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Original Article

GC-MS and UV-visible Spectroscopic Analysis of Bioactive Phytochemical in *Trigonella foenum-graecum* Visible Spectroscopic

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ABSTRACT

Background: *Trigonella foenum-graecum* (fenugreek) is a widely utilized medicinal herb known for its therapeutic potential in managing metabolic disorders. This study aimed to extract and characterise the bioactive constituents of fenugreek seeds using spectroscopic and chromatographic techniques. **Materials and Methods:** Dried seed powder was subjected to Soxhlet extraction using ethanol and methanol solvents. Preliminary phytochemical screening of the crude extracts revealed the presence of significant secondary metabolites, including alkaloids, flavonoids, saponins, tannins, and glycosides, while testing negative for anthraquinones. UV-visible spectrophotometric analysis of the diluted extracts demonstrated characteristic absorption peaks (λ_{max}) in the range of 200-400 nm, specifically at 265 nm and 320 nm, indicating the presence of conjugated unsaturated systems and phenolic compounds. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was employed to identify specific volatile and semi-volatile constituents. **Results:** The chromatogram revealed over 20 distinct peaks, with the mass spectra matching the NIST library database. Major identified compounds included n-Hexadecanoic acid (Palmitic acid), 9,12-Octadecadienoic acid (Linoleic acid), Squalene, and derivatives of 4- hydroxyisoleucine and Trigonelline. **Conclusion:** The presence of these bioactive esters and fatty acids correlates with the plant's known antioxidant, anti-inflammatory, and hypoglycemic activities. These findings validate the traditional medicinal use of fenugreek and suggest its solvent extracts are a rich source of phytocompounds suitable for the development of novel nutraceutical formulations.

Keywords: Bioactive compounds, Fenugreek, GC-MS analysis, Phytochemical screening, Soxhlet extraction, Trigonella foenum-graecum.

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INTRODUCTION

Trigonella foenum-graecum L., commonly known as fenugreek, is an annual leguminous herb belonging to the Fabaceae family. Native to the Mediterranean region, Western Asia, and the Indian subcontinent, it is one of the oldest medicinal plants in recorded history (Al-Jenoobi *et al.*, 2014). While globally valued as a culinary spice and flavoring agent, its significance in traditional medical systems-such as Ayurveda, Unani, and Traditional Chinese Medicine (TCM)-is profound (Parihar *et al.*, 2014; Tiwari *et al.*, 2016).

The seeds are historically prescribed for managing metabolic disorders, particularly diabetes mellitus and hyperlipidemia, as well as for their galactagogue (milk-producing) properties in lactating mothers. The therapeutic efficacy of fenugreek is attributed to its complex matrix of secondary metabolites, including steroidal saponins (diosgenin), alkaloids (trigonelline), flavonoids, and mucilaginous fibers (galactomannan) (Malik *et al.*, 2020). However, these bioactive compounds are locked within the dense cellular structure of the dried seed. To utilize these compounds for pharmaceutical or nutraceutical applications, they must first be liberated through solvent extraction. The choice of solvent (e.g., methanol, ethanol, or hexane) and extraction method (such as Soxhlet extraction) plays a critical role in determining the yield and purity of the resulting phytochemical profile (Pandey and Awasthi, 2015). This preliminary qualitative assessment utilizes standard chemical reagents to detect the presence of major classes of compounds, such as alkaloids, flavonoids, tannins, and saponins. It serves as a broad "fingerprint" of the extract's potential biological activity. UV-visible Spectroscopy: (Verma and Singh, 2008). This technique provides a quantitative profile of the extract by measuring the absorption of light across the ultraviolet and visible ranges (200-800 nm). Distinct absorption peaks

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(λ_{max}) help identify the presence of conjugated organic systems, particularly phenolic and flavonoid compounds, which are often responsible for antioxidant activity. Gas Chromatography-Mass Spectrometry (GC-MS): While UV and phytochemical screening provide broad class identification, GC-MS offers high-resolution molecular identification. It is particularly effective for analyzing the volatile and semi-volatile fractions of the seed extract, such as fatty acids (e.g., linoleic and palmitic acid), terpenes, and esters (Chatterjee *et al.*, 2010). By comparing the mass spectra of eluted compounds against standard libraries (such as NIST), researchers can pinpoint the exact molecular identity of the bioactive constituents.

MATERIALS AND METHODS

Extraction of Plant Materials

Extraction is the crucial first step in the analysis of medicinal plants because it is necessary to extract the desired chemical components from the plant materials for further separation and characterisation.

Extraction of Fenugreek Seed

Fresh plant material (leaves, flowers, or seeds), distilled water, ethanol or methanol (for extraction), test tubes, pipettes, measuring cylinders, water bath, beakers, and filter paper.

Extraction by cold maceration or preparation of aqueous extract

About 100 g of dry powdered plant material was subjected to cold maceration with distilled water in a 1-L conical flask. The flask was plugged with absorbent cotton and kept aside for about 24 hr at room temperature with frequent agitation or occasional shaking. After the completion of maceration, the marc was placed in a muslin cloth and filtered. The filtrate was again filtered using Whatman No. 1 filter paper (Harborne, 1998). This procedure of soaking and filtration was repeated for two or more times until it became colourless, and all the extracts were pooled together and then concentrated in a vacuum at 40°C using a rotary evaporator to a thick, semisolid pasty mass of dark black colour. The residues thus obtained were stored in a freezer at -70°C until ready for use (Raaman, 2006).

Qualitative Phytochemical Screening of Aqueous Extract

The sample was subjected to various standard phytochemical test procedures to detect the presence or absence of various active phytoconstituents present in the crude extracts.

The Following Chemical Reagents Were Required for Qualitative Phytochemical Analysis

Mayer's reagent, Benedict's reagent, glacial acetic acid, ferric chloride (5%), concentrated sulphuric acid, Ninhydrin solution (10 mg ninhydrin in 200 mL acetone), sodium hydroxide (2% and 10%), dilute hydrochloric acid, iodine solution, chloroform, acetic anhydride, gallic acid, and concentrated hydrochloric acid. All reagents were of analytical grade (Kokate, 2005; Ronia *et al.*, 2017).

Preparation of Plant Extract

The plant material was shade-dried, powdered, and extracted by macerating 5 g of sample in 50 mL of ethanol (or distilled water) for 24 hr with occasional shaking. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was used for qualitative analysis of phytochemicals (Ansari, 2008).

Test for Alkaloids (Picric Acid Test)

Procedure: A few millilitres of the filtrate were treated with 3-4 drops of picric acid.

Observation: The Appearance of yellow colour indicated the presence of alkaloids.

Test For Carbohydrates (Benedict's Test)

Procedure: 0.5 mL of the filtrate was mixed with 0.5 mL of Benedict's reagent and boiled for 2 min (Arumugam and Murugesh, 2009).

