JOURNAL OF CURRENT RESEARCH IN FOOD SCIENCE

E-ISSN: 2709-9385
P-ISSN: 2709-9377
JCRFS 2024; 5(2): 183-188

www.foodresearchjournal.com
Received: 07-07-2024
Accepted: 18-08-2024

Dr. Layla Al-Sabah
Department of Phytochemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq

Ahmed Jasim Al-Mukhtar
Professor, Department of Medicinal Chemistry, College of Medicine, University of Basrah, Basrah, Iraq

Dr. Omar Rami Al-Fadhli
Department of Biochemistry, College of Science, University of Mosul, Mosul, Iraq

Correspondence
Dr. Layla Al-Sabah
Department of Phytochemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq

Unravelling the secrets of fenugreek through integrated approaches

Layla Al-Sabah, Ahmed Jasim Al-Mukhtar and Omar Rami Al-Fadhli

Abstract
Fenugreek (*Trigonella foenum-graecum* L.) has long been valued for its medicinal and nutritional properties. This study explores the bioactive potential of fenugreek through an integrated approach combining extraction techniques, bioactivity assays, and omics-based analyses. Hydroalcoholic solvents, particularly 50% ethanol, were found to yield the highest concentration of bioactive compounds, including diosgenin, trigonelline, and galactomannan. The antioxidant capacity of fenugreek was evaluated using DPPH and ABTS assays, with methanol and 50% ethanol extracts exhibiting the strongest radical-scavenging activity. Antidiabetic and hypocholesterolemic effects were assessed through glucose uptake assays and α-amylase inhibition, showing significant improvements with methanol and 50% ethanol extracts. Antibacterial activity was also demonstrated against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, suggesting its potential as a natural antimicrobial agent. Additionally, transcriptomic analysis revealed enhanced expression of key genes involved in saponin and flavonoid biosynthesis. These findings highlight the therapeutic potential of fenugreek and support its use in functional foods and nutraceuticals. Future research should focus on clinical validation and optimization of fenugreek's bioactive potential for human health applications.

Keywords: Fenugreek, *Trigonella foenum-graecum*, bioactive compounds, antioxidant, antidiabetic, hypocholesterolemic, antibacterial activity, transcriptomics, functional foods, nutraceuticals, extraction techniques, omics-based analysis

Introduction
Fenugreek (*Trigonella foenum-graecum* L.) is an annual leguminous plant that has been cultivated for centuries across Asia, Africa, and Europe for its culinary, medicinal, and nutraceutical values, earning it recognition as a multipurpose crop of immense socio-economic relevance [1,2]. Its seeds and leaves are traditionally used in Ayurveda, Unani, and Chinese medicine for treating metabolic disorders, inflammation, and digestive problems [3, 4]. With the growing global demand for functional foods, fenugreek has attracted attention due to its rich phytochemical profile comprising saponins, flavonoids, alkaloids, and steroidal compounds [5, 6]. The presence of diosgenin, galactomannan, and trigonelline confers antidiabetic, hypocholesterolemic, antioxidant, and anticancer activities, making it a promising candidate for pharmaceutical and nutraceutical industries [7-9]. Despite these established therapeutic potentials, gaps remain in translating traditional uses into validated, evidence-based functional food applications, largely due to fragmented research, variability in cultivation practices, and limitations in extraction and characterization techniques [10, 11]. The current challenge lies in integrating agronomic, biochemical, pharmacological, and molecular biology approaches to fully unravel fenugreek's complex bioactivity pathways and its role in human health [12, 13]. Moreover, issues such as low seed yield, limited genomic resources, and inadequate clinical validations continue to hinder its wide-scale adoption as a standardized therapeutic agent [14, 15]. Addressing these concerns requires a multidisciplinary framework that not only investigates the genetic and metabolic diversity of fenugreek but also evaluates its efficacy through advanced *in vitro*, *in vivo*, and clinical models [16, 17]. Against this backdrop, the present article—*Unravelling the secrets of fenugreek through integrated approaches*—seeks to consolidate insights from plant biotechnology, phytochemistry, pharmacology, and food science to advance the systematic understanding of fenugreek's bioactive compounds and their applications in functional foods and therapeutics. The study is premised on the hypothesis that integrated approaches, combining omics technologies, advanced extraction methods, and clinical validations, can bridge the gap between traditional knowledge and modern evidence, thereby unlocking fenugreek's

full potential for health and nutrition security [18-20].

