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# GC-MS AND FT-IR ANALYSIS FOR DETERMINATION OF BIOACTIVE COMPOUNDS IN FENUGREEK (*Trigonella foenum-graecum* L.) var. 'GM-2'

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## ABSTRACT

**The present study focused on the classification and identification of bioactive components, along with functional groups, present in methanolic seed-extract of fenugreek (*Trigonella foenum-grecum* L.) var. Gujarat Methi-2 (GM-2). Seed were analyzed using GC-MS-QP2010 Ultra and FT-IR by alpha ECO-ATR spectrometer. The mass spectra of compounds matched with National Institute of Standards and Technology (NIST) library. GC/MS analysis of methanol extract of var. GM-2 seed revealed the presence of 50 bioactive compounds amongst which 9, 12-octadecadienoic acid (Z, Z)- (32%), mome inositol (12%), cyclopentanol (9%) and 2-methylpyrrolidine (3%) were most dominant. Peaks obtained from FT-IR indicated the presence of various functional groups. The results offer a platform for using var. GM-2 seed as herbal alternative for synthetic antimicrobial and antioxidants sources.**

**Keywords:** FT-IR spectroscopy, Gujarat Methi-2, GC-MS analysis, medicinal properties, *Trigonella foenum-graecum*

## INTRODUCTION

To meet the increasing demand of medicinal plants for use in indigenous systems of medicine and for pharmaceutical industries many medicinal plants need to be cultivated commercially but soil salinity and other forms of pollutants present serious threats to plant production. Fenugreek [*Trigonella foenum-graecum* L.], an annual crop, belongs to the family fabaceae and shows promising medicinal properties (Ahmed *et al.*, 2016). Fenugreek or methi is extensively cultivated in most regions of the world for its medicinal, nutraceutical and pharmaceutical attributes. It is native to South Europe and Asia and grown in many parts of India (Tanvir *et al*., 2014). Its leaves and seeds are consumed in various countries for different purposes like anti-diabetic, anti-fertility, anti-microbial, anti-parasitic and hypo-cholesterolaemic, antiseptic, anti-bronchitis, carminative, aphrodisiac, analgesic, antipyretic anticancer antioxidant, etc. (Chauhan *et al.*, 2011).

India is the largest producer of fenugreek in world with Rajasthan, Gujarat, Uttarakhand, Uttar Pradesh, Maharashtra, Madhya Pradesh, Haryana and Punjab as the foremost producer states. In India fenugreek powder is used as lactation-stimulating agent and protective against ethanol toxicity. Fenugreek seeds are aromatic but bitter having carminative galactogouge, antibacterial and antiviral properties. Rheological properties and emulsions of fenugreek galactomannans seed endosperm have been reported (Wu *et al.*, 2009). Major phytoconstituents found in fenugreek seeds include saponins (fenugreekine, diosgenin, etc.), alkaloids (trigonelline, gentianine, carpaine, etc.), amino acids (4-hydroxyisoleucin, arginine, etc.), proteins and flavonoids. The seeds find extensive use in Indian cuisine to flavor many foods including spice blends, curry powders and tea. The fenugreek seed

possess various medicinal properties like anti-inflammatory, antitumor, cardiotonic, carminative, demulcent, diuretic, emollient, expectorant, febrifuge, galactogogue, hypo-glycemic, hypotensive and laxative action so is frequently used in traditional system of Indian medicine. Fenugreek seeds also contains a substantial amount of fibre (Montgomery, 2009), phospholipids, glycolipids, oleic acid, linolenic acid, linoleic acid (Sulieman *et al.,* 2000; Chatterjee *et al.,* 2010), choline, vitamin A, $B_1$, $B_2$ and C, nicotinic acid and niacin (Leela and Shafeekh, 2008). Despite its exceptional nutritional and medicinal values only a few studies have been made to assess the genetic enhancements and development of fenugreek production agronomically. Fenugreek seeds are a rich source of fiber (50-65 g 100 $g^{-1}$) mainly the non-starch polysaccharides (Montgomery, 2009).