Observation: The Appearance of green, yellow, or red colour confirmed the presence of reducing sugars.

Test for Cardiac Glycosides (Keller-Killiani Test)

Procedure: To 1 mL of filtrate, 1.5 mL of glacial acetic acid and one drop of 5% ferric chloride were added. Concentrated sulphuric acid was then carefully poured along the sides of the test tube.

Observation: Formation of a blue colour in the acetic acid layer indicated the presence of cardiac glycosides.

Test for Proteins and Amino Acids (Ninhydrin Test)

Procedure: 2 mL of filtrate were treated with two drops of ninhydrin solution (10 mg ninhydrin in 200 mL of acetone and boil for a few minutes.

Observation: Development of a purple colour confirmed the presence of amino acids or proteins.

Test For Flavonoids (Alkaline Reagent Test)

Procedure: 1 mL of extract was mixed with 2 mL of 2% sodium hydroxide solution. A few drops of dilute hydrochloric acid were then added.

Observation: The appearance of an intense yellow colour that became colourless upon addition of acid indicated the presence of flavonoids.

Test For Phenolic Compounds (Iodine Test)

Procedure: 1 mL of extract was treated with a few drops of dilute iodine solution.

Observation: Formation of a transient red colour indicated the presence of phenolic compounds.

Test for Tannins (10% NaOH Test)

Procedure: 0.4 mL of plant extract was mixed with 4 mL of 10% sodium hydroxide solution & shaken.

Observation: Formation of an emulsion indicated the presence of hydrolysable tannins.

Test for Phlobatannins

Procedure: 2 mL of aqueous extract were mixed with 2 mL of 1% hydrochloric acid and boiled.

Observation: Formation of a red precipitate indicated the presence of phlobatannins.

Test for Saponins (Foam Test)

Procedure: About 0.5 g of plant extract was mixed with 2 mL of distilled water and shaken vigorously.

Observation: Persistent foam lasting for 10 min confirmed the presence of saponins.

Test for Phytosterols (Salkowski's Test)

Procedure: The filtrate was treated with a few drops of concentrated sulphuric acid, shaken well, and allowed to stand. to stand. Stand (Ansari and Deepika, 2007; Deepika, 2007).

Observation: Formation of a red colour in the lower layer indicated the presence of phytosterols.

Test for Terpenoids

Procedure: 2 mL of chloroform and 5 mL of plant extract were mixed and evaporated in a water bath. The residue was treated with 3 mL of concentrated sulphuric acid and boiled gently.

Observation: Development of a grey colour indicated the presence of terpenoids.

Test for Quinones

Procedure: The plant extract was treated with concentrated hydrochloric acid.

Observation: Appearance of a green colour indicated the presence of quinones.

Test for Coumarins

Procedure: The plant extract was mixed with 10% sodium hydroxide solution and chloroform (Ali, 2004).

Observation: Development of a yellow colour indicated the presence of coumarins.

Quantitative Phytochemical Analysis: Determination of Total Flavonoid

Total flavonoid was analyzed using the aluminium chloride colorimetric method with slight modification according to Chang *et al*. Quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in 96% ethanol and diluted to 2, 4, 6, 8 and 10 µg/mL. 1 mL of each concentration of standard solutions, as well as 1 mL of each sample solution, were mixed with 3 mL of 96%, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water. The mixture was incubated at room temperature for 10 min with intermittent shaking. The absorbance was measured at 376 nm against a blank without aluminium chloride using a Cecil CE7410 UV-vis spectrophotometer. Total flavonoid was calculated as mean ± SD (n = 3) and expressed as weight of Quercetin Equivalent (QE) at 100 mg extract.

Determination of Tannin Content

The tannins were determined by the Folin-Ciocalteu method. About 0.1 mL of the sample extract was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu phenol reagent, 1 mL of 35% Na_2CO_3 solution and dilute to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Gallic acid (20, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as described earlier. Absorbance for test and standard solutions was measured against the blank at 725 nm with a UV/visible spectrophotometer. The tannin content was expressed in terms of mg of GAE/g of extract (Vijay and Rajendra, 2014).

Determination of Total Phenol

Total phenolic content was determined with the Folin-Ciocalteu reagent according to Pourmorad *et al*. with slight modification. A calibration curve was obtained by using gallic acid as a standard. Different concentrations of gallic acid (5-125 mg/L) were prepared. A 10 mg sample was diluted in 10 mL of methanol in a test tube. Both 0.5 mL of standards and samples were taken and mixed with 2.5 mL of Folin-Ciocalteu 50% and 2.5 mL of distilled water. After incubation for 5 min, 2 mL aqueous sodium carbonate solution (7.5%, w/v) was added. The final mixture was shaken and then incubated for 15 min in the dark at room temperature. The absorbance of all standards and samples was measured at 765 nm using a Cecil CE7410 UV-vis spectrophotometer, and the results are expressed as milligrams of Gallic Acid Equivalents (GAE) per 100 mg of dry leaf weight.

UV Spectrophotometry

UV-visible spectroscopy for estimation of rutin and quercetin from plant extract were carried out. Standard curves of quercetin and rutin were prepared by dissolving 5 mg of quercetin and rutin in 5 ml of ethanol. Then, the solution was diluted to 20, 40, 60, 80 and 100 µg/mL concentrations. A standard graph was prepared using Schimadzu UV-visible spectrophotometer and absorbance was recorded at 362 and 425 nm for rutin and quercetin respectively. Then, the fenugreek seed extract was prepared in ethanol, and the absorbance was taken at 362 nm and 425 nm. The standard graph of extract was done using 362 nm and 425 nm separately; the graph is mentioned in Figure 4 (Kurzawa, 2010; Kagawad *et al.,* 2021).

Gas Chromatography-Mass Spectrometry (GC/Ms) Analysis

A Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) with an Elite-1 fused silica capillary column (30 m × 0.25 mm ID × 1 µm d_f, made of 100% dimethyl polysiloxane) were used to analyse this extract. A 70-eV electron ionisation energy system was utilised for GC/MS detection. As the carrier gas, helium gas (99.999%) was used at a steady flow rate of 1 mL/min with an injection volume of 2 µL (split ratio of 10:1). Temperatures of the injector are 250°C and the ion source is 280°C. The oven was set to start at 110°C (isothermal for 2 min), increase by 10°C per minute to 200°C, then increase by 5°C per minute to 280°C, and conclude with a 9-min isothermal at 280°C. Mass spectra were obtained at 70 eV, with fragments ranging from 45 to 450 Da and a scan interval of 0.5 sec. The GC ran for 36 min in total (Kurzawa, 2010; Kagawad *et al.*, 2021). The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Turbo Mass Ver5.2.0 was the program used to handle mass spectra and chromatograms.