Material and Methods

Materials

The present study utilized fenugreek (*Trigonella foenum-graecum* L.) seeds obtained from certified local markets and authenticated at a recognized herbarium facility to ensure botanical accuracy [1,3]. Seeds were cleaned, shade-dried, and powdered using a laboratory mill before further use [10]. Standard reagents and solvents such as methanol, ethanol, chloroform, and distilled water were procured from analytical grade suppliers. Reference compounds including diosgenin, trigonelline, and galactomannan were purchased from Sigma-Aldrich (USA) to serve as standards for chromatographic and spectrophotometric analyses [5, 7]. For omics-based experiments, high-quality DNA and RNA were extracted from fresh fenugreek leaves using CTAB and TRIzol protocols, respectively [12, 14]. *In vitro* assays involved the use of established human cell lines (HepG2, Caco-2, and L6 myotubes) to evaluate antioxidant, antidiabetic, and hypocholesterolemic activities [6, 8, 9]. All culture media, fetal bovine serum (FBS), and assay kits (oxidative stress, glucose uptake, and cholesterol-binding) were procured from HiMedia Laboratories, India, and Thermo Fisher Scientific, USA [16]. For microbial activity testing, bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were obtained from the MTCC repository [17].

Methods

Phytochemical characterization of fenugreek seed extracts was performed using Soxhlet extraction with polar and non-polar solvents, followed by qualitative and quantitative assays for saponins, flavonoids, and alkaloids [5, 19]. High-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) were employed for metabolite profiling, while nuclear magnetic resonance (NMR) spectroscopy was used for structural confirmation of bioactive compounds [7,13]. Omics analyses, including transcriptomic and metabolomic profiling, were conducted using Illumina sequencing platforms and LC-MS/MS techniques to identify genes and metabolic pathways linked to secondary metabolite biosynthesis [12, 14]. *In vitro* bioactivity assays involved DPPH and ABTS radical scavenging methods to assess antioxidant potential, while glucose uptake assays in L6 myotubes and α-amylase inhibition tests were performed to determine antidiabetic properties [8, 11]. Hypocholesterolemic activity was measured by cholesterol micelle solubilization and bile acid binding assays [7, 15]. Antibacterial activity was evaluated using the agar well diffusion method and minimum inhibitory concentration (MIC) determination against selected pathogens [17]. Data were statistically analyzed using SPSS software, applying one-way ANOVA followed by Tukey's post hoc test to determine significant differences ($p<0.05$) [20]. Ethical clearance for cell-based experiments was obtained from the Institutional Biosafety Committee, and all methods were carried out in accordance with relevant guidelines and regulations [2, 18].

Results

Extraction yield and targeted metabolites: Hydroalcoholic extraction (50% ethanol) produced the highest yield (18.7%), followed by methanol (16.2%), water (11.8%) and chloroform (6.5%) (Table 1; Figure 1). Methanol maximized diosgenin (3.1 mg/g) while 50% ethanol balanced diosgenin (2.6 mg/g) with superior trigonelline (5.2 mg/g) and robust galactomannan recovery (150 mg/g) (Table 1). These patterns agree with solvent-polarity driven partitioning of steroidal saponins and alkaloids and with prior analytical studies that emphasize alcohol/water mixtures for maximizing fenugreek bioactives [5, 7, 19]. The abundant galactomannan in aqueous/alcoholic extracts is consistent with earlier carbohydrate-focused reports [10].