Gas chromatography and mass spectroscopy (GC-MS) is a technique employed for screening, identification and quantification of many susceptible bioactive compounds in plant extracts. Gas chromatograph is now-a-day used to separate drugs that might be present in the sample while retention time is meant to identify the characteristic of a drug. The detector used for GC is mass spectrometry (MS). The fragmentation pattern for a drug is always unique and, therefore, very helpful for identifying the characteristic of a drug. The identification of a drug by its retention time and fragmentation patterns along with sample specific information afforded to make GC-MS the foremost confirmation method for analyzing the constituent present in herbal extract (Parthipan *et al.,* 2016). Different solvent extractions provide different types of compounds because of variability in their chemical structure and sensitivity towards the extraction or hydrolysis method (Isfahlan *et al.,* 2010). In recent years GC-MS analysis now-a-days has increasingly been applied for the analysis of bioactive compound presents in medicinal plants. This technique has proved a valuable method for the analysis of non-polar components and fatty acid, volatile essential oil, lipids and alkaloids (Mythili *et al*., 2013).

A method of instrumental analysis Fourier transform infrared spectroscopy (FT-IR) is the most extensively used but well-established tool for the determination of functional groups and polar compounds in a sample based on structural vibration analysis for identification of traditional herbal medicine. This emergent technique is specific, effective, rapid, non-destructive and non-polluting (Fan *et al.,* 2013, Li *et al.,* 2013). Regarding to fingerprint characteristics and extensive applicability for the samples, FT-IR has played an important role in pharmaceutical analysis in the recent years (Adina and Mazura, 2011). The present study was aimed to identify the bioactive compounds and different functional groups in fenugreek var. GM-2 being enriched and fortified by pharmaceutical and nutraceutical properties.

## MATERIALS AND METHODS

The healthy and mature seeds of fenugreek var. 'GM-2' were procured from Vegetable Research Centre Pantnagar Uttarakhand (India). This variety was released by the Centre for Research on Seed Spices, S.D. Agricultural University, Jagudan Gujarat (India). GM-2 is an improved high yielding disease resistant variety. The seeds are collected and washed with distilled water to remove the traces of dust and soil and dried to make the fine powder. Powdered seed (2 g) was soaked in 15 mL methanol for 48 h; centrifuged at 10000 rpm for 10 min and filtered through Whatmann filter paper No.1 along with 2 g anhydrous $Na_2SO_4$ to remove the sediments and traces in the filtrate. It was then subjected to GC-MS analysis.

***Gas chromatography-mass spectrometry analysis***

The GC-MS analysis was performed in a GCMS-QP2010 Ultra Shimadzu system comprising of a gas chromatograph interfaced to a mass spectrometer (GC-MS) at Jawahar Lal Nehru University, New Delhi (India). For this instrument the following conditions were employed: plunger speed (suction): high viscosity compression time: 0.2 sec; plunger speed (injection): high; syringe insertion

speed: high; injection mode: normal; pumping: 5 times; injection port dwell time: 0.0 sec; terminal air gap: no; plunger washing speed: high; washing volume: 6 µL; syringe suction position: 0.0 mm; syringe injection position: 0.0 mm; column oven temperature: 80°C; injection mode: split; flow control mode: linear velocity; injection volume: 1 µL; pressure:81.9 kPa; total flow: 16.3 mL $min^{-1}$; column flow:1.21 mL $min^{-1}$; linear velocity: 40.5 cm $sec^{-1}$; purge flow: 3 mL $min^{-1}$; split ratio: 10; ion source temperature: 230°C; interface temperature: 270°C; solvent cut time: 3.50 min; detector gain mode relative detector gain: +0.00 kV; threshold: 1000; start time: 4.00 min; end time: 50.24 min; ACQ mode: scan event time: 0.20 sec; scan speed: 3333; start m/z: 40; end m/z: 650.