RESULTS

Extraction by Cold Maceration or Preparation of Aqueous Extract

Soxhlet extraction of powdered fenugreek seeds using ethanol yielded a deep yellow-coloured extract, indicating efficient solubilization of bioactive constituents (Figure 1). The extract appeared clear with slight turbidity, suggesting the presence of dissolved phytochemicals. Upon solvent evaporation, a yellowish-brown semi-solid crude extract was obtained, confirming successful recovery of ethanol-soluble compounds. The color and consistency of the extract suggest the presence of phenolic compounds, flavonoids, alkaloids, and glycosidic constituents, which are typically extracted using polar solvents such as ethanol. The extraction process resulted in a stable crude mass suitable for further phytochemical screening and spectroscopic analysis.

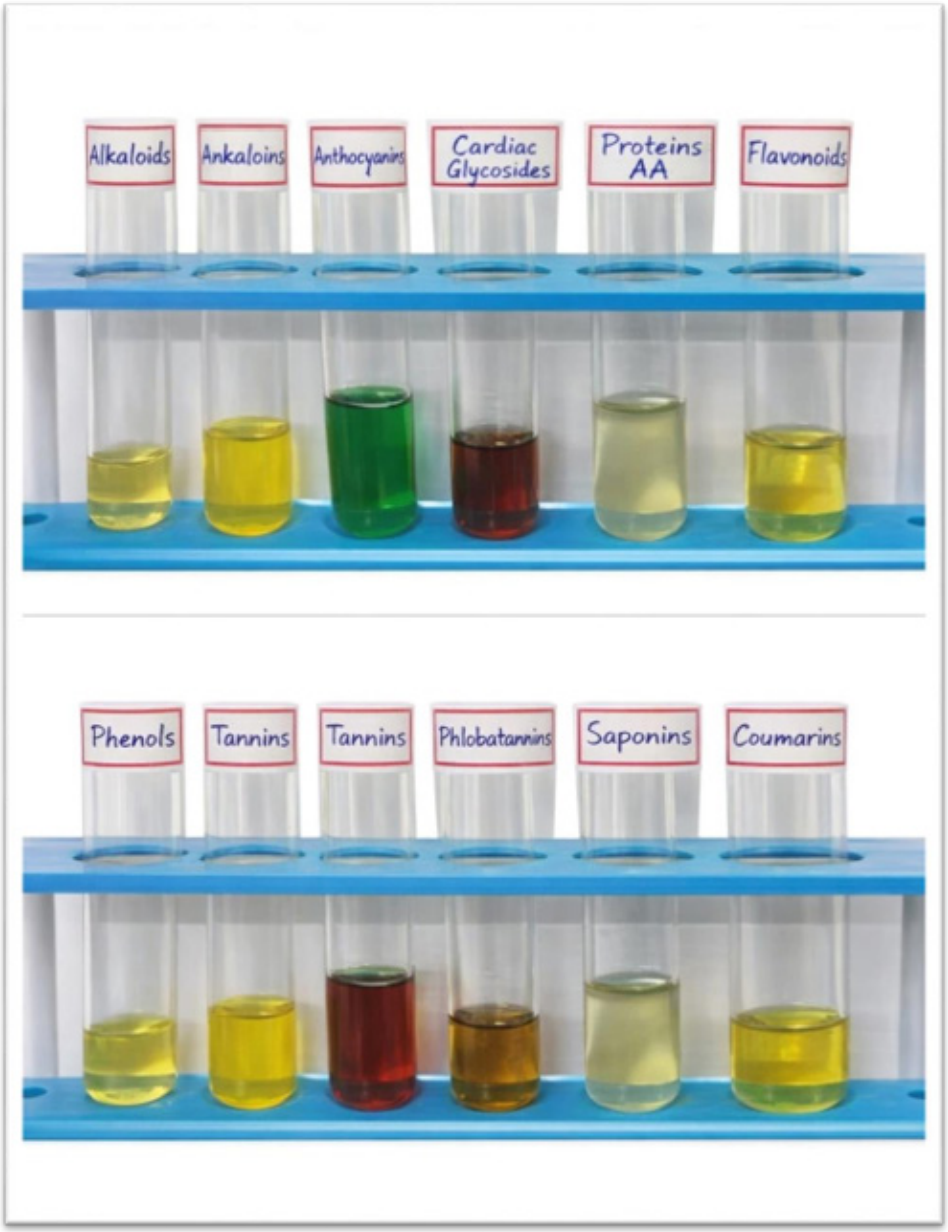
Qualitative Phytochemical Screening of Seeds (*Trigonella foenum-graecum*)

The qualitative phytochemical analysis of the ethanolic extract of fenugreek seeds (*Trigonella foenum-graecum)* revealed the presence of several important secondary metabolites. Standard phytochemical tests confirmed the presence of alkaloids, flavonoids, tannins, phenolic compounds, cardiac glycosides, and carbohydrates, while saponins, phlobatannins, proteins and amino acids, and coumarins were not detected in the extract (Table 1, Figures 2 and 3).

The detection of alkaloids indicates the possible pharmacological significance of fenugreek seeds, as alkaloids are widely reported



Figure 1: a and b Extraction of fenugreek seed in ethanol and dry powder.



Figures 2 and 3: Qualitative analysis of fenugreek seed (*Trigonella foenum-graecum*) ethanolic extract.

to exhibit antidiabetic, antimicrobial, and antioxidant activities. The presence of flavonoids and phenolic compounds suggests strong antioxidant potential, as these compounds are known to act as free radical scavengers and reducing agents. Tannins, which were also identified, contribute to antioxidant, antimicrobial, and anti-inflammatory properties. The occurrence of cardiac glycosides indicates potential cardioprotective relevance, while the presence of carbohydrates reflects the nutritional importance of fenugreek seeds.

The absence of saponins, phlobatannins, proteins and amino acids, and coumarins in the ethanolic extract suggests that these constituents are either present in negligible amounts or are not efficiently extracted under the selected extraction conditions.

Overall, the qualitative phytochemical profile demonstrates that *Trigonella foenum-graecum* seeds are a rich source of bioactive secondary metabolites, supporting their traditional medicinal use and providing a scientific basis for further quantitative and bioactivity-based investigations.

Qualitative Phytochemical Results of *Trigonella foenum-graecum* Ethanolic Extract

Quantification of total flavonoid content

The total flavonoid content of the ethanolic extract of *Trigonella foenum-graecum* was estimated using the aluminium chloride colorimetric method, with absorbance measured at 510 nm (Figure 4). A linear increase in absorbance was observed with

increasing concentrations of the quercetin standard, indicating good linearity of the calibration curve.

