol
34	24.029	616421	0.34	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester
35	24.459	1148022	0.63	1-Hydroxy-2,2,6,6-tetramethyl-3-piperidinomethyl-4-piperid
36	26.334	2250865	1.23	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester
37	26.823	804454	0.44	2-Ethylbutyric acid, eicosyl ester
38	27.499	411987	0.23	1,2-benzenedicarboxylic acid
39	28.813	2946244	1.62	9,12-octadecadienoic acid (z,z)-, 2-hydroxy-1-
40	29.122	796893	0.44	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymet
41	30.761	3548165	1.95	Oxalic acid, 3,5-difluorophenyl nonyl ester
42	30.844	1128708	0.62	1,1,1,5,5,5-hexafluoro-4-[[3-(trifluoromethyl)
43	33.760	11119436	6.10	(2,14-Dioxocyclotetradecyl)acetic acid methyl ester
44	33.936	3833674	2.10	2-[2-Carboxyethyl]-3-methyl-tetrahydrofuran[4,5-a]androst
45	34.093	865129	0.47	9,12,15-octadecatrien-1-ol
46	34.231	997550	0.55	1,1,1,5,5,5-hexafluoro-4-[[3-(trifluoromethyl)
47	36.665	824794	0.45	DL-.alpha.-tocopherol
48	39.360	606890	0.33	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin
49	42.278	3107503	1.70	Gamma.-sitosterol
50	44.959	427451	0.23	Longifolenbromid-i

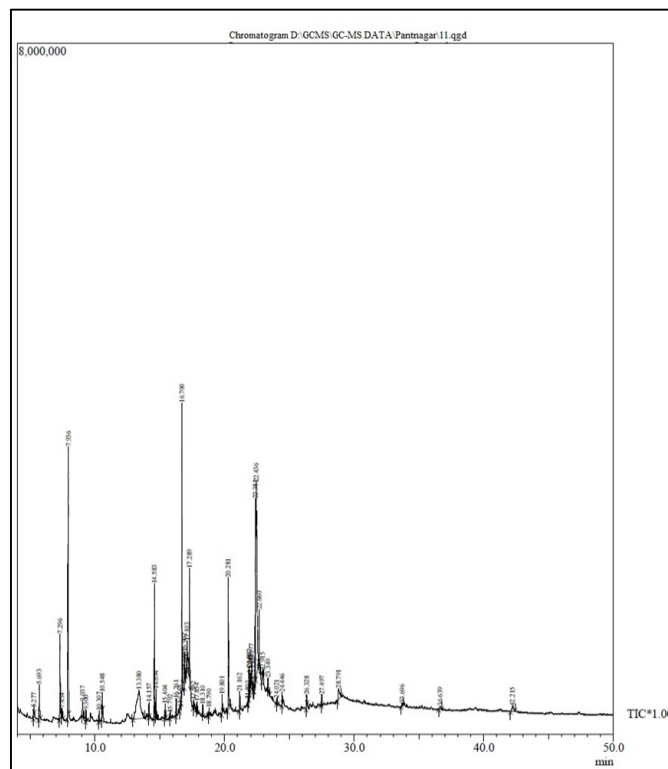
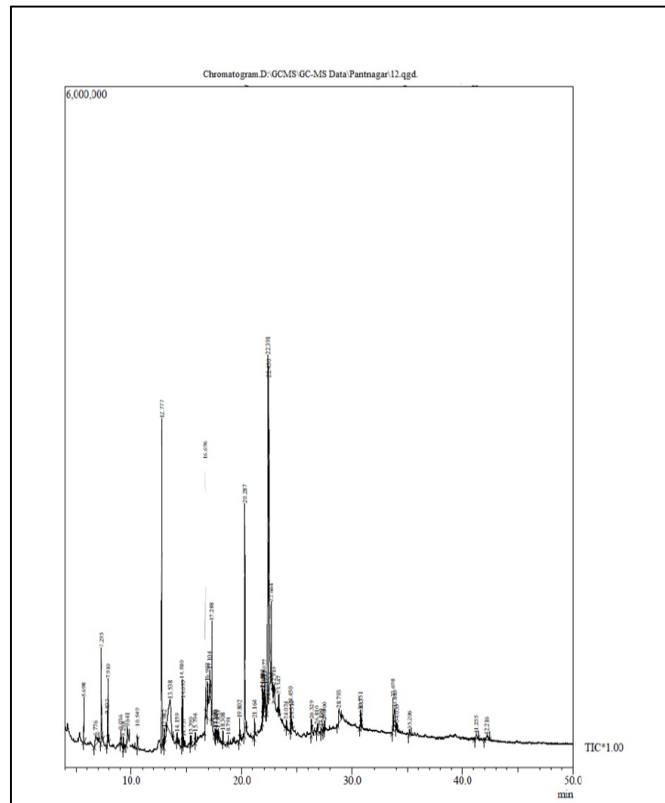