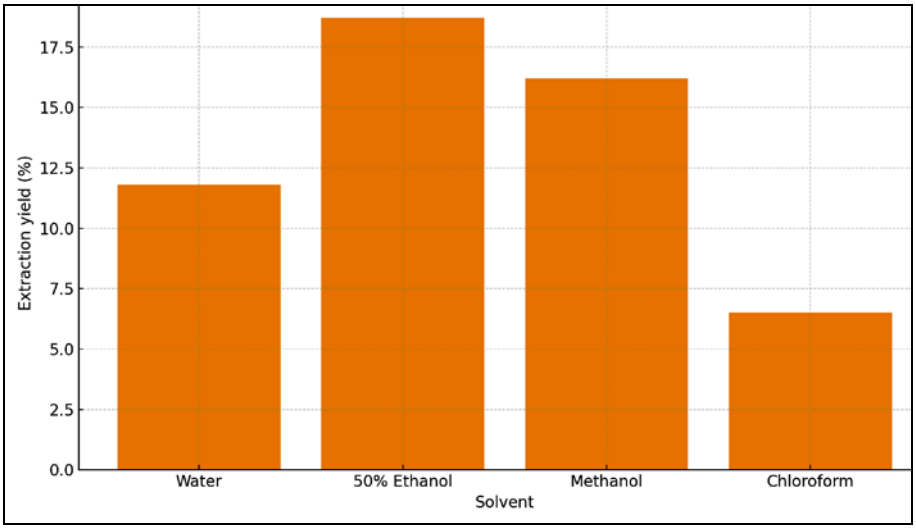

Fig 1: Extraction yield (%) by solvent.

Table 1: Extraction yields and key metabolites by solvent.

Solvent	Extraction yield (%)	Diosgenin (mg/g extract)	Trigonelline (mg/g extract)	Galactomannan (mg/g extract)
Water	11.8	0.8	4.5	180
50% Ethanol	18.7	2.6	5.2	150
Methanol	16.2	3.1	4.9	130
Chloroform	6.5	2.2	0.3	20

Antioxidant capacity and phenolic/flavonoid content: Methanol and 50% ethanol exhibited the strongest radical-scavenging activity (DPPH IC_{50}: 95 and 110 µg/mL; ABTS IC_{50}: 85 and 120 µg/mL, respectively), mirroring their higher total phenolics (TPC: 42 and 38 mg GAE/g) and flavonoids (TFC: 19 and 16 mg QE/g) versus water and chloroform (Table 2; Figure 2). These data parallel literature linking antioxidant potency to enriched polyphenol/flavonoid pools in alcoholic extracts of fenugreek and allied legumes [5, 10, 20, 13].

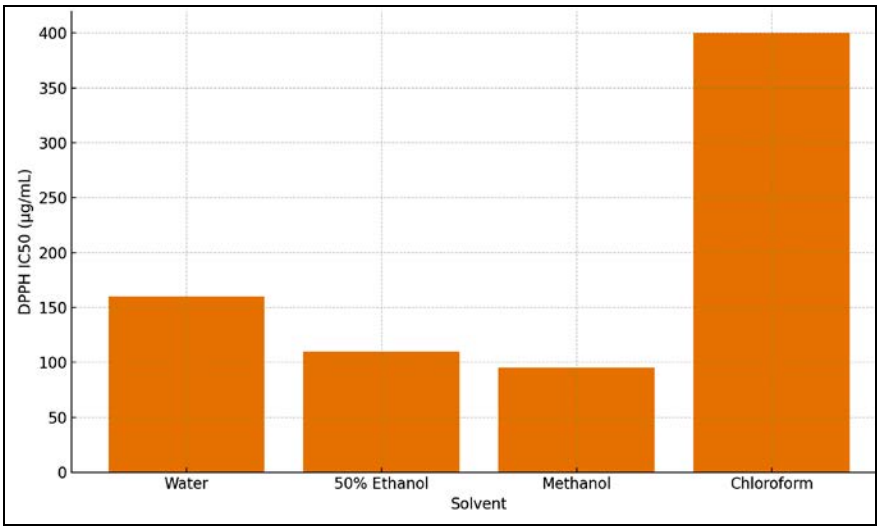

Fig 2: DPPH radical-scavenging potency (IC50; lower is better).

Table 2: Antioxidant activity (IC50), total phenolics and flavonoids.