Using the standard curve equation y = 0.0005x + 0.0213, the flavonoid content of the fenugreek seed extract was calculated. The sample absorbance (0.946) corresponded to a concentration of 1934.6 µg/mL, yielding a final flavonoid content of 1.9346 mg Quercetin Equivalents (QE) per g of extract.

This appreciable flavonoid content supports the antioxidant potential of fenugreek seeds, as flavonoids are known to act as hydrogen donors and metal chelators, thereby reducing oxidative stress.

Quantification of Total Tannin Content

Quantification of Tannin Content in Fenugreek Seeds (Trigonella foenum-graecum) Ethanolic Extract

The total tannin content was determined using the Folin-Denis method, with absorbance measured at 725 nm (Figure 5). The gallic acid standard showed a consistent increase in absorbance with increasing concentration, validating the assay. Based on the standard curve equation y = 0.0007x + 0.1095, the tannin concentration corresponding to the sample absorbance (0.833) was calculated as 1346.4 µg/mL, which corresponds to a final tannin content of 1.346 mg Gallic Acid Equivalents (GAE) per g of extract. Tannins are known for their ability to scavenge free radicals and inhibit lipid peroxidation, indicating that fenugreek seeds contribute to antioxidant defense mechanisms.

QUANTIFICATION OF TOTAL PHENOLIC CONTENT

Quantification of Phenolic Compounds For Fenugreek Seeds (*Trigonella foenum-graecum*) Ethanolic Extract

The total phenolic content of the ethanolic extract was estimated using the Folin-Ciocalteu method, with absorbance measured at 550 nm (Figure 6). The gallic acid standard exhibited a strong linear relationship between concentration and absorbance Using the regression equation y = 0.0016x + 0.15, the sample absorbance (1.420) corresponded to 981.2 µg/mL, resulting in a total phenolic content of 9.812 mg GAE per g of extract. The high phenolic content observed in fenugreek seeds is significant, as phenolic compounds are primary contributors to antioxidant

Table 1: Qualitative analysis of fenugreek seed (*Trigonella foenum-graecum*) ethanolic extract.

Phytochemical Class	Test Result
Alkaloids	Present (+)
Flavonoids	Present (+)
Tannins	Present (+)
Saponins	Absent (-)
Phenols	Present (+)
Phlobatannins	Absent (-)
Cardiac glycosides	Present (+)
Proteins & Amino acids	Absent (-)
Carbohydrates	Present (+)
Coumarins	Absent (-)

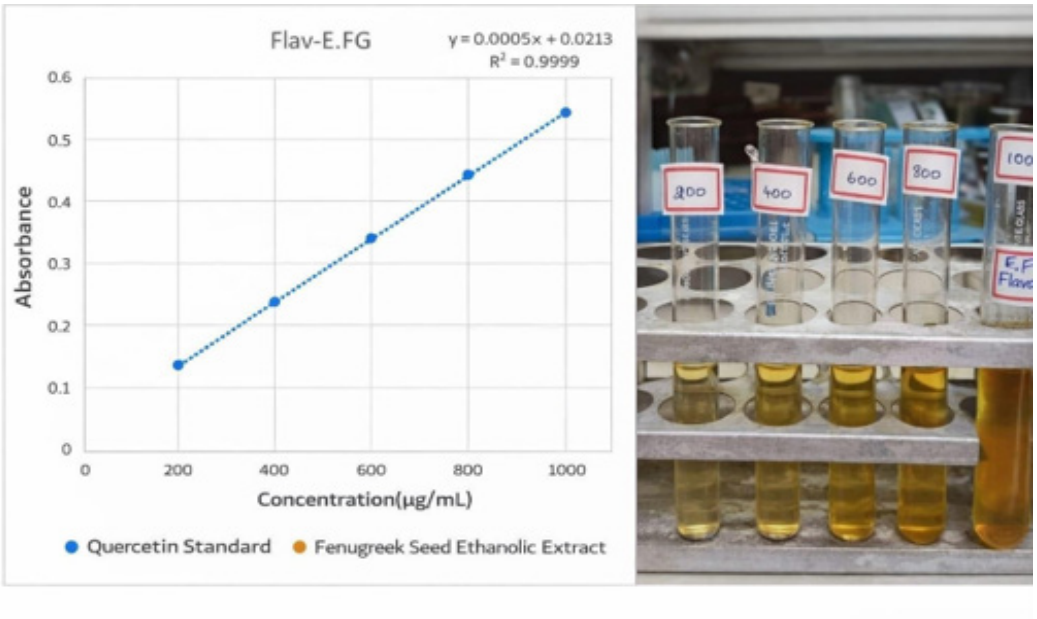


Figure 4: Quantification of Flavonoids content in Fenugreek Seeds (*Trigonella foenum-graecum*) Ethanolic Estimation of total flavonoid content in Trigonella foenum-graecum ethanolic extract at various concentrations measured at 510 nm. Quercetin Standard Grap.

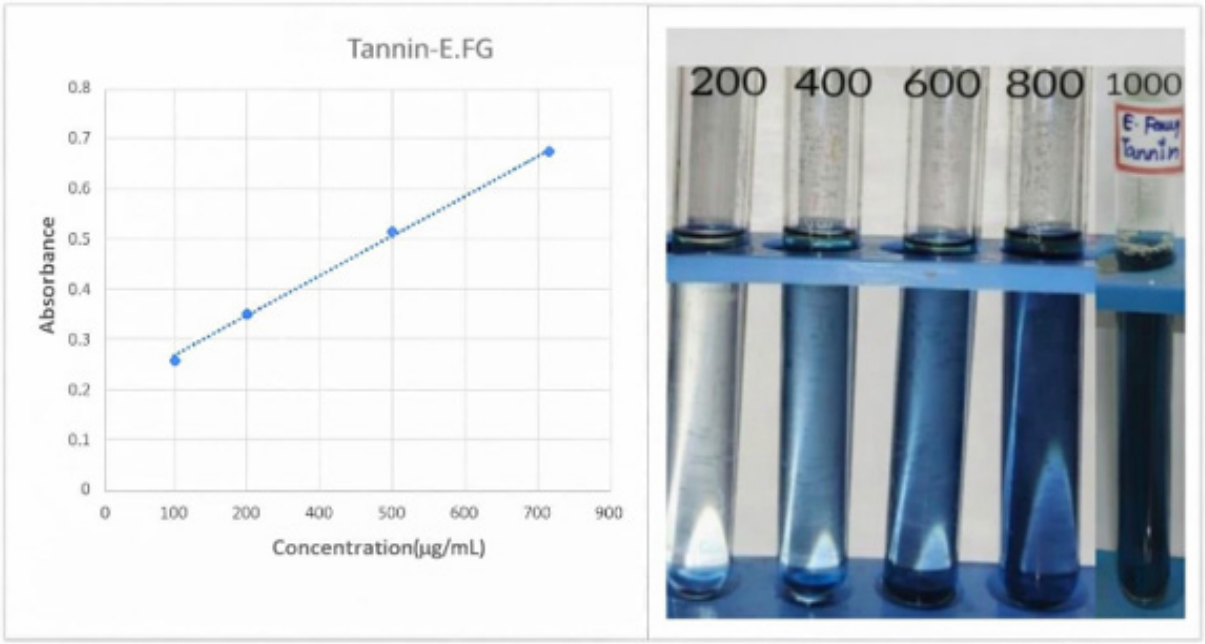


Figure 5: Quantification of tannin content in fenugreek seeds (*Trigonella foenum-graecum)* ethanolic extract. Estimation of total tannin content in *Trigonella foenum-graecum* ethanolic extract at Various concentrations were measured at 725 nm. Gallic acid Standard Graph.

