Phytochemical Profiling and *in vitro* Antioxidant, Cytotoxic, and Digestive Enzyme Inhibitory Activities of Ethanolic Fenugreek (*Trigonella foenum-graecum* L.) Seed Extract

Nada H. Aljarba [a], Mohsen A. Khormi [b], Hamida Hamdi [c,d], Luluah M. Al Masoudi [c], Noha T. Al Thagafi [c], Ashwaq T. Althobaiti [c], Rowa K. Zarah,[c] Nuha Anajirih [e], and Mohamed K. Y. Soliman [f]

Trigonella foenum-graecum L. (fenugreek) is a medicinal and culinary plant valued for its rich phytochemical composition and diverse biological activities. This study investigated the phytochemical profile and *in vitro* biological activities of an ethanolic fenugreek seed extract using chromatographic and biological assays. GC–MS analysis tentatively identified nineteen volatile constituents, with 2-methyl-2-butenal (36.65%) and p-cymene (17.96%) as the major compounds, while HPLC revealed a phenolic-rich extract. The extract exhibited concentration-dependent antioxidant activity, reaching 85.75 ± 1.73% and 71.79 ± 1.95% scavenging in the DPPH and ABTS assays, respectively, at 1000 µg/mL. MTT evaluation demonstrated preferential cytotoxicity toward HepG2 and Caco-2 cancer cells, with IC_{50} values of 124.65 and 164.39 µg/mL, respectively, compared with 480.8 µg/mL for normal WI-38 cells. The extract also showed moderate pancreatic lipase inhibitory activity (IC_{50} = 116.74 µg/mL) compared with orlistat (IC_{50} = 17.83 µg/mL) and dose-dependently inhibited α-amylase and α-glucosidase. Collectively, these findings demonstrate the extract's *in vitro* antioxidant activity, preferential cytotoxicity toward the tested cancer cell lines, and inhibitory effects on pancreatic lipase and carbohydrate-digesting enzymes.

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*Contact information: a: Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; b: Department of Biology, College of Science, Jazan University, P.O. Box. 114, Jazan 45142, Kingdom of Saudi Arabia; c: Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; d: Zoology Department, Faculty of Science, Cairo University, Giza 12613, Egypt; e: Department of Emergency Medical Services, Faculty of Health Sciences, Al Qunfudhah, Umm Al-Qura University, 21912, Makkah, Saudi Arabia; f: Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City 11884, Cairo, Egypt; * Corresponding authors: Mohamed.k.yousef@azhar.edu.eg*

INTRODUCTION

Medicinal plants remain important sources of structurally diverse bioactive metabolites that may support the development of safer therapeutic and preventive strategies, particularly for chronic metabolic disorders and cancer (Yildirim *et al*. 2025). The increasing incorporation of herbal medicinal resources into daily diets reflects a growing interest in nutraceutical-rich natural products with potential health-promoting properties. Medicinal plants have therefore attracted considerable attention for their

potential roles in the prevention and management of diet-related disorders, including inflammation, hypertension, cardiovascular disease, diabetes, and cancer (Hina *et al*. 2025).

Trigonella foenum-graecum L. (fenugreek) is a well-known herb that is widely used in culinary practices and traditional medicine. It is also recognized as an important medicinal species because of its valuable nutritional and functional properties (Ansari *et al*. 2024). Fenugreek seeds have been associated with several pharmacological activities, *e.g.* antioxidant, antidiabetic, hypolipidemic, anti-inflammatory, and anticarcinogenic effects (Sun *et al*. 2021). Consequently, extracts and powdered preparations derived from different parts of the plant are widely used in food products and pharmaceutical formulations. Fenugreek seeds are particularly rich in bioactive constituents, including steroidal saponins, alkaloids, and polyphenols, which may contribute to their reported antioxidant, antidiabetic, and hypolipidemic properties (Akhtar *et al*. 2025).

Oxidative stress is strongly associated with the progression of metabolic diseases and several malignancies, and phenolic compounds are widely recognized for their ability to neutralize reactive species through hydrogen-atom and electron-transfer mechanisms. Recent literature has emphasized the importance of phenolic structure–activity relationships in determining antioxidant performance and other biological activities, supporting the investigation of polyphenol-rich plant extracts as potential functional ingredients (Jain *et al*. 2025). Furthermore, comprehensive reviews of phenolic compounds have highlighted their relevance to human health and their importance as a major class of plant secondary metabolites with diverse biological functions (Galić *et al*. 2025).