***Identification of components***

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

***FT-IR spectroscopy***

FT-IR spectra was recorded in the region of 3500-1000 $cm^{-1}$ on alpha ECO-ATR spectrometer (Bruker), maintained in an air-conditioned room (21°C, 50% relative humidity).

***Analysis conditions***

For the experiment, each spectrum was calculated from a total of 32 co-added scans with a resolution of 4 $cm^{-1}$, prior to data analysis. The FT-IR spectra were baseline corrected and smoothed with their absorbance normalized at 1700 $cm^{-1}$. Pure potassium bromide (KBr) background spectrum was recorded before sample analysis. Seed powder (1 mg) was incorporated into 100 mg spectroscopic grade KBr powder (1% w/w) and pressed into a 1.0 mm transparent disk by 8 MPa pressure for transmission infrared spectroscopy. The organic functional groups of var. GM-2 seeds were analyzed using an alpha ECO-ATR spectrometer (Bruker) in frequency range of 3500 $cm^{-1}$ to 1000 $cm^{-1}$ as per the method of Florence and Jeeva (2015) with some minor modifications. Each sample was scanned with three replicates. The scans of each sample were examined for consistence and average spectrum of each sample used for further analyses.

## RESULTS AND DISCUSSION

In present study methanol was used as an extracting solvent due to its high polarity. Same solvent was also used by Raman *et al.* (2012) for GC-MS analysis of *Eupatorium odorataum*; Sah *et al.* (2015) in *Rumex vesicarius* and Parveen *et al*. (2016) in *Cassia angustifolia*. The methanolic seed fraction of cv. GM-2 revealed 47 bioactive compounds out of which 2-methylpyrrolidine, mome inositol, pentadecanoic acid, 9,12-octadecadienoic acid (Z, Z), (2, 14-dioxocyclo-tetradecyl) acetic acid methyl ester, dl-α-tocopherol and γ-sitostero were major compounds. Along with these, esters, amino acids and tocopherols were the compounds of special interest as they are involved in various biological activities. For instance, tocopherols exhibit anti-inflammatory, antioxidant and antimicrobial properties (Table 1). Similar results in fenugreek methanolic seed extract such as fatty acids, essential oils and alkaloids have been reported by Praveen *et al.* (2016). Haque *et al.* (2015) reported the presence of 14 different compounds in fenugreek of which 86% was essential oils. Parthipan *et al.* (2016) reported 46 phytochemical compounds in *Cassia angustifolia* by GC-MS analysis. The prevailing compounds were vitamin E, penta-deconoic acid and squalene which are used in cosmetics and possess antioxidants, antibiotic and anti-bacterial properties.

Amongst the identified phytochemicals, alkaloids show antimicrobial and anti-inflammatory activities. The deconate salts and esters of various drugs were also present of which decanoic acid is