Fig 2: GC-MS chromatogram of methanolic seed fraction in genotype AM-1 of *Trigonella foenum-graecum*.

**Table 2:** Bioactive compounds present in methanolic extract in genotype AM-1 of *Trigonella foenum-graecum*.

Peak	RT	Area	Area (%)	Name of compounds
1	5.277	203604	0.28	3-cyclobutene-1,2-dione, 3,4-dihydroxy-
2	5.693	1172793	1.61	1-hexanol, 2-ethyl-
3	7.296	2921686	4.02	2-methylpyrrolidine
4	7.454	276562	0.38	formamide, n-octyl-
5	7.936	10902879	15.00	2-methylpyrrolidine
6	9.037	543489	0.75	2-oxonanone
7	9.300	288977	0.40	6-octen-1-ol, 3,7-dimethyl-
8	10.307	237108	0.33	2,2-dimethyl-1-aza-spiro[2.4] heptane
9	10.548	513866	0.71	2-pentenoic acid, 4-methyl-
10	13.380	6922320	9.52	6,6-dideutero-nonen-1-ol-3
11	14.157	328065	0.45	2h-pyran-2-on, tetrahydro-4-(2-methyl-3-met
12	14.583	2960433	4.07	2-(4-methyl-3-cyclohexen-1-yl)-1-propanol
13	14.694	626056	0.86	1-oxaspiro[4,5] decane 4-carbonitrile, 2-oxo-
14	15.404	424620	0.58	1,2-benzenedicarboxylic acid, diethyl ester
15	15.792	112984	0.16	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten
16	16.261	371358	0.51	methyl (3-oxo-2-pentylcyclopentyl)acetate
17	16.565	131061	0.18	1-(4-isopropylphenyl)-2-methylpropyl acetic
18	16.700	7151065	9.84	2-penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0(2
19	16.902	737817	1.01	bergamot, z-.alpha.-trans-
20	17.000	77739	0.11	1h-benzocycloheptene, 2,4a,5,6,7,8,9,9a-octah
21	17.103	609557	0.84	2-penten-1-ol, 2-methyl-5-(2-methyl-3-methyl
22	17.289	2500303	3.44	2-penten-1-ol, 2-methyl-5-(2-methyl-3-methyl
23	17.592	231868	0.32	2-penten-1-ol, 2-methyl-5-(2-methyl-3-methyl
24	17.854	205777	0.28	Lancelot, cis
25	18.310	218260	0.30	19,19-dimethoxy-3-oxoandrost-1-en-17-yl ace
26	18.790	175316	0.24	dodecanal dimethylacetal
27	19.801	415905	0.57	hexadecanoic acid, methyl ester
28	20.281	4140582	5.69	pentadecanoic acid
29	21.162	597603	0.82	naphthalene, 1-(2-nitro-2-propenyl)-
30	21.803	80689	0.11	piperidin-1-yl-acetic acid (2,2-dichloro-3-phenyl-cyclopropy
31	21.882	579896	0.80	9,12-octadecadienoic acid (z,z)-, methyl ester
32	21.943	498170	0.69	10-octadecenoic acid, methyl ester
33	22.011	222643	0.31	17-octadecenoic acid, methyl ester
34	22.077	431339	0.59	2-pentyl-3-methyl-2-cyclopenten-1-one
35	22.217	291650	0.40	octadecanoic acid, methyl ester
36	22.381	7420691	10.21	9,12-octadecadienoic acid (z,z)-
37	22.436	12437171	17.11	cis-vaccenic acid
38	22.660	1325918	1.82	octadecanoic acid
39	22.915	273734	0.38	12,15-octadecadienoic acid, methyl ester
40	23.349	423101	0.58	10,12-hexadecadien-1-ol
41	24.021	122253	0.17	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester
42	24.446	288594	0.40	octanamide, n-(2-hydroxyethyl)-
43	26.328	26.328	0.34	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester
44	27.497	257245	0.35	1,2-benzenedicarboxylic acid
45	28.791	909389	1.25	ethyl (9z,12z)-9,12-octadecadienoate
46	33.696	137159	0.19	6-acetoxycitronellal
47	36.639	176134	0.24	vitamin E
48	42.215	581115	0.80	gamma.-sitosterol