Type 2 diabetes mellitus is characterized by impaired insulin sensitivity and persistently elevated blood glucose levels. One strategy for controlling postprandial hyperglycemia involves inhibiting the carbohydrate-digesting enzymes α-amylase and α-glucosidase, thereby delaying starch digestion and glucose release. Accordingly, the simultaneous targeting of these enzymes remains an active area of research and may contribute to the development of dietary strategies and complementary interventions for diabetes management (Liu *et al*. 2025). Pancreatic lipase is another important digestive enzyme because its inhibition can reduce the hydrolysis and intestinal absorption of dietary fats. It is therefore commonly investigated as a therapeutic target in the screening of natural products with potential anti-obesity activity (Hou *et al*. 2022). Given its diverse phytochemical composition, fenugreek is a promising candidate for investigation as a natural source of bioactive compounds capable of modulating these metabolic enzymes (Ammari *et al*. 2026).

The present study aimed at comprehensively characterizing the phytochemical composition of the ethanolic extract of *T. foenum-graecum* seeds using GC–MS and HPLC analyses and to evaluate its antioxidant, cytotoxic, pancreatic lipase-inhibitory, and antidiabetic activities through *in vitro* assays.

MATERIALS AND METHODS

Extraction Procedure of Fenugreek Flour

The tested material (fenugreek seeds) was sourced from the Agricultural Research Center (ARC), Giza, Egypt. The seeds were finely ground to pass through a 250 μm sieve. Ethanolic extraction was performed by macerating 375 g of fenugreek flour in 1.5 L ethanol for 24 h. This process was repeated two additional times. After collection, the extracts were

combined, filtered, and evaporated under low-pressure conditions at 45 °C, yielding 44.7 g of green solid residue. The resulting extract was employed for further analysis of major phytochemicals contents, as well as for assessing biological activities. For further analysis, the extract was reconstituted in distilled water (Abdalla *et al*. 2023). The 44.7 g of dried extract corresponded to an extraction yield of 11.9% (w/w) relative to the initial dry seed weight. The extraction yield was calculated using the following equation: extraction yield (%) = (weight of the dried extract/initial dry seed weight) × 100. The dried extract was stored in an airtight amber glass container at 4 °C and protected from light until further analysis. Immediately before biological testing, the extract was reconstituted in distilled water to prepare a stock solution and subsequently diluted to the required working concentrations for each assay.

GC–MS Analysis

The volatile fraction of the fenugreek extract was analyzed using a Trace GC Ultra–ISQ system (Thermo Scientific, USA). Before analysis, the sample was diluted in methanol, dried over anhydrous sodium sulfate, and filtered through a 0.45-µm syringe filter. The system was equipped with a TG-5MS fused-silica capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1 mL/min, and a 1-µL aliquot was injected at 250 °C in split mode. The oven temperature was initially set at 70 °C, increased to 280 °C at 5 °C/min, held for 2 min, and then increased to 300 °C at 10 °C/min. The mass spectrometer was operated in electron ionization mode at 70 eV. The ion-source and transfer-line temperatures were maintained at 200 and 250 °C, respectively, and mass spectra were recorded in full-scan mode over an *m*/*z* range of 40 to 550. Compounds were tentatively identified by comparison with the Wiley 09 mass spectral library, based on the highest spectral match and agreement of the major fragment ions with the library spectrum (Alfattah *et al*. 2025).

HPLC Analysis

Phenolic compounds present in the fenugreek extract (100 µg/mL) were analyzed by high-performance liquid chromatography (HPLC). The analysis was executed using an Agilent 1260 chromatographic system (Agilent Technologies, Santa Clara, CA, USA) equipped with a reversed-phase C18 column (100 mm × 4.6 mm, 5 µm particle size). Chromatographic separation was accomplished at a flow rate of 0.6 mL/min using a ternary solvent system consisting of acetonitrile, methanol, and aqueous phosphoric acid solution (0.2%). A gradient elution was employed, starting with a high proportion of acetonitrile and gradually altering the solvent composition over a 30-min run. UV detection was monitored at a wavelength of 284 nm, with the column temperature maintained at 30 °C and an injection volume of 20 µL. Phenolic constituents were identified by comparing their retention times with authentic standards, and their concentrations were calculated using external calibration curves prepared from the corresponding reference compounds.