**Table 1: Bioactive compounds present in seed extract of fenugreek var. 'GM-2'**

| S. No | Retention time (min) | Name of the compound | Molecular Formula | Molecular weight | % area |
|---|---|---|---|---|---|
| 1 | 4.192 | 2-Cyclopenten-1-one, 2-hydroxy- | $C_5H_6O_2$ | 98 | 1.19 |
| 2 | 5.327 | Oxirane, 2-butyl-3-methyl-, cis- | $C_7H_{14}O$ | 114 | 0.40 |
| 3 | 5.695 | 1-Hexanol, 2-ethyl- | $C_8H_{18}O$ | 130 | 0.53 |
| 4 | 6.250 | 2,4-Dihydroxy-3,3-dimethylbutanoic acid .ga | $C_6H_{10}O_3$ | 130 | 0.37 |
| 5 | 7.312 | 2-Methylpyrrolidine | $C_5H_{11}N$ | 85 | 3.08 |
| 6 | 7.838 | 2-Butenoic acid, 2,3-dimethyl- | $C_6H_{10}O_2$ | 114 | 0.54 |
| 7 | 9.041 | 2-Oxonanone | $C_8H_{14}O_2$ | 142 | 0.60 |
| 8 | 9.400 | 2,3-Dihydro-benzofuran | $C_8H_8O$ | 120 | 0.34 |
| 9 | 9.633 | 3-Amino-4,5-dimethyl-2(5H)-furanone | $C_6H_9NO_2$ | 127 | 0.68 |
| 10 | 12.431 | 1-Isopropyl-2,2-dimethyl-propylideneamine | $C_8H_{17}N$ | 127 | 0.63 |
| 11 | 12.778 | Dihydro-nor-dicyclo-pentadienyl acetate | $C_{12}H_{16}O_2$ | 192 | 2.22 |
| 12 | 13.799 | Cyclopentanol | $C_5H_{10}O$ | 86 | 9.42 |
| 13 | 14.159 | 7-Hexadecenal, (z)- | $C_{16}H_{30}O$ | 238 | 0.08 |
| 14 | 14.583 | 2-(4-Methyl-3-cyclohexen-1-yl)-1-propanol | $C_{10}H_{17}DO$ | 155 | 0.99 |
| 15 | 14.698 | 2h-Pyran-2-on, tetrahydro-4-(2-methyl-1-pro | $C_9H_{14}O_2$ | 154 | 0.87 |
| 16 | 15.380 | 9-Methyl-6-oxo-9-azabicyclo[3.3.1]non-2-yl a | $C_{11}H_{17}NO_3$ | 211 | 0.38 |
| 17 | 15.796 | 2-hydroxy-2,4,4-trimethyl-3-(3-methyl-buta-1 | $C_{14}H_{22}O_2$ | 222 | 0.12 |
| 18 | 17.678 | Mome inositol | $C_7H_{14}O_6$ | 194 | 12.22 |
| 19 | 18.696 | 2,6,10-Trimethyl,14-ethylene-14-pentadecne | $C_{20}H_{38}$ | 278 | 0.10 |
| 20 | 19.259 | 11-Octadecenal (spectrum disagrees) | $C_{18}H_{34}O$ | 266 | 0.10 |
| 21 | 19.804 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270 | 0.32 |
| 22 | 20.326 | Pentadecanoic acid | $C_{15}H_{30}O_2$ | 242 | 8.00 |
| 23 | 21.488 | Heptadecanoic acid | $C_{17}H_{34}O_2$ | 270 | 0.21 |