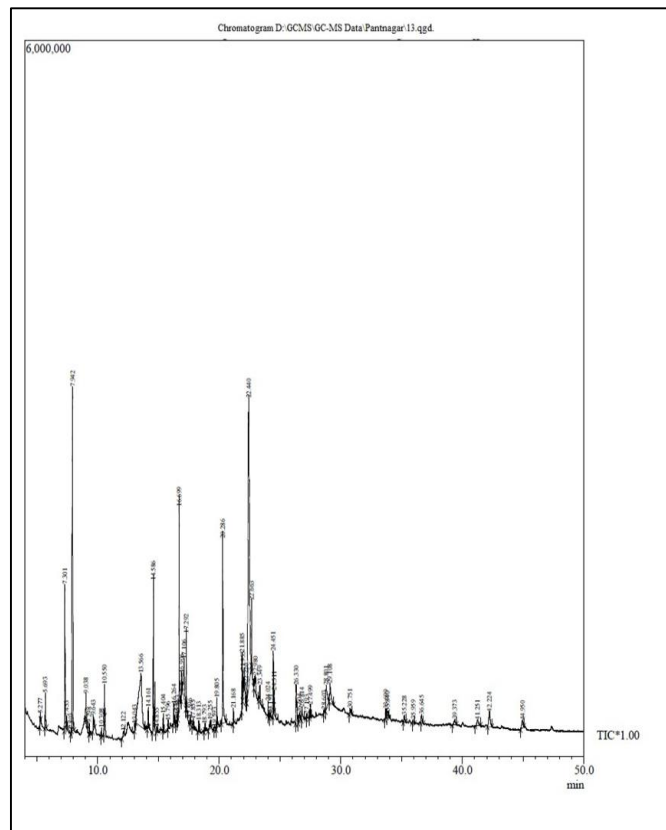


**Fig 3:** GC-MS chromatogram of methanolic seed fraction of genotype PR of *Trigonella foenum-graecum*.

**Table 3:** Bioactive compounds present in methanolic extract in genotype PR of *Trigonella foenum-graecum*.

Peak	RT	Area	Area (%)	Name of compound
1	5.698	1140036	1.61	1-hexanol, 2-ethyl-
2	6.776	762051	1.08	cyclopentane, 1-acetyl-1,2-epoxy-
3	7.295	2479557	3.50	2-methylpyrrolidine
4	7.833	594268	0.84	2-butenoic acid, 2,3-dimethyl-
5	7.910	1092062	1.54	2-methylpyrrolidine
6	9.036	325709	0.46	2-oxonanone
7	9.298	226486	0.32	6-octen-1-ol, 3,7-dimethyl-
8	9.641	629865	0.89	3-amino-4,5-dimethyl-2(5h)-furanone
9	10.549	283078	0.40	2-pentenoic acid, 4-methyl-
10	12.777	6900098	9.74	dihydro-nor-dicyclo-pentadienyl acetate
11	12.982	268040	0.38	indan-1,3-diol monoacetate
12	13.538	4277370	6.04	Cytidine
13	14.159	158969	0.22	bicyclo[3.1.1]heptane-2-methanol, 6,6-dimethyl-, acetate
14	14.580	1069509	1.51	2-(4-methyl-3-cyclohexen-1-yl)-1-propanol
15	14.695	729797	1.03	1-oxaspiro[4,5] decane 4-carbonitrile, 2-oxo-
16	14.757	105297	0.15	benzene, 1,2,3-trimethoxy-5-(2-propenyl)-
17	15.390	231167	0.33	9-methyl-6-oxo-9-azabicyclo[3.3.1]non-2-yl a
18	15.794	146059	0.21	menthol, 1'-(butyn-3-one-1-yl)-, (1s,2s,5r)-
19	16.696	4979262	7.03	2-penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0(2
20	16.902	554706	0.78	bergamotol, z.-alpha.-trans-
21	17.104	422985	0.60	2-penten-1-ol, 2-methyl-5-(2-methyl-3-methyl
22	17.288	1821248	2.57	2-penten-1-ol, 2-methyl-5-(2-methyl-3-methyl
23	17.590	131754	0.19	2-penten-1-ol, 2-methyl-5-(2-methyl-3-methyl
24	17.680	255929	0.36	tetradecanoic acid
25	17.853	126868	0.18	lanceol, cis
26	18.308	212696	0.30	19,19-dimethoxy-3-oxoandro-1-en-17-yl ace
27	18.791	160138	0.23	dodecanal dimethylacetal