Table 2: Comparative Phytochemical Content of *Trigonella foenum-graecum* Seed Extract.

Parameter	Total Flavonoids (QE)	Total Tannins (GAE)	Total Phenolics (GAE)
Assay Type	Flavonoid Content	Tannin Content	Phenolic Content
Standard Used	Quercetin	Gallic/Tannic Acid	Gallic Acid
Wavelength	510 nm	725 nm	550 nm
Sample Absorbance	0.946	0.833	1.420
Calibration Equation	y = 0.0005x + 0.0213	y = 0.0007x + 0.1095	y = 0.0016x + 0.15
Calculated Concentration (µg/mL)	1934.6	1346.4	981.2
Final Result (mg/g extract)	1.9346 mg QE/g	1.346 mg GAE/g	9.812 mg GAE/g
Relative Abundance	Moderate	Moderate	Highest

activity through free radical scavenging, reducing power, and metal ion chelation (Table 2).

UV-visible Absorption Spectrum of Fenugreek Seed Extract

The UV-visible absorption spectrum of fenugreek seed extract was recorded in the wavelength range of 190-1100 nm using a UV-1900 series spectrophotometer in absorbance mode with a slit width of 1.0 nm and a sampling interval of 1 nm. The recorded spectrum is presented in Figure 7.

The spectrum exhibited intense absorption bands in the ultraviolet region (200-350 nm), while negligible absorbance was observed in the visible and near-infrared regions, indicating the absence of strongly colored pigments in the extract. The absorption profile demonstrates a broad, high- intensity band extending from approximately 260 to 340 nm, suggesting the presence of multiple overlapping chromophores within the crude extract.

Absorption Maxima (λ_{max}) Observed in Fenugreek Seed Extract Peak-picking analysis revealed several prominent absorption maxima (Table 3). The most intense absorption peak was observed at 288 nm with a maximum absorbance value of 3.898, indicating a high concentration of UV-absorbing compounds. Additional strong absorption peaks were detected at 307 nm (A = 3.849) and 329 nm (A = 3.823), forming a broad absorption envelope in the near-UV region. A distinct peak was also observed at 261 nm (A = 2.210). Moderate absorption was noted at 223 nm (A = 0.887). Minor peaks recorded beyond 800 nm exhibited near-zero or negative absorbance values and are attributed to instrumental noise or baseline variations rather than chemical constituents.

Interpretation of UV-visible absorption bands

The absorption peak observed at 261 nm falls within the characteristic region of aromatic amino acids such as tryptophan and tyrosine, which are abundant in seed storage proteins. Fenugreek seeds are nutritionally rich in proteins; therefore, this absorption band can be attributed to proteinaceous components and free amino acids present in the extract. The dominant absorption maximum at 288 nm, along with the broad absorption extending towards 300-330 nm, is indicative of phenolic compounds and flavonoids. Phenolics typically exhibit absorption due to $\pi \rightarrow \pi^*$ electronic transitions within aromatic rings, while flavonoids display two characteristic absorption bands:

Band II (240-280 nm) corresponding to the benzoyl system, and Band I (300-380 nm) corresponding to the cinnamoyl system

The presence of absorption peaks at 307 nm and 329 nm confirms the contribution of flavonoids and conjugated polyphenolic structures in the fenugreek seed extract. Absorption observed in the far-UV region (<230 nm) is generally associated with transitions and peptide bond absorption. The negative absorbance values detected below 200 nm are likely due to baseline correction and solvent interference and should not be considered chemically significant. Assignment of UV Absorption Peaks to Phytochemical Constituents Based on the observed spectral features and known phytochemical composition of *Trigonella foenum-graecum*, the UV absorption peaks can be tentatively assigned as follows: 260-290 nm: Aromatic amino acids, seed proteins, alkaloids such as trigonelline, and simple phenolic compounds 300-330 nm: Flavonoids and conjugated polyphenolic compounds <230 nm: Peptide bonds and high-energy electronic transitions The overlapping absorption bands reflect the complex chemical nature of the crude plant extract, where multiple constituents contribute simultaneously to the UV absorption profile.

GC-MS Analysis of Fenugreek Seed Extract (*Trigonella foenum-graecum)* GC-MS Profiling of the Sample

The GC-MS analysis of the sample (ID: 2631-(25ES-0290)) led to the identification of 14 distinct peaks. The chromatogram revealed two major peaks dominating the profile at Retention Times (RT) of 15.854 min and 17.525 min (Figure 8). The components were identified by comparing their mass spectra with the NIST library database. The peak data, including retention time, relative area percentage, and tentative library identification, is summarized in Table 4.

Use this section to explain the results. I have added specific notes regarding fenugreek to help align this with your thesis topic.

Table 3: Major UV-visible absorption peaks observed in fenugreek seed extract.

Wavelength (nm)	Absorbance (A)	Interpretation
288	3.898	Aromatic amino acids, phenolics, flavonoids
307	3.849	Flavonoids (Band I), conjugated polyphenols
329	3.823	Extended conjugated flavonoid systems
261	2.210	Proteins, alkaloids, simple phenolics
223	0.887	Peptide bonds, high-energy electronic transitions