| 24 | 21.883 | 9,12-Octadecadienoic acid (z,z)-, methyl ester | $C_{19}H_{34}O_2$ | 294 | 0.71 |
| 25 | 21.944 | 9-Octadecenoic acid (z)-, methyl ester | $C_{19}H_{36}O_2$ | 296 | 0.41 |
| 26 | 22.079 | 2-Cyclopenten-1-one, 2-methyl-3-(2-pentenyl) | $C_{11}H_{18}O$ | 166 | 0.22 |
| 27 | 22.219 | Methyl stearate | $C_{19}H_{38}O_2$ | 298 | 0.16 |
| 28 | 22.459 | 9,12-Octadecadienoic acid (Z,Z)- | $C_{18}H_{32}O_2$ | 280 | 32.68 |
| 29 | 22.700 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 1.63 |
| 30 | 22.936 | 6-Nonen-1-ol, (E)- | $C_9H_{18}O$ | 142 | 0.15 |
| 31 | 23.361 | 10,12-Hexadecadien-1-ol | $C_{16}H_{30}O$ | 238 | 0.32 |
| 32 | 24.029 | 3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester | $C_{12}H_{23}NO_2$ | 213 | 0.34 |
| 33 | 24.459 | 1-Hydroxy-2,2,6,6-tetramethyl-3-piperidinomethyl-4-piperid | $C_{15}H_{28}N_2O_2$ | 268 | 0.63 |
| 34 | 26.334 | 3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester | $C_{12}H_{23}NO_2$ | 213 | 1.23 |
| 35 | 26.823 | 2-Ethylbutyric acid, eicosyl ester | $C_{26}H_{52}O_2$ | 396 | 0.44 |
| 36 | 27.499 | 1,2-Benzenedicarboxylic acid | $C_{24}H_{38}O_4$ | 398 | 0.23 |
| 37 | 28.813 | 9,12-Octadecadienoic acid (z,z)-, 2-hydroxy-1- | $C_{21}H_{38}O_4$ | 354 | 1.62 |
| 38 | 30.761 | Oxalic acid, 3,5-difluorophenyl nonyl ester | $C_{17}H_{22}F_2O_4$ | 328 | 1.95 |
| 39 | 30.844 | 1,1,1,5,5,5-Hexafluoro-4-{[3-(trifluoromethyl) | $C_{12}H_6F_9NO$ | 351 | 0.62 |
| 40 | 33.760 | (2,14-Dioxocyclotetradecyl) acetic acid methyl ester | $C_{17}H_{28}O_4$ | 296 | 6.10 |
| 41 | 33.936 | 2-[2-Carboxyethyl]-3-methyl-tetrahydrofurano[4,5-a]androst | $C_{25}H_{40}O_4$ | 404 | 2.10 |
| 42 | 34.093 | 9,12,15-Octadecatrien-1-ol | $C_{18}H_{32}O$ | 264 | 0.47 |
| 43 | 36.665 | dl-α-Tocopherol | $C_{29}H_{50}O_2$ | 340 | 0.45 |
| 44 | 39.360 | 2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin | $C_{13}H_{22}O_3$ | 226 | 0.33 |
| 45 | 42.278 | γ-Sitosterol | $C_{29}H_{50}O$ | 414 | 1.70 |
| 46 | 44.959 | Longifolenbromid-i | $C_{15}H_{23}Br$ | 282 | 0.23 |