28	19.802	361119	0.51	hexadecanoic acid, methyl ester
29	20.287	5156170	7.28	pentadecanoic acid
30	21.164	377892	0.53	naphthalene, 1-(2-nitro-2-propenyl)-
31	21.881	463230	0.65	9,12-octadecadienoic acid (z,z)-, methyl este
32	21.943	394108	0.56	cis-13-octadecenoic acid, methyl ester
33	22.011	243290	0.34	16-octadecenoic acid, methyl ester
34	22.077	396934	0.56	2-pentyl-3-methyl-2-cyclopenten-1-one
35	22.217	289205	0.41	octadecanoic acid, methyl ester

36	22.391	11611745	16.40	9,12-octadecadienoic acid (z,z)-
37	22.450	12948286	18.29	cis-vaccenic acid
38	22.664	1394931	1.97	octadecanoic acid
39	22.919	278984	0.39	10,13-octadecadienoic acid, methyl ester
40	23.347	463280	0.65	10,12-hexadecadien-1-ol
41	24.024	148999	0.21	fumaric acid, 2-dimethylaminoethyl heptadecyl ester
42	24.450	412468	0.58	octanamide, n-(2-hydroxyethyl)-
43	24.510	214511	0.30	octanamide, n-(2-hydroxyethyl)-
44	26.329	209887	0.30	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester
45	26.810	161878	0.23	oxalic acid, 3,5-difluorophenyl tetradecyl ester
46	27.190	145865	0.21	octadecanoic acid, methyl ester
47	27.363	64495	0.09	Pregnane-3,11,20-triol, (3.alpha., 11.beta., 20.beta.)-
48	27.500	165091	0.23	1,2-benzenedicarboxylic acid
49	28.793	873134	1.23	ethyl (9z,12z)-9,12-octadecadienoate #
50	30.751	423099	0.60	1-tetradecanol
51	30.832	145625	0.21	1,1,1,5,5,5-hexafluoro-4-[3-(trifluoromethyl
52	33.698	1232884	1.74	9,12-octadecadienoic acid (z,z)-, 2,3-dihydroxypropyl ester
53	33.883	946663	1.34	4-cyanobenzoic acid, undec-10-enyl ester
54	34.033	272677	0.39	6,9,12,15-docosatetraenoic acid, methyl ester
55	35.206	251905	0.36	spirost-5-en-3-yl acetate #
56	41.255	391370	0.55	spirost-5-en-3-ol, (3.beta.,25r)-
57	42.216	282265	0.40	murolan-3,9(11)-diene-10-peroxy



