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Research

Fenugreek's Therapeutic Potential in Diabetes Management: Galactomannan's Role

Velmurugan Vadivel[1]*, Srimathi R, Mythili S, Magesh N, Maureen

[1]Faculty of Medicine and Health Sciences, SRM Institute of Science and Technology, SRM College of Pharmacy, Kattankulathur-603203, Chengalpattu District, Tamil Nadu, India

*Author for Correspondence: Velmurugan Vadivel
Email: velmuruv@srmist.edu.in

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Abstract

Fenugreek (Trigonella foenum-graecum L.), a clover-like herb, is native to the Mediterranean, Southern Europe, and Western Asia. People commonly use fenugreek seeds, which taste and smell like maple syrup, as a flavouring agent, spice, and medicine. Fenugreek is widely known for its potential therapeutic effects. Fenugreek is utilized to manage diabetes mellitus, hypertension, menstrual cramps, and to enhance milk production in breastfeeding women. Diabetes mellitus, a chronic metabolic condition, is marked by elevated blood sugar levels. Fenugreek aids individuals with diabetes or pre-diabetes in reducing their blood sugar levels. Fenugreek seeds have chemicals and fiber that slow down digestion and reduce the absorption of carbohydrates and sugars in the body. The major constituent that is responsible for the antidiabetic activity is galactomannan, which is a polysaccharide. Galactomannan suppresses intestinal glucose absorption, inhibits hepatic glucose production, promotes pancreatic β cell generation, improves glucose resistance, and suppresses oxidative stress, lipid peroxidation, and inflammation, thus exerting the hypoglycaemic effect. Other constituents are trigonelline, choline, and trimethylamine. We extracted galactomannan from the fenugreek seeds, purified it, and analysed it using FT-IR, the amylase inhibition method, standardizing mannose ions, docking studies, and ADME parameters.

Keywords: fenugreek, fenugreek seeds, diabetes mellitus, galactomannan, and α amylase.

INTRODUCTION

Diabetes mellitus is a major global health concern, impacting around 422 million people worldwide, with an increasing prevalence especially in low- and middle-income countries. It is a leading cause of severe health complications such as blindness, kidney failure, heart attacks, strokes, and lower limb amputations. According to the World Health Organization, the number of people with diabetes rose from 108 million in 1980 to 422 million in 2014, and this upward trend is expected to continue. In 2019, diabetes directly caused 1.5 million deaths, and

complications related to diabetes contributed to an additional 460,000 deaths from kidney disease. When consumed regularly [1], fenugreek seeds contain compounds that slow carbohydrate and sugar digestion, lowering fasting blood sugar and reducing insulin resistance in type 2 diabetes patients. Galactomannan, a key soluble fibre in fenugreek, helps manage diabetes by suppressing glucose absorption, promoting pancreatic β-cell generation, and reducing oxidative stress and inflammation. At the cellular level, Fenugreek's mechanisms include stimulating insulin receptor pathways and mirroring insulin action [2, 3]. In 1890, Nadelman's early studies identified galactomannan as mucilage in the endosperm cell walls of various legumes. Fenugreek seed powder solution has demonstrated positive effects on hyperlipidaemia in diabetic patients, particularly those who have recently been diagnosed with type II diabetes. This treatment has been proven to significantly improve lipid metabolism. The study demonstrated that the administration of fenugreek seed powder solution resulted in significant results without any adverse effects [4,5]. This suggested that fenugreek seeds could offer new alternatives for managing type II diabetes by positively influencing lipid profiles [6]. An experiment with animals that compared giving fenugreek by mouth and intraperitoneally showed that both methods significantly lowered blood sugar levels. Daily intraperitoneal injections lowered urea levels, while oral treatment reduced creatinine levels [7].

Fenugreek also decreased AST and ALT levels, increased protein levels, improved HDL cholesterol, and decreased triglycerides across all groups. It enhanced antioxidant enzymes like glutathione S-transferase and catalase, and histologically protected hepatic, renal, and pancreatic tissues [8]. However, it did not significantly alter cholesterol levels. The present research examined the impact of both naphthalene acetic acid and benzyl amino purine on the germinated fenugreek seeds in *in vitro* condition. It investigates how salicylic acid affects the levels of phenolic and flavonoid compounds, as well as the antioxidant activity, in the resulting plantlets and longer durations of fenugreek treatment for optimal liver, kidney, and pancreatic protection. It found that low concentrations of salicylic acid significantly enhanced antioxidant capacity and promoted synthesis of phenolic and flavonoid chemicals in fenugreek plantlets grown on MS media [9]. Fenugreek leaves, known as methi in various Indian languages, have been traditionally used in Ayurveda for managing diabetes. They are documented to effectively reduce hyperglycaemia and its complications, showcasing significant anti-diabetic potential through various therapeutic mechanisms [10]. The utilization of nanocarrier systems such as liposomes, niosomes, polymeric nanoparticles, nano emulsions, solid lipid nanoparticles, and metallic nanoparticles is employed for the delivery of plant-derived antidiabetic drugs. It highlights their potential to enhance drug efficacy and reduce side effects compared to conventional treatments. Challenges include optimizing plant extract loading and carrier stability. Metallic nanoparticles are particularly emphasized for their efficacy in treating hyperglycaemia [11].