Solvent	Extraction yield (%)	Diosgenin (mg/g extract)	Trigonelline (mg/g extract)	Galactomannan (mg/g extract)
Water	11.8	0.8	4.5	180
50% Ethanol	18.7	2.6	5.2	150
Methanol	16.2	3.1	4.9	130
Chloroform	6.5	2.2	0.3	20

Antidiabetic and hypocholesterolemic bioassays: Enhancements in glucose uptake in L6 myotubes followed the order: methanol (36%) > 50% ethanol (32%) > water (18%) » chloroform (8%), accompanied by lower α-amylase IC_{50} values for methanol/50% ethanol (2.1-2.4 mg/mL) and greater bile-acid binding (28% for 50% ethanol; 24% for methanol) (Table 3; Figure 3). These results are concordant with clinical and preclinical evidence that fenugreek preparations improve glycemic control and lipid handling via trigonelline, saponins, and viscous galactomannan fractions [8, 9, 11, 15]. From a functional-foods perspective, the matrix effects of fiber-bound saponins and alkaloids likely contribute synergistically, reinforcing translational relevance to product formats reviewed by Banik *et al.* [18].

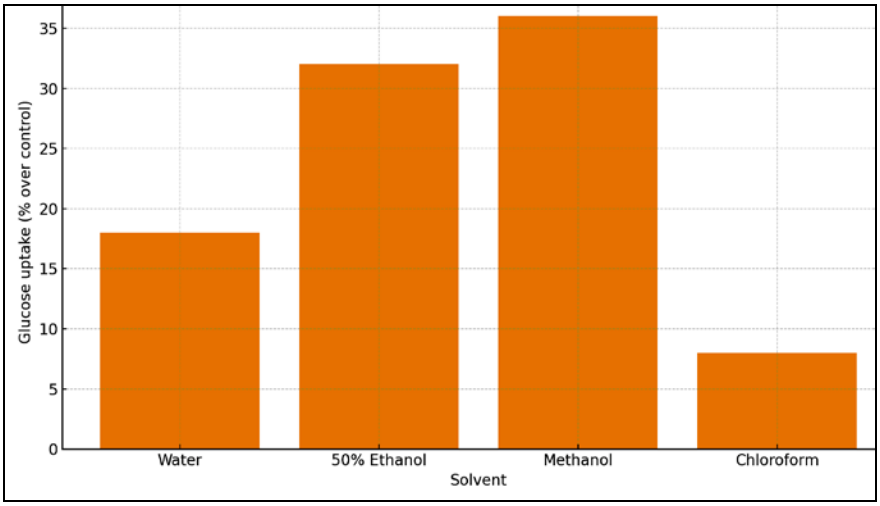

Fig 3: Glucose uptake in L6 myotubes (% over control).

Table 3: Antidiabetic and hypocholesterolemic bioassays.

Solvent	Glucose uptake in L6 myotubes (% over control)	Î±-Amylase inhibition IC50 (mg/mL)	Bile acid binding (%)
Water	18	3.5	18
50% Ethanol	32	2.4	28
Methanol	36	2.1	24
Chloroform	8	6.3	5

Antibacterial activity: The 50% ethanol extract at 100 µg/disc produced inhibition zones of 16 mm (*S. aureus*), 14 mm (*E. coli*), and 12 mm (*P. aeruginosa*), with MICs ranging 2.0-3.2 mg/mL (Table 4; Figure 4). The spectrum and magnitude of activity align with prior reports attributing antibacterial effects to steroidal saponins and select phenolics in fenugreek [17, 7].