Anticancer Activity Test

HepG2 (HB-8065), Caco-2 (HTB-37), and WI-38 (CCL-75) cell lines were employed in this study together with the normal lung fibroblast cell line WI-38. Cells were seeded into 96-well plates at a density of 1×10^5 cells/mL (100 µL/well) followed by incubation at 37 °C for 24 h to enable adherence. The culture medium was then removed, and the cells were washed twice with washing buffer. The samples were diluted stepwise in RPMI medium supplemented with 2% serum to obtain final concentrations ranging from

15.62 to 1000 µg/mL and then added to the wells, while untreated controls received medium only. Cell viability was evaluated using the MTT assay, in which metabolically active cells reduced 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (5 mg/mL in PBS) to formazan crystals. The crystals were dissolved in 200 µL DMSO with gentle shaking (150 rpm, 5 min), and absorbance was recorded at 560 nm. Cell viability was expressed as a percentage of the control based on absorbance value (Tolosa *et al*. 2014; Soliman *et al*. 2025). Cell viability was calculated as follows:

$$\text{Cell viability (\%)} = (\text{Absorbance treated} / \text{Absorbance untreated control}) \times 100 \quad (1)$$

Antioxidant Activity Evaluation

DPPH radical scavenging assay

The antioxidant effectiveness of the fenugreek extract was examined through its ability to neutralize 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals using a modified protocol. A freshly prepared DPPH ethanolic solution (0.2 mg/mL; 0.135 mM) was distributed into test tubes (1.0 mL per tube). The extract was prepared at different concentrations ranging from 7.81 to 1000 µg/mL and mixed with the DPPH solution. The reaction mixtures were gently mixed and kept at room temperature in the absence of light for 30 min to allow the reaction to proceed. After the incubation period, absorbance values were measured at 517 nm using a microplate spectrophotometer (Epoch 2, BioTek, USA), with ethanol used as the blank. Ascorbic acid was included as a standard antioxidant for comparison(Soliman *et al*. 2026).

ABTS radical scavenging assay

The free radical scavenging ability of the fenugreek extract was assessed using an ABTS-based method with slight procedural modifications. The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation was generated by mixing equal volumes of ABTS solution (7 mM) and potassium persulfate (2.5 mM), followed by incubation in the dark at room temperature for 16 h. The activated ABTS solution was diluted with ethanol to obtain an initial absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 100 µL of the fenugreek extract at each tested concentration (7.81 to 1000 µg/mL) was mixed with 100 µL of the diluted ABTS working solution and incubated for 10 min at room temperature in the dark. Ascorbic acid was used as the positive antioxidant control, and absorbance was measured at 734 nm using a microplate reader(Khormi *et al*. 2025).

Assessment of Pancreatic Lipase Inhibition Potential

The anti-obesity potential of fenugreek seed extract was evaluated by assessing its capacity to suppress pancreatic lipase activity using a modified colorimetric approach. Orlistat was employed as a reference inhibitor, while 4-nitrophenyl butyrate was used as the reaction substrate. The extract was tested at a series of concentrations ranging from 7.81 to 1000 µg/mL. Pancreatic lipase was freshly dissolved in potassium phosphate buffer (0.1 M, pH 7.0) to obtain a final enzyme concentration of 5 mg/mL. For each assay, the reaction mixture was assembled by sequential addition of buffer, enzyme solution, and the extract or standard inhibitor. The enzymatic reaction was initiated by introducing the substrate solution (15 mM). The total reaction volume comprised 220 µL buffer, 35 µL enzyme, 5 µL extract or orlistat, and 5 µL substrate. The reaction mixtures were incubated at 37 °C for 10 min, after which lipase activity was determined by measuring the release of 4-nitrophenol at 405 nm using a microplate reader. Enzyme inhibition was expressed as

a percentage reduction in absorbance relative to the control reaction conducted without inhibitors (Kim *et al*. 2010).

Antidiabetic Activity

α-Glucosidase inhibitory assay

The assay was performed using α-glucosidase from *Saccharomyces cerevisiae* (Sigma-Aldrich, St. Louis, MO, USA) and p-nitrophenyl-α-D-glucopyranoside (pNPG) as the substrate. The fenugreek extract and acarbose reference standard were tested at concentrations ranging from 1.95 to 1000 µg/mL. The enzyme solution (1 U/mL) was incubated with the extract or acarbose at 37 °C for 20 min. The reaction was initiated by adding pNPG (10 mM), incubated at 37 °C for 30 min, and stopped with 1 M sodium carbonate. Absorbance was measured at 405 nm (Li *et al*. 2024; Soliman *et al*. 2025).