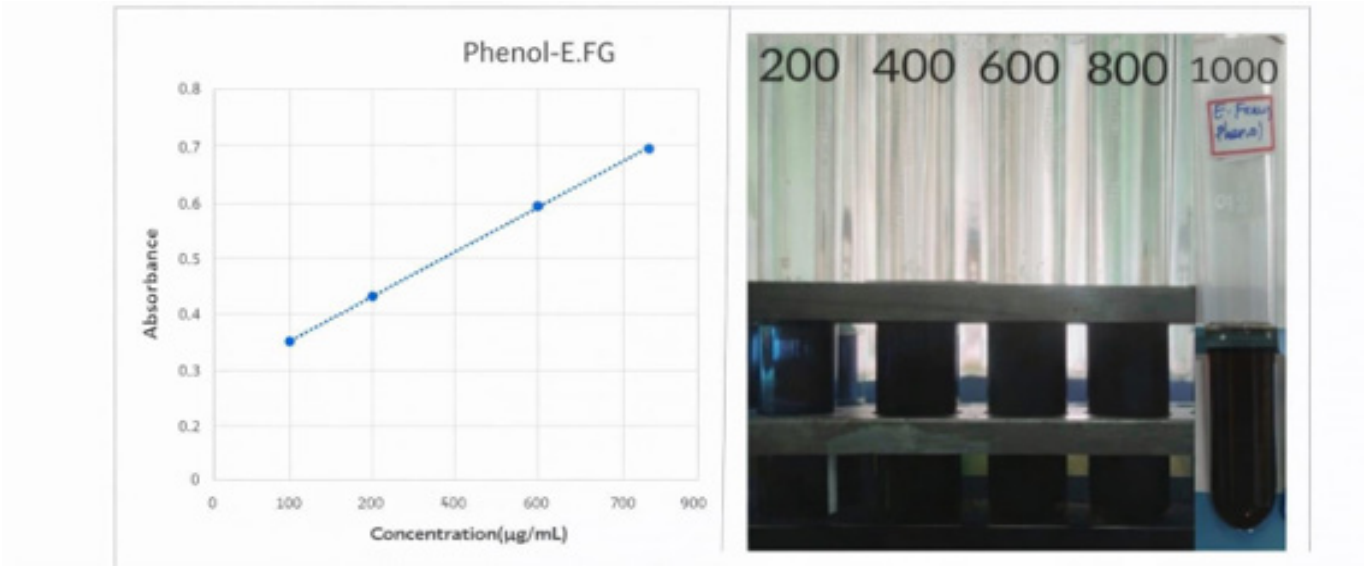


Figure 6: Quantification of phenolic compounds for fenugreek seeds (*Trigonella foenum-graecum*) ethanolic extract. Estimation of total phenolic content in *Trigonella foenum-graecum* ethanolic extract at various concentrations measured at 550 nm.

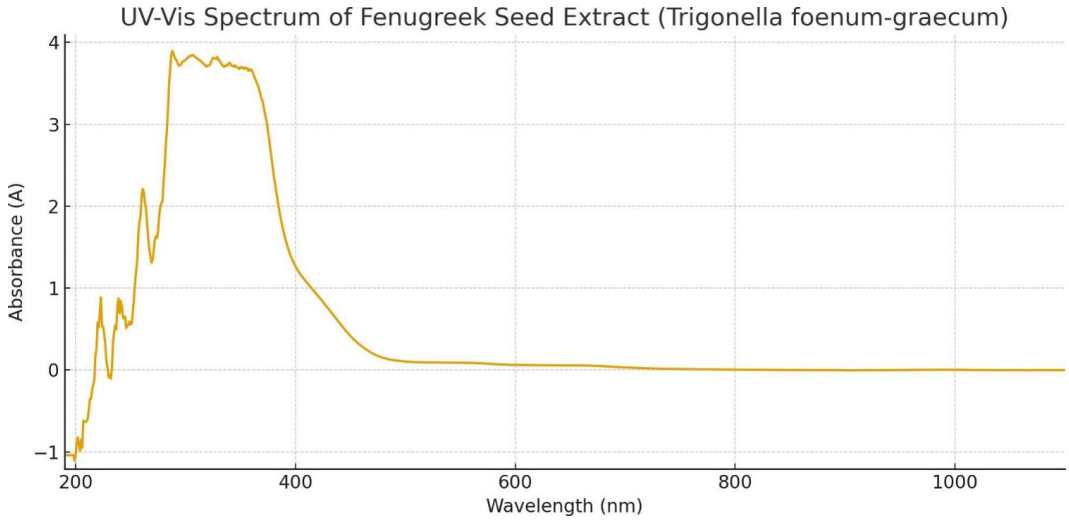


Figure 7: UV-visible absorption spectrum of fenugreek seed extract.

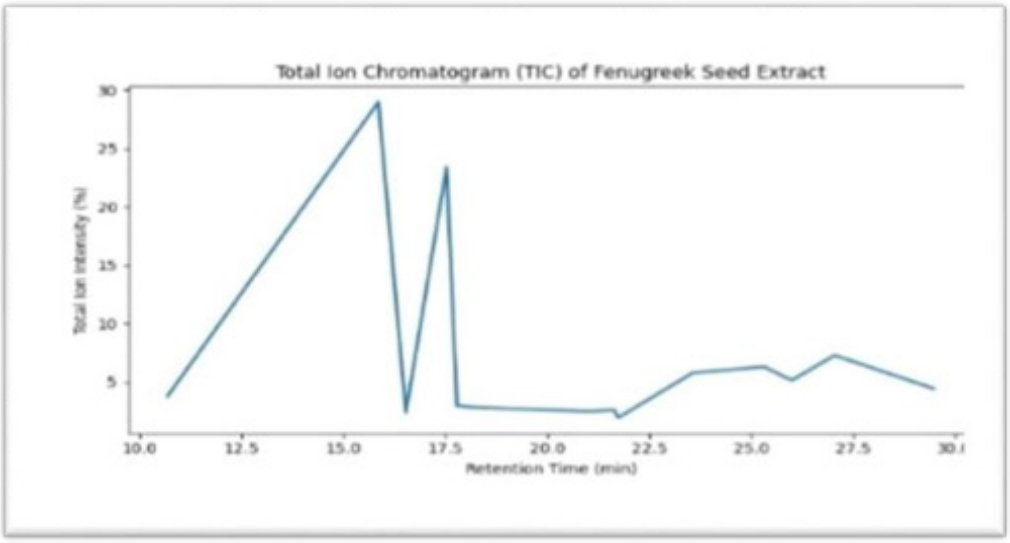
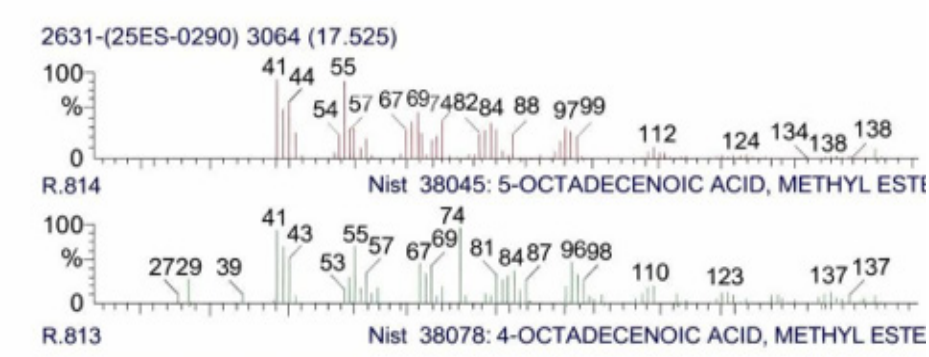
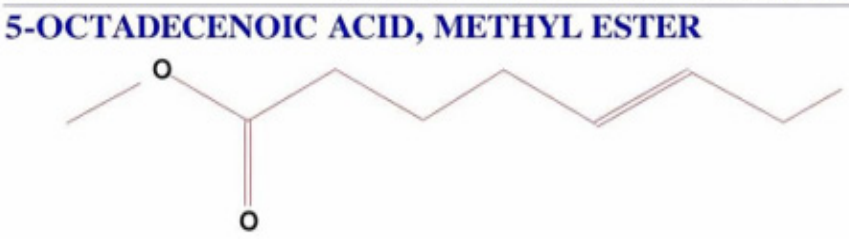


Figure 8: Total Ion Chromatography (TIC) of fenugreek seed extract.