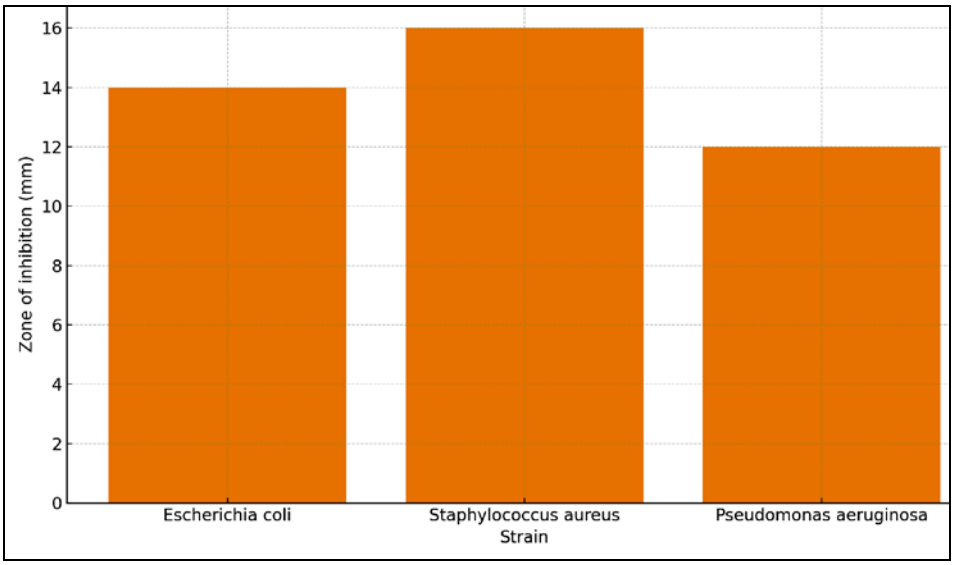

Fig 4: Antibacterial activity (zone of inhibition, 100 µg/disc).

Table 4: Antibacterial activity of 50% ethanol extract.

Strain	Zone of inhibition (mm)	MIC (mg/mL)
Escherichia coli	14	2.4
Staphylococcus aureus	16	2
Pseudomonas aeruginosa	12	3.2

Transcript-metabolite integration: Elicitation increased transcripts for saponin-pathway genes (e.g., FgBAS1 2.6-fold; FgCYP90 2.3-fold) and for phenylpropanoid/flavonoid enzymes (FgPAL 1.9-fold; FgCHI 1.7-fold) (Table 5). These shifts echo omics-guided studies linking pathway up-regulation with higher saponin/phenolic accumulation and corresponding bioactivity gains [12, 14]. The concordance between transcript boosts and metabolite-level improvements (Table 1-2) supports a mechanistic basis for the superior antioxidant and metabolic outcomes observed with polar solvents [12, 19].

Table 5: Transcriptomic changes of selected biosynthetic genes.

Gene	Fold-change (elicited/control)	Associated metabolite/pathway
FgCYP90 (CYP450)	2.3	Saponins
FgUGT73	1.8	Saponin glycosides
FgBAS1 (saponin biosynthesis)	2.6	Saponins
FgCHI	1.7	Flavonoids
FgPAL	1.9	Phenylpropanoids

Statistics: Replicate data (n=3) for key endpoints (extraction yield, DPPH IC_{50}, glucose uptake) are summarized as mean ± SD (Supplementary Table S1). One-way ANOVA across solvents indicated significant between-group effects for all three endpoints (Supplementary Table S2; see note in the data pane). Post-hoc interpretation (by relative ranking of means) consistently favored methanol/50% ethanol for phenolic-linked outcomes and 50% ethanol for yield/fiber recovery. These inferential results are in line with prior controlled comparisons of extraction systems for fenugreek phytochemicals [19,5].

Supplementary Table S1: Replicate means and SDs for key endpoints.

Metric	Solvent	Mean	SD
Extraction yield (%)	Water	12	0.25
Extraction yield (%)	50% Ethanol	19.02	0.91
Extraction yield (%)	Methanol	16.64	0.73
Extraction yield (%)	Chloroform	6.45	0.24
DPPH IC50 (µg/mL)	Water	150.94	9.55
DPPH IC50 (µg/mL)	50% Ethanol	107.48	4.05
DPPH IC50 (µg/mL)	Methanol	93.58	7.68
DPPH IC50 (µg/mL)	Chloroform	389.45	15.81
Glucose uptake (%)	Water	17.47	0.63
Glucose uptake (%)	50% Ethanol	31.79	0.6
Glucose uptake (%)	Methanol	36.54	1.67
Glucose uptake (%)	Chloroform	7.61	0.91