α-Amylase inhibitory assay

The assay was performed using α-amylase from porcine pancreas (Sigma-Aldrich, St. Louis, MO, USA). The fenugreek extract and acarbose were tested at concentrations ranging from 1.95 to 1000 µg/mL. The extract or acarbose was incubated with α-amylase (2 U/mL) at 37 °C for 20 min. After adding 1% potato starch, the reaction was continued at 37 °C for 30 min and stopped with DNSA reagent. The mixtures were heated at 90 °C for 10 min, cooled, and measured at 540 nm (Kashtoh and Baek 2023). For both assays, sample blanks were prepared without enzyme. The control contained enzyme and substrate without the extract or acarbose. The control contained substrate and buffer without enzyme

Statistical Analysis

Data were analyzed using GraphPad Prism 8.0 and are presented as mean ± SD from 3 independent tests, each in triplicate. One-way ANOVA followed by Tukey's test was used for concentration comparisons within each group, while two-way ANOVA followed by Tukey's test evaluated concentration × cell-line or treatment effects. GC–MS and HPLC data were analyzed descriptively. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

GC–MS Profiling of Fenugreek Ethanolic Extract

The volatile constituents of the fenugreek ethanolic extract were investigated using GC–MS analysis. The analysis tentatively identified nineteen compounds based on their retention behavior and comparison of their mass spectra with the Wiley 09 spectral library (Fig. 1 and Table 1). The detected constituents belonged mainly to monoterpenes, sesquiterpenes, and aromatic derivatives. The extract was dominated by 2-methyl-2-butenal, accounting for 36.6% of the total peak area. Plant-derived volatile aldehydes have previously been investigated for various biological properties (Hu *et al*. 2025); however, the identity and biological contribution of 2-methyl-2-butenal were not independently confirmed in the present study. Similarly, p-cymene, γ-terpinene, and α-terpinene are commonly reported plant monoterpenes with literature-described biological properties (Baginska *et al*. 2023). Sesquiterpenes, including germacrene D and δ-cadinene, were also tentatively detected, and related sesquiterpene classes have been investigated for anti-inflammatory and cytotoxic properties (Teng *et al*. 2025).

Nevertheless, the individual compounds were not isolated or experimentally tested; therefore, the biological activities of the whole extract cannot be attributed to specific

constituents. Cyclotrisiloxane and the reported phthalate derivative may represent laboratory or analytical-system contaminants, and no biological relevance was assigned to them without further confirmation.

Table 1. GC–MS Profile of Volatile Constituents Detected in Fenugreek Ethanolic Extract

Peak	RT	Area	Area Sum %	Peak Name
1	2.841	11306134.92	36.65	2-Methyl-2-butenal
2	3.836	474755.57	1.54	Cyclotrisiloxane, hexamethyl-
3	7.212	280972.94	0.91	Geranyl isovalerate
4	7.67	279336.39	0.91	1-Octen-3-ol
5	8.214	1428639.55	4.63	α-Terpinene
6	8.425	5541447.45	17.96	*p*-Cymene
7	8.54	563371.78	1.83	D-Limonene
8	9.043	1077306.05	3.49	β-Ocimene
9	9.352	1720804.74	5.58	γ-Terpinene
10	11.595	1552208.77	5.03	Benzyl nitrile
11	12.019	846293.32	2.74	Isomenthone
12	12.54	429748.24	1.39	Levomenthol
13	18.073	826189.33	2.68	Copaene
14	19.217	269271.31	0.87	Caryophyllene
15	20.076	524422.28	1.7	Humulene
16	20.751	1671838.12	5.42	Germacrene D
17	21.111	318349.43	1.03	Bicyclogermacrene
18	21.746	1223653.29	3.97	δ-Cadinene
19	23.406	249447.35	0.81	Phthalic acid, 7-bromoheptyl octyl ester

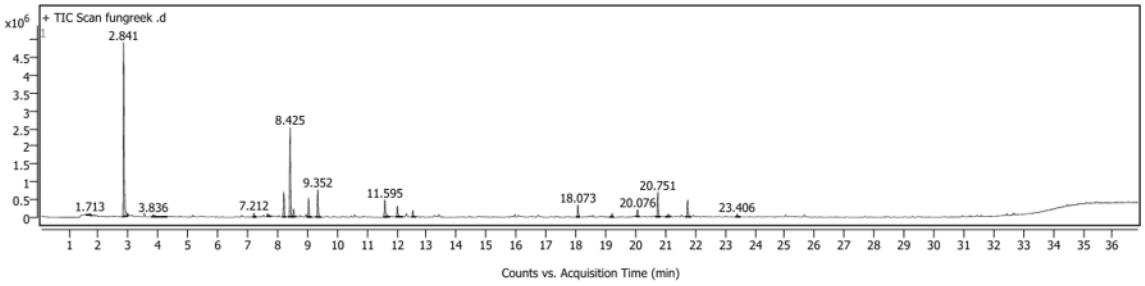

Fig. 1. GC–MS-derived total ion chromatogram showing the chemical profile of the ethanolic fenugreek extract

Analysis of Phenolic Composition of Fenugreek Extract by HPLC

The phenolic composition in the ethanol-based fenugreek extract was determined using HPLC analysis. The results revealed a rich profile of phenolic acids and flavonoids with variable concentrations (Table 2). Within the identified chemical constituents, epicatechin gallate was the most abundant constituent, reaching 1220 µg/g. This was followed by sinapic acid (890 µg/g), and vanillic acid (250 µg/g). In addition, considerable amounts of rutin (266 µg/g), protocatechuic acid (26.9 µg/g), chlorogenic acid (26.3 µg/g), and catechin (22.3 µg/g) were detected. Other phenolic acids such as gallic, syringic,

cinnamic, and *p*-coumaric acids were present in moderate to low concentrations, along with flavonoids including quercetin and chrysin.