a fatty acid which forms salt or ester with drugs and thus increases lipophilic character and affinity for fatty tissue (Paulpriya *et al.*, 2014). It also contains inositol which is responsible for anti-alopecic, anti-cirrhotic, anti-neuropathic, cholesterolytic, lipotropic and sweetener properties (Kumar *et al.*, 2014). Esters are also present in seed extracts and have hypocholesterolemic, nematicide antiarthritic, hepatoprotective, antiandrogenic, hypocholesterolemic nematicide, antihistaminic, anti-

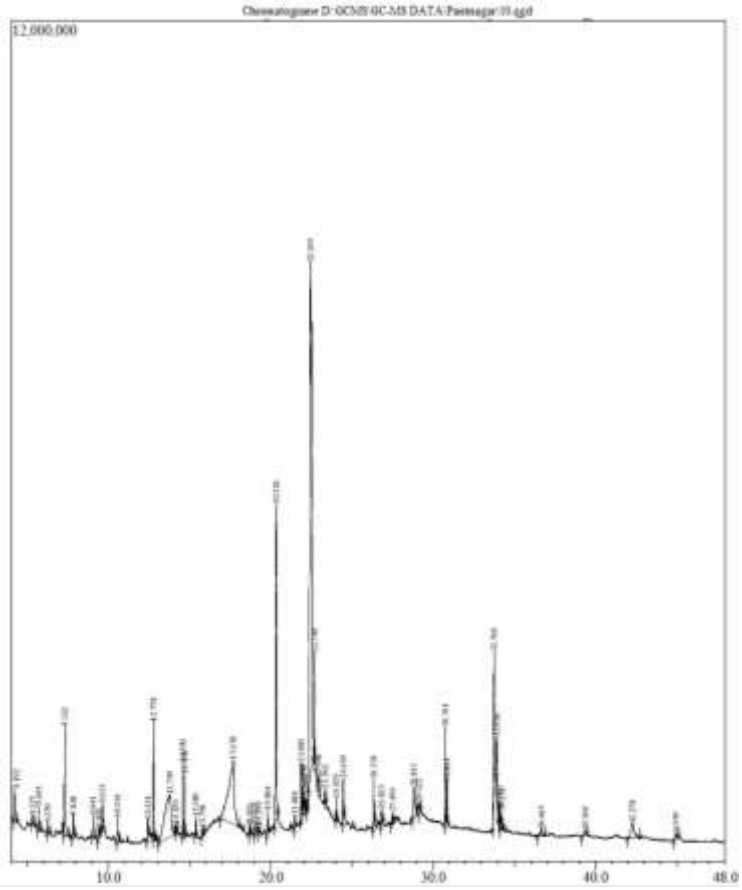

**Fig. 1: GC-MS chromatogram of methanolic seed fraction of *Trigonella foenum-graecum* var. GM-2**

coronary, insectifuge, anti-eczemic, antiacne properties (Priya *et al.,* 2011). Alpha tochopherol or vitamin E, natural antioxidant inhibits the activity of protein kinase an enzyme involved in cell proliferation and differentiation in smooth muscle cell. It is reported to be involved in immune system, cell signaling, regulation of gene expression and other metabolites, and possess anti-inflammatory activity (Maya *et al.,* 2012).

The present study identified various functional groups and polar compounds in GM-2 seed samples based on the structural vibration analysis. The fingerprint technique of FTIR spectroscopy has been applied due its rapidity and as a non-destructive analytical tool in the spectral region of 3500 $cm^{-1}$ to 1000 $cm^{-1}$. Further, it reveals the presence of alcohols, aldehydes, phenols, alkyl halides, alkanes, alkynes, carboxylic acids, aromatics, amines nitro-compounds. GM-2 seeds have a complex chemical system. As expected, the conventional IR spectrum revealed the chemical composition that allowed the characterization of herb as a fingerprint (Fig. 2).

On the basis of spectral data obtained from FTIR distinct fingerprint features particularly in the range of 2500 - 2000 $cm^{-1}$ and 2000 - 1500 $cm^{-1}$, a broad band was observed at 1500 - 1000$cm^{-1}$, two mid-strong bands at 1040 $cm^{-1}$ and 995 $cm^{-1}$ and an intense band at 2362 $cm^{-1}$. Through comparative analysis of IR spectra of var. GM-2 seeds many different absorption bands were observed in differencing in position and intensity.

In detail, the strong peak at 2362 $cm^{-1}$ is assigned to asymmetric and symmetric C–H stretching vibrations. The peak at 1631 $cm^{-1}$ is assigned to the stretching vibration of C=O groups of conjugated and aromatic rings. This indicates that different type of carbonyl compounds existed in var. GM-2 seeds. The bands centered at 1631 and 1507 $cm^{-1}$ belong to the stretching vibrations of aromatic ring. The peak at 1337 $cm^{-1}$ is due to the stretching vibration of aliphatic C–H stretching groups in methyl and phenol O–H. The peaks at 1220 and 1040 $cm^{-1}$ were mainly attributed to the stretching vibration of C–O for starch. These bands are characteristic of cellulosed materials (fibres) which show the characteristic absorptions spectrum for polysaccharides and

**Table 2: Dominated peaks in *Trigonella foenum-graecum* L. var. GM-2**

| S. No. | Band ($cm^{-1}$) | Vibration mode |
|---|---|---|
| 1 | 2362 | C-H stretching |
| 2 | 1631 | C=O stretching |
| 3 | 1507 | Aromatic skeletal |
| 4 | 1337 | C-H aliphatic |
| 5 | 1220 | C-C, C-O stretching |
| 6 | 1040 | O-H stretching |
| 7 | 995 | C-N stretching |
| 8 | 760 | C-Br stretching |