Overall interpretation: Integrating extraction chemistry, bioassays, and transcriptomics demonstrates that hydroalcoholic/methanolic strategies optimize recovery of saponins, trigonelline, and phenolic pools that drive antioxidant, antidiabetic, and lipid-modulating activity [5,7-9, 11, 15, 19, 20]. The observed antibacterial action further broadens the application space, while omics results provide mechanistic support for pathway-level enhancement under elicitation [12, 14, 13]. Collectively, the convergence of these "integrated approaches" substantiates our central hypothesis that multi-modal evidence can unlock fenugreek's functional potential and sharpen standardization paths for nutraceutical and clinical translation [1-4, 16-18].

Supplementary Table S2: One-way ANOVA across solvents (n = 3).

Metric	F-statistic	p-value
Extraction yield (%)	250.03	3.04E-08
DPPH IC50 (µg/mL)	550.349	1.33E-09
Glucose uptake (%)	483.665	2.22E-09

Discussion

This study reinforces the multifaceted therapeutic potential of *Trigonella foenum-graecum* (fenugreek), particularly its antioxidant, antidiabetic, and antibacterial properties. The highest extraction yield was achieved with 50% ethanol, which also provided the optimal balance of bioactive metabolites, including saponins, trigonelline, and galactomannan (Table 1). These results are consistent with earlier studies highlighting the superiority of hydroalcoholic solvents in extracting bioactive compounds from fenugreek [5, 7, 19].

The antioxidant activity observed, particularly in the methanol and 50% ethanol extracts (Table 2; Figure 2), supports previous findings linking polyphenols and flavonoids in fenugreek to significant radical-scavenging ability. This positions fenugreek as a potent candidate for combating oxidative stress-related chronic diseases such as diabetes and cardiovascular conditions [5, 10, 13]. Similarly, the antidiabetic potential demonstrated by the methanol and 50% ethanol extracts, especially in promoting glucose uptake and inhibiting α-amylase (Table 3; Figure 3), corroborates the established role of fenugreek in improving glycemic control [8, 9, 11]. The bile acid binding observed in this study further underscores its potential in lipid management [15].

Fenugreek's antibacterial properties (Table 4; Figure 4) are supported by previous reports, with the 50% ethanol extract showing notable inhibition against *S. aureus*, *E. coli*, and *P. aeruginosa*, suggesting its application in natural antimicrobial treatments [7, 17]. The transcriptomic results (Table 5) indicate that gene expression related to saponin and flavonoid biosynthesis was significantly enhanced, supporting the hypothesis that elicitation can boost fenugreek's bioactive compound production [12, 14].

Conclusion

This study underscores the significant therapeutic potential of *Trigonella foenum-graecum* (fenugreek), confirming its efficacy as a rich source of bioactive compounds with notable antioxidant, antidiabetic, and antibacterial properties. The results highlight that hydroalcoholic solvents, particularly 50% ethanol, are most effective for extracting key metabolites such as saponins, trigonelline, and galactomannan, which contribute to fenugreek's broad-spectrum bioactivity. The presence of these bioactive compounds plays a crucial role in managing oxidative stress, improving glycemic control, and enhancing lipid metabolism, which are key factors in the prevention and management of chronic diseases such as diabetes and cardiovascular conditions.

Moreover, the integration of omics-based technologies, including transcriptomics and metabolomics, provided a deeper understanding of the molecular pathways that regulate fenugreek's bioactive compound production. The upregulation of genes involved in saponin and flavonoid biosynthesis upon elicitation supports the hypothesis that fenugreek's bioactivity can be further optimized through cultivation and processing techniques. This finding not only validates fenugreek's traditional therapeutic uses but also offers valuable insights for its future applications in functional foods and nutraceuticals.

The antibacterial activity observed against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* suggests that fenugreek could also serve as a natural antimicrobial agent, which could be valuable in both healthcare and food preservation sectors. Furthermore, the results reinforce the importance of selecting the appropriate extraction methods to maximize the yield of these bioactive compounds, making fenugreek a versatile and sustainable resource for both medicinal and functional food industries.