Table 2. Retention Times (RT) and Concentrations of Phenolic Acids and Flavonoids Identified in the Ethanolic Extract of *Trigonella foenum-graecum* Using HPLC Analysis

Compound	RT (min)	Concentration (µg/g)	Compound	RT (min)	Concentration (µg/g)
Epicatechin gallate	25.58	1223.25	Sinapic acid	24.61	889.66
Rutin	27.26	265.64	Vanillic acid	18.34	249.50
Epicatechin	17.72	62.09	Protocatechuic acid	8.00	26.86
Chlorogenic acid	14.82	26.34	Catechin	13.72	22.32
Cinnamic acid	36.79	15.24	Syringic acid	16.73	11.61
Gallic acid	4.54	3.07	Chrysin	54.26	2.94
p-Hydroxybenzoic acid	12.33	2.46	*p*-Coumaric acid	28.52	2.23
Apigenin-7-glucoside	33.00	2.08	Caffeic acid	16.04	1.83
Quercetin	37.89	1.24	—	—	—

The abundance of phenolic acids and flavonoids in fenugreek extract highlights its strong antioxidant and therapeutic potential. Polyphenols such as epicatechin derivatives and rutin are widely recognized for their potential to counteract reactive oxygen species and protect cells from oxidative injury, which may explain the antioxidant activity observed in this study (Hu *et al*. 2025). Notably, sinapic acid, one of the major detected phenolics, has been recently reported to possess significant anti-inflammatory, antidiabetic, and anticancer activities through modulation of oxidative pathways and enzyme inhibition (Yasser *et al*. 2025). Likewise, chlorogenic acid and catechin are well-documented natural antioxidants with protective effects against metabolic disorders, supporting the biological relevance of fenugreek as a functional medicinal plant (Nunes *et al*. 2025).

Biological Activity Studies

Cytotoxic effects of fenugreek ethanolic extract on cancer cell lines

The fenugreek ethanol extract was studied for its anticancer properties using the MTT colorimetric assay against human HepG2 and Caco-2 cell lines, with the normal human lung fibroblast WI-38 included for comparison (Fig. 2). Upon treatment with the extract resulted in a concentration-dependent decline in cell survival in both cancer cell models, indicating a pronounced cytotoxic response at increasing extract concentrations. At the maximum dose (1000 µg/mL), HepG2 cells showed a marked decrease in viability to nearly 3%, while Caco-2 viability was reduced to approximately 6%, confirming strong cytotoxic activity. The calculated IC_{50} values further demonstrated the anticancer effectiveness of the sample test. HepG2 cells exhibited the highest sensitivity to the extract (IC_{50} = 125 µg/mL), whereas Caco-2 cells showed moderate susceptibility (IC_{50} = 164 µg/mL). Conversely, the normal WI-38 cells were substantially less affected, with an IC_{50} of 481 µg/mL, indicating a selective anticancer effect. The anticancer response elicited by the fenugreek extract in HepG2 and Caco-2 cells may stem from its rich polyphenolic

composition and terpenoid constituents, as confirmed by HPLC and GC–MS analyses. Bioactive constituents of fenugreek, particularly flavonoids, phenolic acids, and steroidal saponins, have been shown to inhibit cancer cell proliferation and promote apoptosis through oxidative stress modulation and mitochondrial pathway interference (Ahmed *et al*. 2025). The stronger activity against HepG2 cells compared with Caco-2 may reflect differences in cellular metabolism and sensitivity to plant-derived bioactive compounds. Similar findings have been reported for fenugreek-derived extracts, which demonstrated selective cytotoxicity toward cancer cells (Akhtar *et al*. 2025). Similarly, a recent study showed that fenugreek polyphenol-rich extracts displayed selective anticancer effects while exerting minimal toxicity toward normal cell (Salam *et al*. 2023). Importantly, the higher IC50 value obtained for WI-38 normal cells indicates that the extract is less harmful to non-cancerous cells, supporting its potential as a safer natural anticancer candidate. This selective behavior is a desirable feature in the development of plant-based chemotherapeutic agents.