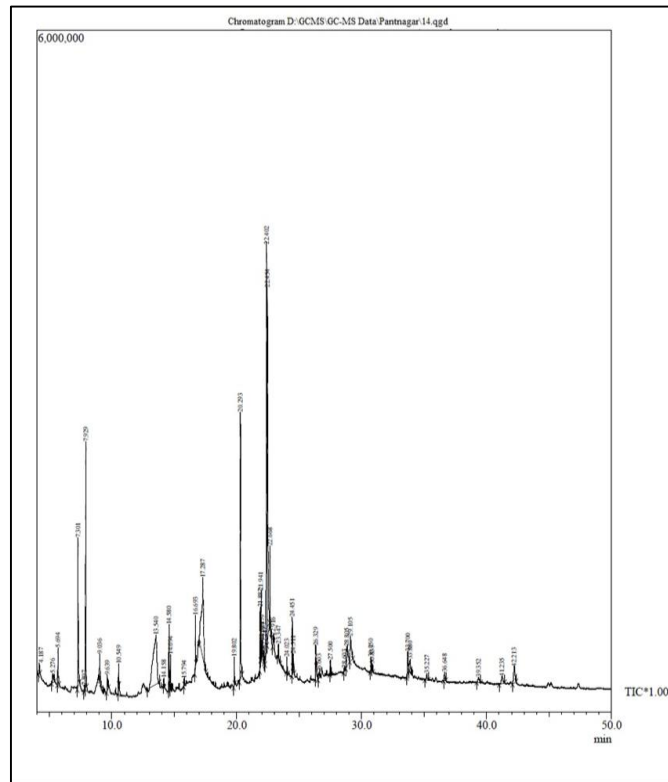
**Fig 4:** GC-MS chromatogram of methanolic seed fraction of genotype AFG-1 of *Trigonella foenum-graecum* L.

**Table 4:** Bioactive compounds present in methanolic extract in genotype AFG-1 of *Trigonella foenum-graecum*.

Peak	RT	Area	Area (%)	Name of compounds
1	5.277	143146	0.17	3-cyclobutene-1,2-dione, 3,4-dihydroxy-
2	5.693	855802	1.04	1-hexanol, 2-ethyl-
3	7.301	3658970	4.44	2-methylpyrrolidine
4	7.453	192722	0.23	formamide, n-octyl-
5	7.803	66006	0.08	aziridine, 1-(1-butenyl)-, (e)-
6	7.942	10928354	13.25	2-methylpyrrolidine
7	9.038	676183	0.82	2-oxonanone
8	9.298	204972	0.25	6-octen-1-ol, 3,7-dimethyl-
9	9.643	496575	0.60	3-amino-4,5-dimethyl-2(5h)-furanone
10	10.308	149827	0.18	2,2-dimethyl-1-aza-spiro[2.4]heptane
11	10.550	686936	0.83	2-pentenoic acid, 4-methyl-