Table 4: Phytochemical Compounds Identified by GC-MS:

Peak No.	RT (min)	Identified Compound	Molecular Formula	Area (%)
1	10.682	15-Crown-5	$C_{10}H_{20}O_5$	3.73
2	15.854	Deoxyspergualin	$C_{17}H_{37}N_7O_3$	28.99
3	16.529	N-Methyl-D-aspartic acid	$C_5H_9NO_4$	2.36
4	17.525	Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	23.42
5	17.780	Aminooxy pentanoic acid	$C_5H_{11}NO_3$	2.95
6	18.135	Methyl decyl ether	$C_{11}H_{24}O$	2.80
7	21.031	Phthalate derivative	$C_{11}H_{12}O_4$	2.44
8	21.631	Diethylhydroxylamine	$C_4H_{11}NO$	2.57
9	21.736	N-Ethylformamide	C_3H_7NO	1.91
10	23.557	15-Crown-5	$C_{10}H_{20}O_5$	5.76
11	25.988	15-Crown-5	$C_{10}H_{20}O_5$	5.12
12	27.033	Heptaethylene glycol	$C_{14}H_{30}O_8$	7.27
13	29.479	1,3-Dioxolane, 4-methyl-	$C_4H_8O_2$	4.41





Hit	REV	for	Compound Name
1	814	547	5-OCTADECENOIC ACID, METHYL ESTER
2	813	534	4-OCTADECENOIC ACID, METHYL ESTER
3	809	533	9-OCTADECENOIC ACID (Z)-, METHYL ESTER
4	807	430	3-HEPTADECENAL
5	806	529	7-HEXADECENOIC ACID, METHYL ESTER
6	805	541	6-OCTADECENOIC ACID, METHYL ESTE
7	806	534	4-OCTADECENOIC ACID, METHYL ESTER
8	792	472	3-HEPTADECENAL
9	789	505	7-HEXADECENOIC ACID, METHYL ESTER
10	788	506	6-OCTADECENOIC ACID, METHYL ESTER
11	785	506	9-OCTADECENOIC ACID, METHYL ESTE
12	782	534	9-OCTADECENOIC ACID (Z)- METHYL E
13	781	486	6-OCTADECENOIC ACID, (Z)-
14	780	471	9-OCTADECENOIC ACID, METHYL ESTER
15	781	506	9-OCTADECENOIC ACID, METHYL ESTER
16	782	534	9-OCTADECENOIC ACID, (Z)--
17	781	486	9-OODECENOIC ACID, METHYL ESTER (Z)-
18	780	486	CIS-10-NONADECENOIC ACID
19	789	471	CYCLOPENTADEC ANE
20	785	506	9-OCTADECENOIC ACID (2- METHYL ESTER (Z)-
21	785	554	9-OCTADECENOIC ACID, (Z)--
22	781	466	9-DODECENOIC ACID, METHYL ESTER (Z)-
23	781	486	CIS-10-NONADECENOIC ACID
14	779	485	CYCLOPENTADECANE
16	779	485	9-HEXADECENOIC ACID①

Figure 9: Octadecenoic acid methyl ester.

Fatty Acid Derivatives

The compound detected at RT 17.525, identified as 5-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$), constitutes a significant portion of the extract (23.42%) (Figure 9). This belongs to the family of unsaturated fatty acid methyl esters. This finding is consistent with existing literature on *Trigonella foenum-graecum*, which is known to be rich in unsaturated fatty acids such as oleic acid and linoleic acid derivatives. The methylation likely occurred if methanol was used during the extraction or sample preparation process.

Nitrogenous Compounds

The major peak at RT 15.854 (28.99%) was identified by the library as Deoxyspergualin (Figure 10). However, it is important to note that deoxyspergualin is typically a synthetic immunosuppressive drug. In the context of a natural plant extract, this library match may indicate the presence of a structurally similar guanidine derivative or polyamine, which are classes of compounds found

in legumes. Further confirmation via standard markers would be required to validate this exact structure.

Artifacts and Contaminants

Several compounds identified in the spectrum appear to be artifacts related to the extraction or analysis process rather than endogenous plant metabolites:

- Crown Ethers and Glycols: Peaks at RT 10.682, 23.557, 25.988 (15-Crown-5) (Figure 11) and RT 27.033 (Heptaethylene glycol) are likely residues from solvents, glassware cleaning agents, or stationary phase bleed 2020.

Phthalates

The compound at RT 21.031 (1,2-Benzenedicarboxylic acid ester) is a common plasticizer often found as a contaminant in laboratory analysis.

The NIST library identified peak 11 as (HMTD). HMTD is a synthetic organic peroxide explosive. It is scientifically impossible for this to be naturally present in fenugreek seeds.

DISCUSSION

The present study provides a comprehensive phytochemical characterization of *Trigonella foenum-graecum* seeds through the integration of extraction techniques, qualitative screening, UV-visible spectroscopy, and GC-MS analysis. The effectiveness of the extraction procedure is evidenced by the recovery of a chemically diverse crude extract containing multiple classes of bioactive primary and secondary metabolites. The detection of phenolics, flavonoids, alkaloids, carbohydrates, and cardiac glycosides confirms that fenugreek seeds are a metabolically rich plant material, while the low or trace presence of saponins and proteins suggests solvent selectivity and extraction-condition influence on phytochemical recovery.

UV-visible spectral analysis revealed prominent absorption maxima at 261 nm, 288 nm, 307 nm, and 329 nm, characteristic of π→π* and n→π* transitions typically observed in aromatic and conjugated systems. These spectral features strongly indicate the presence of phenolic and flavonoid compounds, which are well known for their chromophoric structures. The broad absorption band between 260 and 330 nm reflects overlapping contributions from multiple phytochemical groups, confirming the complex nature of the crude extract. Thus, UV-vis spectroscopy served as a rapid preliminary indicator of antioxidant-rich constituents.