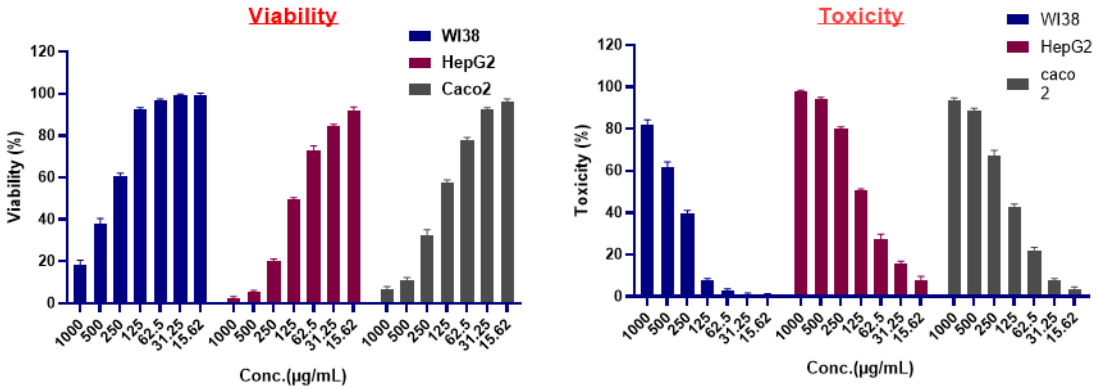

Fig. 2. Effect of fenugreek ethanolic extract on cell viability and cytotoxicity percentages in HepG2, Caco-2, and WI-38 cell lines at different concentrations, as determined by the MTT assay

Table 3. DPPH and ABTS Radical Scavenging Activities (%) of Fenugreek Extract Compared with Ascorbic Acid

Concentration (µg/mL)	DPPH Radical Scavenging (%)		ABTS Scavenging (%)	
	Fenugreek	Ascorbic Acid	Fenugreek	Ascorbic Acid
1000	85.75 ± 1.73	96.10 ± 1.10	71.79 ± 1.95	96.45 ± 0.32
500	76.75 ± 1.55	85.00 ± 1.51	60.16 ± 1.31	82.29 ± 0.89
250	56.26 ± 1.14	72.68 ± 1.45	53.13 ± 1.23	72.34 ± 1.07
125	35.37 ± 1.00	63.73 ± 1.52	40.93 ± 0.90	64.42 ± 1.12
62.5	20.22 ± 1.23	51.84 ± 1.33	31.00 ± 0.67	54.27 ± 0.79
31.25	9.67 ± 0.52	32.39 ± 1.56	20.32 ± 0.43	43.55 ± 1.57
15.62	6.13 ± 0.59	19.68 ± 0.99	15.61 ± 0.68	29.09 ± 1.45
7.81	3.60 ± 0.18	5.96 ± 0.73	9.49 ± 0.20	13.03 ± 0.99

Antioxidant Activity of Fenugreek Ethanolic Extract

In-vitro antioxidant potential determined by DPPH and ABTS assays

The two assays are commonly employed to evaluate the hydrogen-donating and electron-transfer capacity of plant-derived antioxidants. These findings are in line with recent evidence suggesting that fenugreek seeds possess considerable antioxidant potential because of their high phenolic and flavonoid content (Ammari *et al*. 2026). The ability of fenugreek to counteract oxidative radicals was assessed using two established *in vitro*

assays, namely DPPH and ABTS radical scavenging methods. As detailed in Table 3, the extract demonstrated a progressive enhancement in free radical neutralization with increasing concentrations in both assays. At the greatest intensity (1000 µg/mL), the extract achieved 85.75 ± 1.73% inhibition in the DPPH assay and 71.79 ± 1.95% in the ABTS assay. In contrast, the reference standard ascorbic acid showed higher scavenging efficiencies (96.10 ± 1.10% for DPPH and 96.45 ± 0.32% for ABTS) at the same quantities, confirming its stronger antioxidant potency. The observed dose-dependent enhancement of antioxidant activity is consistent with previous reports that fenugreek extracts possess substantial radical scavenging potential attributed to their rich phytochemical profile, particularly polyphenols, flavonoids, and tannins. A recent study reported significant DPPH scavenging activity in fenugreek seed acetone extracts, confirming the high antioxidant capacity of fenugreek phytoconstituents and their role in neutralizing free radicals (Bakhtiar and Mirjalili 2025; Kamil *et al*. 2025). In addition to the effectiveness of fenugreek microgreen extracts in both DPPH and ABTS scavenging assays, correlating with high levels of total phenolic and antioxidant contents was reported by (Çoban 2025).