12	12.122	199632	0.24	3-azabicyclo[3.2.1]octane, 1,8,8-trimethyl-, (1r)-
13	13.043	63234	0.08	.alpha.-guaiene
14	13.566	8586452	10.41	6,6-dideutero-nonen-1-ol-3
15	14.161	425261	0.52	2h-pyran-2-on, tetrahydro-4-(2-methyl-3-met
16	14.586	2600958	3.15	2-(4-methyl-3-cyclohexen-1-yl)-1-propanol
17	14.855	148621	0.18	dodecanoic acid, silver(1+) salt
18	15.404	478103	0.58	1,2-benzenedicarboxylic acid, diethyl ester
19	15.796	133276	0.16	3a(1h)-azulenol, 2,3,4,5,8,8a-hexahydro-6,8a-d
20	16.264	391880	0.48	methyl (3-oxo-2-pentylcyclopentyl)acetate
21	16.443	95391	0.12	5-methylene-1,3a,4,5,6,6a-hexahydropentalen-1-ol
22	16.563	100074	0.12	1-(4-isopropylphenyl)-2-methylpropyl acetat
23	16.699	4195004	5.09	2-penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0(2
24	16.906	434105	0.53	bergamotol, z.alpha.-trans-
25	17.106	2089650	2.53	mome inositol
26	17.292	1562745	1.89	2-penten-1-ol, 2-methyl-5-(2-methyl-3-methyl
27	17.596	133173	0.16	2-penten-1-ol, 2-methyl-5-(2-methyl-3-methyl
28	17.855	238312	0.29	lanceol, cis
29	18.313	249099	0.30	19,19-dimethoxy-3-oxoandrost-1-en-17-yl acet
30	18.793	134634	0.16	19,19-dimethoxy-3-oxoandrost-1-en-17-yl acet
31	19.255	359040	0.44	1,2-epoxyundecane
32	19.593	138902	0.17	2h-oxecin-2-one, 3,4,7,8,9,10-hexahydro-4-hydroxy-10-met
33	19.805	457844	0.56	hexadecanoic acid, methyl ester
34	20.286	4654542	5.64	pentadecanoic acid
35	21.168	360948	0.44	naphthalene, 1-(2-nitro-2-propenyl)-
36	21.885	793546	0.96	9,12-octadecadienoic acid (z,z)-, methyl ester
37	21.953	790900	0.96	9,12,15-octadecatrienoic acid, methyl ester
38	22.220	288341	0.35	methyl stearate
39	22.440	19923751	24.16	cis-vaccenic acid
40	22.663	1317238	1.60	octadecanoic acid
41	22.980	928798	1.13	e,z-1,3,12-nonadecatriene
42	23.349	639204	0.77	9,12-octadecadienoic acid (z,z)-
43	24.024	265523	0.32	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester
44	24.191	128925	0.16	hexadecanoic acid, 1-(hydroxymethyl)-1,2-e
45	24.451	1012014	1.23	1-hydroxy-2,2,6,6-tetramethyl-3-(1-piperidiny
46	24.511	418017	0.51	palmidrol
47	26.330	1314405	1.59	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester
48	26.604	260805	0.32	2-hydroxy-3-[(9e)-9-octadecenoyloxy]propyl
49	26.814	493795	0.60	2-ethylbutyric acid, eicosyl ester
50	27.193	166966	0.20	octadecanoic acid, methyl ester
51	27.499	255437	0.31	1,2-benzenedicarboxylic acid
52	28.603	141162	0.17	5,5-dimethyl-1,3-dioxane-2-ethanol, tert-butyl dimethylsilyl
53	28.801	1608286	1.95	ethyl (9z,12z)-9,12-octadecadienoate
54	29.108	1289650	1.56	trilinolein
55	30.751	303595	0.37	oxalic acid, 3,5-difluorophenyl nonyl ester
56	33.699	315433	0.38	9,12-octadecadienoic acid (z,z)-, 2,3-dihydroxypropyl ester
57	33.840	352217	0.43	glycine,n-isobutoxycarbonyl-, undec-10-enyl ester
58	35.228	288602	0.35	spirost-5-en-3-yl acetate #
59	35.959	161219	0.20	murolan-3,9(11)-diene-10-peroxy
61	36.645	386031	0.47	vitamin E
62	39.373	291374	0.35	5-(7a-isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-meth
63	41.251	419904	0.51	spirost-5-en-3-ol, (3.beta.,25r)-
64	42.224	1142459	1.39	gamma.-sitosterol