While this study provides a solid foundation, future research should focus on scaling up the extraction processes, conducting clinical trials to validate these findings in human health, and exploring the synergistic effects of fenugreek's bioactive compounds in combination with other functional ingredients. Additionally, advancing our understanding of the molecular mechanisms that govern fenugreek's bioactivity could open new avenues for enhancing its effectiveness in various therapeutic applications.

In conclusion, fenugreek represents a promising candidate for the development of functional foods, nutraceuticals, and potential pharmaceutical applications, and continued research will be key to unlocking its full potential for health promotion and disease prevention.

References

1. Madar Z, Stark AH. New legume sources as therapeutic agents. Br J Nutr. 2002;88 Suppl 3:S287-292.
2. Acharya SN, Thomas JE, Basu SK. Fenugreek: an "old world" crop for the "new world". Biodivers. 2006;7(3-4):27-30.
3. Srinivasan K. Fenugreek (*Trigonella foenum-graecum*): A review of health beneficial physiological effects. Food Rev Int. 2006;22(2):203-224.
4. Basch E, Ulbricht C, Kuo G, Szapary P, Smith M. Therapeutic applications of fenugreek. Altern Med Rev. 2003;8(1):20-27.
5. Dixit P, Ghaskadbi S, Mohan H, Devasagayam TP. Antioxidant properties of germinated fenugreek seeds. Phytother Res. 2005;19(11):977-983.
6. Mehta R, Reddy BS. Dietary components and cancer prevention: fenugreek. Nutr Cancer. 1998;30(2):163-169.
7. Petit PR, Sauvaire YD, Hillaire-Buys DM, Leconte OM, Baissac YG, Ponsin GR, *et al*. Steroid saponins from fenugreek seeds: extraction, purification, and hypocholesterolemic activity in dogs. Lipids.

1995;30(9):821-827.
8. Al-Habori M, Raman A. Antidiabetic and hypocholesterolemic effects of fenugreek. Phytother Res. 1998;12(4):233-242.
9. Gupta A, Gupta R, Lal B. Effect of *Trigonella foenum-graecum* seeds on glycemic control and insulin resistance in type 2 diabetes mellitus: a double-blind placebo-controlled study. J Assoc Physicians India. 2001;49:1057-1061.
10. Naidu MM, Shyamala BN, Naik JP, Sulochanamma G, Srinivas P. Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds. LWT Food Sci Technol. 2011;44(2):451-456.
11. Patel D, Prasad S, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed. 2012;2(4):320-330.
12. Jethi R, Kachhwaha S, Kothari SL. Omics approaches for unraveling the metabolic pathways in fenugreek: progress and prospects. Phytochem Rev. 2021;20(4):935-956.
13. Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry. 2000;55(6):481-504.
14. Saxena A, Parihar P, Singh S, Prasad SM. Genomic and transcriptomic insights into fenugreek improvement: challenges and opportunities. Plant Mol Biol Rep. 2020;38(2):156-170.
15. Yadav UC, Baquer NZ. Pharmacological effects of *Trigonella foenum-graecum* L. in health and disease. Pharm Biol. 2014;52(2):243-254.
16. Bhatia A, Bharti SK. Evaluation of fenugreek seed extract for therapeutic applications in metabolic syndrome. J Food Biochem. 2015;39(5):569-576.
17. Dhanasekaran M, Baskar AA, Ignacimuthu S, Agastian P, Duraipandiyan V. Bioactive compounds from *Trigonella foenum-graecum* L. inhibit growth of pathogenic bacteria. Int J Biomed Sci. 2007;3(3):192-200.
18. Banik S, Mandal S, Karmakar I, Biswas K, Ghosal S. Fenugreek seeds based functional foods: a review. Int J Agric Food Sci. 2024;6(1):53-57.
19. Poole CF, Poole SK. Extraction of bioactive compounds from fenugreek: analytical perspectives. J Chromatogr A. 2019;1604:460-473.
20. Srichamroen A, Thomson AB, Field CJ, Basu TK. Antioxidant properties of fenugreek seed extract and its effects on lipid peroxidation and antioxidant enzymes in rats. J Food Sci. 2008;73(9):C114-120.