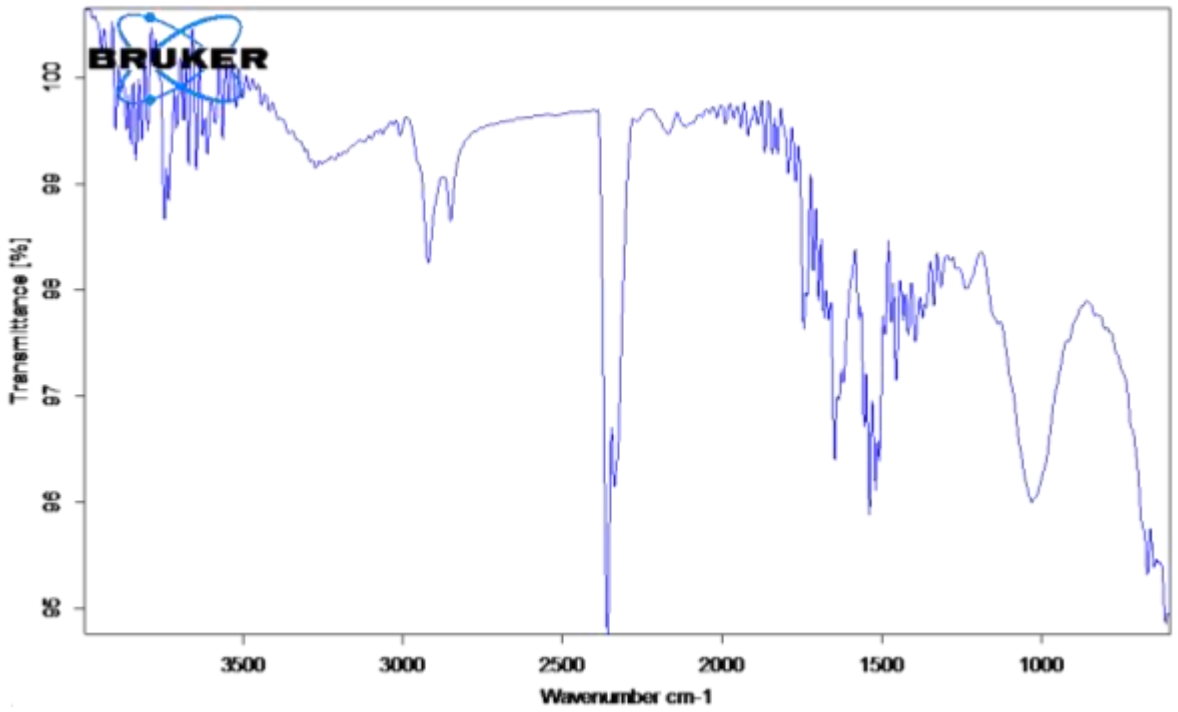


**Fig. 2: FT-IR spectra of var. GM-2 seeds in the range of 3500-100 cm$^{-1}$:** The major compounds present in the seed extract of Gujarat methi-2 are identified by GC-MS mome inositol (RT 17.68) which is used for diabetic nerve pain, high cholesterol, panic disorder, insomnia, cancer and depression. Pentadecanoic acid (RT: 20.32) 9, 12-octadecadienoic acid (Z, Z)-(RT: 22.4) having the anti-inflammatory and anti- arthritic, has reported by earlier workers. It is used in pharmaceutical dosage forms; stearic acid is used as an emulsifying agent, solubilizing agent, tablet and capsule lubricant.3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester (RT: 26.33) (2,14-dioxocyclotetradecyl) acetic acid methyl ester (RT:33.76) and octadecanoic acid (RT-22.7) a natural component of cocoa butter and shea butter. Several esters and other compound which are reported to be the part of medicinal virtues are also present in the extract, exhibiting the antioxidant, and anti-inflammatory, anticancer, anti-diabetic activities.

coumarin. The peak at 995 cm$^{-1}$ is assigned to C-N stretching, while peaks at 760 cm$^{-1}$ was mainly attributed to the variable angle vibration and bending vibration of halogens containing bromine groups. Above all the substantial information on the chemical composition of var. GM-2 seeds can be attained from its IR. So, fenugreek var. GM-2 can be a potent source of several bioactive compounds which can be used in the pharmaceutical and nutraceutical industries. FTIR is also reported by earlier researchers for identification of different functional groups in *T. foenum-graecum* seed and *Tamarindus indica* by Verma *et al.* (2014) and Florence and Jeeva (2015) in *Gmelina asiatica* leaves (Gopukumar *et al.*, 2016) in *Ficus benghalensis.*

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