**Fig 5:** GC-MS chromatogram of methanolic seed fraction of genotype FGK-75 of *Trigonella foenum-graecum*.

**Table 5:** Bioactive compounds present in methanolic extract of genotype FGK-75 of *Trigonella foenum-graecum*.

Peak	RT	Area	Area (%)	Name of compound
1	4.187	309122	0.38	2-Cyclopenten-1-one, 2-hydroxy-
2	5.276	453713	0.56	Piperazine, 3-butyl-2,5-dimethyl-
3	5.694	937998	1.16	1-hexanol, 2-ethyl-
4	7.301	3734515	4.62	2-methylpyrrolidine
5	7.807	71447	0.09	Diallyl maleate
6	7.929	6773738	8.38	2-methylpyrrolidine
7	9.036	643776	0.80	2-oxonanone
8	9.639	493100	0.61	3-Amino-4,5-dimethyl-2(5H)-furanone
9	10.549	444723	0.55	2-pentenoic acid, 4-methyl-
10	13.540	8898772	11.00	6,6-dideutero-nonen-1-ol-3
11	14.158	146815	0.18	Bicyclo[3.1.1]heptane-2-methanol, 6,6-dimethyl-, acetate
12	14.580	1073867	1.33	2-(4-methyl-3-cyclohexen-1-yl)-1-propanol
13	14.694	605187	0.75	1-Oxaspiro[4,5]decane 4-carbonitrile, 2-oxo-
14	15.794	113376	0.14	2-hydroxy-2,4,4-trimethyl-3-(3-methyl-buta-1
15	16.693	1012502	1.25	2-penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0(2
16	17.287	7757226	9.59	Mome inositol
17	19.802	478727	0.59	Hexadecanoic acid, methyl ester
18	20.293	6060457	7.49	Pentadecanoic acid
19	21.882	872692	1.08	9,12-octadecadienoic acid (z,z)-, methyl este
20	21.941	1123943	1.39	9-Octadecenoic acid (Z)-, methyl ester
21	22.011	193548	0.24	9-octadecenoic acid (z)-, methyl ester
22	22.079	382527	0.47	2-pentyl-3-methyl-2-cyclopenten-1-one
23	22.217	295385	0.37	Methyl stearate
24	22.402	13293100	16.44	9,12-octadecadienoic acid (z,z)-
25	22.454	12865304	15.91	Cis-Vaccenic acid
26	22.668	1439991	1.78	Octadecanoic acid
27	22.916	229028	0.28	9-octadecyne
28	23.347	445665	0.55	10,12-hexadecadien-1-ol
29	24.023	290160	0.36	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester
30	24.451	957207	1.18	1-hydroxy-2,2,6,6-tetramethyl-3-(1-piperidinyl
31	24.511	394565	0.49	Palmidrol
32	26.329	443960	0.55	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester
33	26.603	207241	0.26	Glycine,N-isobutoxycarbonyl-, undec-10-enyl ester
34	27.500	270179	0.33	1,2-benzenedicarboxylic acid
35	28.603	109585	0.14	14-Aza-10,12-dioxatetracyclo[6.5.1.0(2,7).0(9,13)]tetradeca-
36	28.805	653757	0.81	Ethyl (9z,12z)-9,12-octadecadienoate #

37	29.105	1457178	1.80	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester
38	30.750	340965	0.42	1-tetradecanol
39	30.839	114952	0.14	Decane, 1,1'-oxybis-
40	33.700	982701	1.22	4-Cyanobenzoic acid, undec-10-enyl ester
41	33.880	680866	0.84	4-Cyanobenzoic acid, undec-10-enyl ester
42	35.227	298947	0.37	Spirost-5-en-3-yl acetate
43	36.648	369047	0.46	Vitamin E
44	39.352	347716	0.43	7-(2-hydroxy-1-methylethyl)-1,4a-dimethyl-2
45	41.235	588348	0.73	Diosgenin
46	41.213	1206538	1.84	Stigmast-5-en-3-ol, (3.beta.)-