Although the antioxidant efficacy of fenugreek extract in this study appeared stronger than some previously reported values (often influenced by solvent type and extraction method), the trend of increasing activity with concentration has been universally observed across multiple investigations. For example, HPLC-DAD-MS characterization of fenugreek seed flavonoids revealed notable antioxidant activity in DPPH and ABTS assays, with IC_{50} values in the hundreds of µg/mL range, further confirming the role of phenolics and flavonoid glycosides in radical scavenging. The differences between the DPPH and ABTS assay responses may stem from the specificity of these methods toward different types of antioxidant compounds; ABTS typically responds to both hydrophilic and lipophilic antioxidants, whereas DPPH may be more selective to certain hydrogen donors. Such variations are commonly reported in comparative antioxidant studies of plant extracts (Khenifi *et al*. 2023).

Pancreatic Lipase Suppression Potential (Anti-Obesity Potential)

The pancreatic enzyme, in a manner similar to lipase, plays a crucial role in lipid digestion by facilitating the breakdown of triglycerides into fatty acids that can be readily absorbed. Therefore, inhibition of this enzyme is a well-established therapeutic strategy for obesity management (Oliveira *et al*. 2022). In this study, fenugreek extract displayed moderate yet consistent lipase inhibition, supporting its promise as a naturally derived agent for obesity management. The fenugreek extract intensified in a concentration-dependent manner, attaining a maximum value of 63.06 ± 2.05% at the highest tested dose. The inhibitory effect gradually declined with decreasing concentration, reaching 23.12 ± 0.75% at 7.81 µg/mL (Fig. 3a). The calculated IC_{50} value for fenugreek extract was 117 µg/mL, indicating moderate lipase inhibitory potential. In contrast, orlistat demonstrated significantly higher inhibitory activity across all tested concentrations, achieving 98.52 ± 0.23% inhibition at 1000 µg/mL and exhibiting a much lower IC_{50} value of 17.8 µg/mL, confirming its superior potency ($P < 0.05$). Although the inhibitory effect of fenugreek extract was lower than that of orlistat, such differences are expected since orlistat is a synthetic, highly specific lipase inhibitor (Hou *et al*. 2022). In contrast, plant-derived inhibitors often exert milder enzyme suppression but are favored due to their lower gastrointestinal side effects and broader health benefits, including antioxidant and metabolic regulatory effects (Zarei *et al*. 2025). The observed lipase inhibition may be attributed to the phytochemical constituents of fenugreek, particularly polyphenols,

flavonoids, and saponins, which have been widely reported to interfere with digestive enzymes (Rana *et al*. 2022). Polyphenols are known to inhibit pancreatic lipase through direct interaction with the enzyme's active site or by altering enzyme conformation, thereby reducing substrate accessibility (Fiore *et al*. 2025).

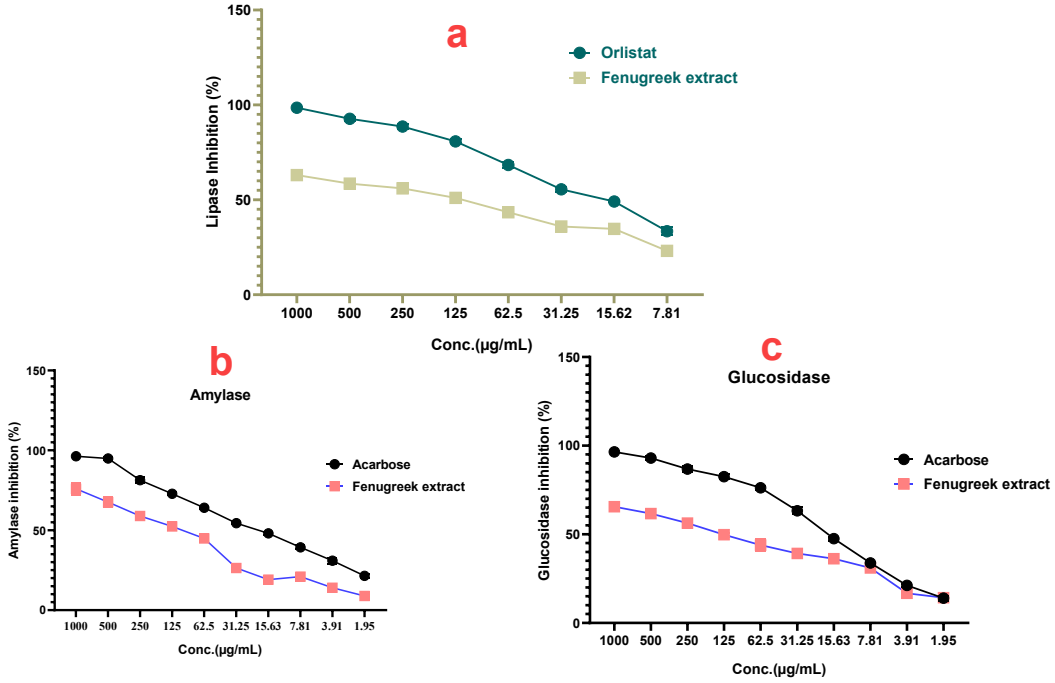

Fig. 3. Inhibitory effects of ethanolic fenugreek extract on digestive enzymes: (a) Lipase, (b) α-amylase, and (c) α-glucosidase activities