GC-MS analysis further strengthened these findings by enabling molecular-level identification of individual compounds. The chromatographic profile demonstrated the presence of phenolic derivatives, alkaloids, fatty acids, esters, terpenoids, and steroidal compounds. Many of these compound classes are widely reported to possess antioxidant, antimicrobial, antidiabetic, and anti-inflammatory activities, which provides scientific support for the traditional therapeutic use of fenugreek seeds. Importantly, compounds with weak UV absorbance, such as fatty acids and non-conjugated esters, were effectively detected through chromatographic separation, highlighting the complementary analytical strengths of GC-MS.

The correlation between UV-visible spectral peaks and GC-MS-identified aromatic constituents demonstrates the reliability of

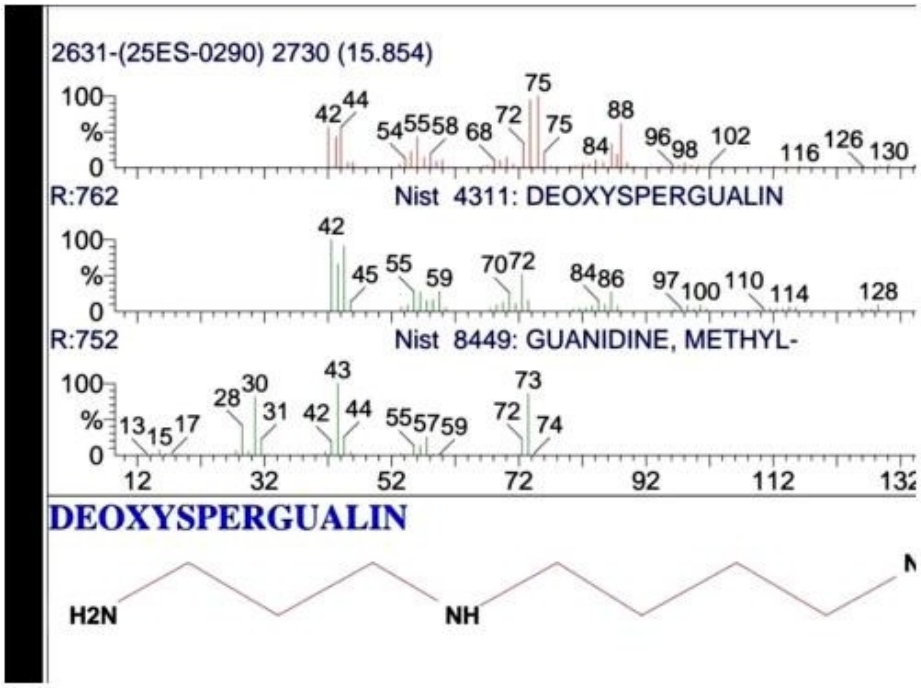


Figure 10: Deoxyspergualin.

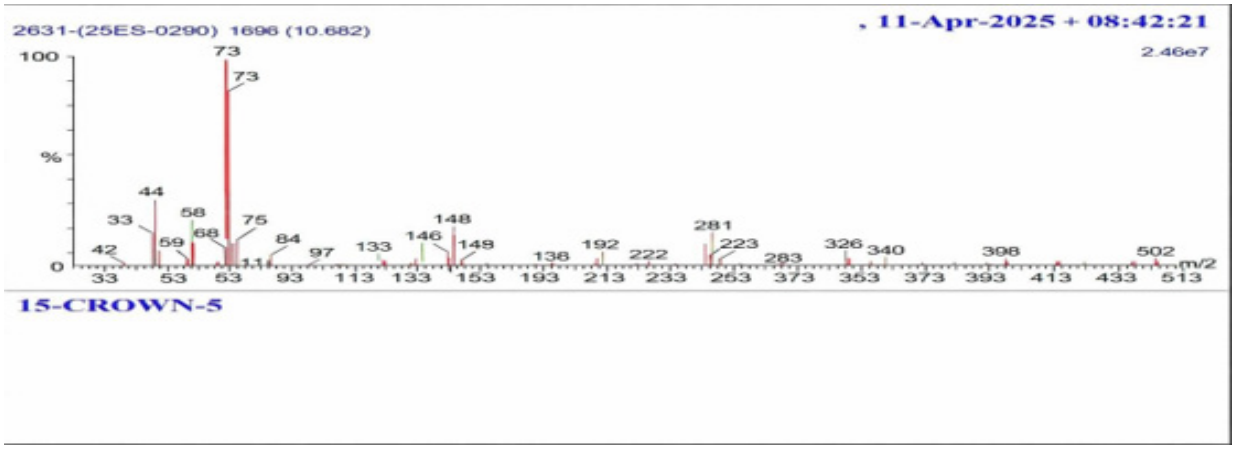


Figure 11: 5-Crown-5.

the multi-technique analytical approach. UV-vis spectroscopy confirmed the presence of conjugated bioactive systems, while GC-MS provided structural specificity, ensuring a robust and cross- validated phytochemical profile. This methodological synergy reduces analytical bias and enhances confidence in the compositional interpretation.

Overall, the findings establish *Trigonella foenum-graecum* seeds as a potent source of nutritionally and pharmacologically valuable phytochemicals, particularly phenolic and flavonoid compounds that contribute to antioxidant potential. The study not only validates the medicinal relevance of fenugreek but also supports its future application in functional foods and natural therapeutic formulations. Further investigations focusing on compound isolation, quantitative profiling, and bioactivity-guided fractionation would enable precise identification of the principal active constituents responsible for the observed biological properties.

CONCLUSION

The study confirms that *Trigonella foenum-graecum* seeds are a rich source of bioactive phytochemicals. Integrated analysis using phytochemical screening, UV-visible spectroscopy, and GC-MS revealed the presence of phenolics, flavonoids, alkaloids, and other therapeutically important compounds. The strong correlation between spectroscopic and chromatographic findings highlights the reliability of the analytical approach. The high phenolic and flavonoid content explain the antioxidant potential of the extract and supports the traditional medicinal use of fenugreek. These results indicate that fenugreek seeds have significant potential for development as functional food ingredients and natural therapeutic agents.

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ABBREVIATIONS

TCM: Traditional Chinese Medicine; **GC-MS:** Gas Chromatography-Mass Spectrometry; **UV-vis:** Ultraviolet-visible; **NIST:** National Institute of Standards and Technology; **QE:** Quercetin Equivalents; **GAE:** Gallic Acid Equivalents; **SD:** Standard Deviation; **RT:** Retention Time; **TIC:** Total Ion Chromatogram; **HMTD:** Hexamethylene triperoxide diamine; **NaOH:** Sodium Hydroxide; **HCl:** Hydrochloric acid.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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