**Table 6:** Major bioactive compounds present in five different genotypes of *Trigonella foenum-graecum* L.

Compound	RT	M.W.	Chemical formula	Genotypes					Activity
				AFG-1	AM-1	GM-2	FGK-75	PR	
1-hexanol, 2-ethyl	5.69	130	C8H18O	1.04	1.61	-	1.16	1.61	Flavour additive, fragrant
2-methylpyrrolidine	7.94	85	C5H11N	13.25	15.00	3.08	8.38	3.50	NA
6,6-dideutero-nonen-1-ol-3	13.56	120	C9H16D2O	10.41	9.52	-	11.00	-	Essential oil
2-penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0(2	16.69	220	C15H24O	5.09	9.84	-	1.25	7.03	Flavouring agent
bergamotol, z.alpha.-trans-	16.90	220	C15H24O	0.53	1.01	-	-	0.78	Olfactory properties
2-penten-1-ol, 2-methyl-5-(2-methyl-3-methyl	10.55	114	C6H10O2	1.89	3.44	-	-	7.03	Perfume industry
pentadecanoic acid	20.29	282	C15H30O2	5.64	5.69	0.12	7.49	7.28	Antibacterial, antifungal
9,12-octadecadienoic acid (z,z)-	22.39	280	C18H32O2	-	10.21	32.68	16.44	16.40	Anti-inflammatory, Nematicide
cis-vaccenic acid	22.44	282	C18H34O2	24.16	17.11	-	15.91	18.29	Hypolipidaemic, antihypertensive
octadecanoic acid	22.66	284	C18H36O2	1.60	1.82	-	1.78	1.97	Antibacterial, antifungal, antitumor
ethyl (9z,12z)-9,12-octadecadienoate	28.80	308	C20H36O2	1.95	1.25	-	-	1.23	NA
dihydro-nor-dicyclo-pentadienyl acetate	12.77	192	C12H16O2	-	-	2.22	-	9.74	Antiperspirant
Cytidine	13.53	243	C9H13N3O5	-	-	-	-	6.04	Glutamatergic antidepressant drug
Trilinolein	29.10	878	C57H98O6	1.56	-	-	-	-	Cardioprotective, circulatory disease
Pentadecanoic acid	20.28	242	C15H30O2	-	-	8.00	7.49	7.28	Agricultural chemicals
spirost-5-en-3-ol, (3.beta.,25r)	41.25	414	C27H42O3	0.51	-	-	-	0.55	Anticancerous, anti-inflammatory
mome inositol	17.10	194	C7H14O6	-	-	12.22	9.59	-	Antiallopecic, anticirrhotic, lipotropic
Palmdrol	24.51	299	C18H37NO2	0.51	-	-	0.49	-	Chronic diseases
dl-alpha-tochopherol	36.66	430	C29H50O2	-	-	0.45	-	-	Anti-inflammatory, antioxidants, anti-microbial
Diosgenin	41.23	414	C27H42O3	-	-	-	0.73	-	Cortico-steroid, anti-inflammatory
Vitamin E	36.64	430	C29H50O2	0.47	0.24	-	0.46	-	Antioxidant, antiageing
gamma.-sitosterol	42.22	414	C29H50O	1.39	0.80	1.70	-	-	Anti-diabetic, Anti-ageic, Anticancer, antimicrobial, anti-inflammatory, antiarrhoeal and antiviral
Retinol	44.95	286	C20H30O	0.59	-	-	-	-	Immune system maintenance, antiageing
Pregnane -3,11,20-triol	27.36	336	C21H36O3	-	-	-	-	0.09	Steroid
Naphthalene, 1-(2-nitro-2-propenyl)	21.16	213	C13H11NO2	0.44	0.82	-	-	0.53	Fumigant
Lanceol-cis	17.85	220	C15H24O	0.29	0.28	-	-	0.18	Essential oil

## Conclusion

These genotypes can be exploited by the pharmaceutical industries in the preparation of herbal medicines. Further enhancement of some important compounds reported to have potent biological effects can be done in these genotypes. The findings of the present study would help to ascertain the potential of above mentioned genotypes of fenugreek as a health promoting spice and as a medicinal plant along with preparation of herbal medicinal formulation and nutritional fortification. These compounds are reported for the first time in these genotypes in the present study. The findings of the present study also supplement the future findings in nutritional, pharmaceutical and therapeutic industries.

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## Conflict of interest

We declare that we have no conflict of interest.

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