Antidiabetic Potential of Fenugreek Extract through Inhibition of both α-Amylase and α-Glucosidase

Strategies for managing hyperglycemia commonly focus on the inhibition of key carbohydrate-digesting enzymes, particularly α-amylase and α-glucosidase, because of their central roles in dietary starch hydrolysis and glucose absorption, particularly α-amylase and α-glucosidase, due to their central role in dietary starch hydrolysis and glucose absorption. Pharmacological inhibitors of these enzymes are clinically effective in delaying carbohydrate digestion and suppressing postprandial blood glucose excursions (Lan *et al*. 2023).

The outcome results indicated that both acarbose and fenugreek extract displayed a dose-responsive decrease feature against α-amylase and α-glucosidase. Fenugreek extract demonstrated moderate but notable inhibitory effects, achieving 75.9% restraint of α-amylase and 65.6% restraint of α-glucosidase at a concentration of 1,000 µg/mL. In comparison, at the same concentration acarbose showed pronounced enzyme inhibition, reaching 96.3% inhibition of α-amylase and 96.5% inhibition of α-glucosidase confirming its strong inhibitory efficacy (Figs. 3b and 3c). For α-amylase, the IC_{50} value of the fenugreek extract was 105 µg/mL, which was significantly higher than that of acarbose (20.5 µg/mL), indicating lower inhibitory potency of the extract. Similarly, for α-glucosidase, the IC_{50} value of the fenugreek extract (129 µg/mL) was markedly higher than that of acarbose (18.3 µg/mL).

Previous studies have reported that fenugreek-derived bioactive compounds, including flavonoids, saponins, and phenolic constituents, can interact with carbohydrate-hydrolyzing enzymes and suppress their catalytic activity (Laıla *et al*. 2024). α-Amylase catalyzes the hydrolysis of complex starch into oligosaccharides, while α-glucosidase further cleaves α-1,4-glycosidic linkages to release absorbable glucose units (Li *et al*. 2022). Therefore, restraint of these enzymes plays a crucial role in controlling postprandial hyperglycemia. The moderate enzyme suppression observed for fenugreek extract suggests a potential advantage in minimizing gastrointestinal side effects commonly associated with strong synthetic inhibitors such as acarbose, while still contributing to glycemic regulation (Alu'datt *et al*. 2024).

Comparative analysis revealed that the fenugreek extract exerted slightly stronger a suppressive impact on α-amylase than on α-glucosidase. This selective inhibition profile may be advantageous, as excessive suppression of α-amylase by synthetic suppressive agent such as acarbose has been associated with gastrointestinal side effects (Abadi *et al*. 2023). The observed enzyme inhibitory activity of fenugreek supports its traditional use in diabetes management and aligns with previous reports demonstrating its ability to reduce postprandial hyperglycemia through modulation of carbohydrate metabolism (Chehregosha *et al*. 2025).

Cooperatively, the present findings support the potential application of fenugreek extract as a natural antidiabetic agent by adjusting of carbohydrate-digesting enzymes and fenugreek extract exhibits potential to function as a food ingredient or complementary therapeutic agent for the management of type 2 diabetes mellitus (Abduallah *et al*. 2024).

CONCLUSIONS

1. Gas chromatography-mass spectrometry (GC–MS) and high preformance liquid chromatography (HPLC) analyses demonstrated that the ethanolic fenugreek seed extract contained diverse volatile and phenolic compounds.
2. The extract exhibited concentration-dependent antioxidant activity in the *in vitro* DPPH and ABTS assays.
3. The MTT assay demonstrated *in vitro* cytotoxic effects against HepG2 and Caco-2 cells, with lower cytotoxicity toward normal WI-38 cells under the tested conditions.
4. The extract inhibited pancreatic lipase, α-amylase, and α-glucosidase *in vitro*, although its inhibitory activity was lower than that of the reference inhibitors orlistat and acarbose.
5. Overall, these findings provide preliminary *in vitro* evidence of the antioxidant, cytotoxic, pancreatic lipase inhibitory, and carbohydrate-digesting enzyme inhibitory activities of fenugreek extract. Further mechanistic, toxicological, and *in vivo* studies are required before any therapeutic or nutraceutical applications can be proposed.

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