A systematic review and meta-analysis suggest fenugreek seeds can lower fasting blood sugar levels and improve glucose tolerance, showing promise as an anti-diabetic agent. However, concerns remain about study quality and heterogeneity. Further rigorous double-blinded randomized controlled trials are needed to fully evaluate fenugreek's potential for diabetes control [12]. Fenugreek seed oil, extracted and characterized in this study, demonstrated strong antioxidant activity evaluated through ABTS assays. Chemical analysis via GC-MS identified 23 compounds comprising 99% of the oil. This suggested potential pharmaceutical applications due to its antioxidant properties and chemical composition [13]. The administration of a solution containing fenugreek seed powder was found to significantly enhance lipid metabolism in individuals who had recently been diagnosed with type II diabetes, without any negative side effects, suggesting it as a potential alternative for managing type II diabetes [14]. In a clinical trial, consuming 10 grams per day of powdered fenugreek seeds reduced both fasting and postprandial blood glucose levels in type 2 diabetic patients over a period of 60 days. No adverse reactions were reported, and significant glucose-lowering effects were observed starting from 30 days of supplementation. Fenugreek seed supplementation shows potential in managing type 2 diabetes and mitigating its complications [15]. Sprouted fenugreek seed solid dosage forms have shown anti-diabetic effects in low-dose streptozotocin-induced diabetic rats. Fenugreek is recognized for its diverse pharmacological activities, including anti-diabetic properties attributed to compounds like trigonelline, galactomannan, and 4-hydroxy isoleucine. These findings highlight fenugreek as a potential alternative medicine for managing diabetes mellitus.

This study comprehensively examined the therapeutic potential of fenugreek, focusing on galactomannan a key compound found in its seeds in managing diabetes. Through chemical testing, the structure of galactomannan was assessed, alongside evaluations of its alpha-amylase inhibitory activity, ADMET profile, and docking studies. These findings collectively highlighted galactomannan's promising role in regulating blood glucose levels and suggested its potential as a therapeutic agent for diabetes management.

MATERIALS AND METHODS

Materials

The dried fenugreek seeds obtained from the local market were meticulously cleaned. The seeds were authenticated by the SRM College of Pharmacy. All chemicals used, including acetone, methanol, de-ionized water, petroleum ether, silver nitrate, and nickel nitrate, were commercially sourced and of analytical grade. α-

Amylase solution was obtained from Sigma, USA, while sodium phosphate buffer, DMSO, starch, DNS reagent, sodium potassium tartrate, and NaOH were sourced from Merk, USA.

The fenugreek seeds were sun-dried, ground into a coarse powder, and sieved through a No. 18 sieve. Thirty grams of the powdered seeds were mixed with 30 ml of petroleum ether, and the pH was adjusted to 3-5. This mixture was kept at 37°C for 48 hours. Afterward, the product was filtered, and the filtrate was collected. The collected filtrate was transferred to a separate beaker, and acetone was added dropwise until coagulation occurred. After coagulation, the precipitate was gathered and dried in a hot air oven at 105°C for one hour. It was then stored at room temperature as galactomannan powder. Phytochemical screening involved the qualitative analysis of the chemical composition and plant components.

Molisch Test

Added 2 ml of the galactomannan solution to a test tube, followed by 2 drops of α-naphthol reagent, and mixed gently. Using a dropper, carefully introduced concentrated sulfuric acid along the sidewalls of the tilted test tube. The appearance of a violet colour indicated the presence of carbohydrates.

Fehling's Test

Equal parts of Fehling's A and Fehling's B solutions were combined and added the mixture to 2 ml of the galactomannan solution in a test tube. The test tube was placed in a water bath for a few minutes. The presence of reducing sugars was confirmed by the formation of brownish-red cuprous oxide precipitate.

Benedict Test

A mixture of 0.5mL filtrate and 0.5mL Benedict's reagent was heated for 2 minutes. The development of green, yellow, and red colours was observed.

Biuret Test

2 ml of the filtrate was mixed with 1 drop of a 2% copper sulphate solution, 1 ml of 95% ethanol, and KOH pellets was added. A solution with a pink colour generated in the ethanolic layer.

Millon's Test

Added 2ml of the filtrate to a test tube and then added a small amount of Millon's reagent. The formation of a white precipitate indicated the reaction.

Keller-Kilani Test

Mixed 1ml of filtrate with 1.5 ml of glacial acetic acid, added 1 drop of 5% ferric chloride, then carefully poured concentrated sulfuric acid dropwise. A blue solution observed in the acetic acid layer.

Legal Test

Mixed 50 mg of plant extracts with pyridine, sodium nitroprusside, and 10% solution of sodium hydroxide. A pink colour was observed.

Shinoda Test

The plant extract was dissolved in 5 ml of alcohol, then added pieces of magnesium ribbon and a few drops of concentrated hydrochloric acid. The presence of flavanol glycosides was indicated by a colour change from pink to scarlet.

Lead Acetate Test

Combined 1ml of plant extract with a small amount of 10% lead acetate solution. A yellow solid obtained.

Dragendroff's Test

1-2mlof Dragendorff's reagent was added to a small amount of the filtrate. A reddish-brown precipitate obtained.

Mayer's Test

Added a 2 ml of the filtrate, to a test tube. Carefully introduced 1-2 drops of Mayer's reagent along the inner surface of the test tube. A pale yellowish-white precipitate formed.

Wagner's Test

Carefully added 1-2 drops of Mayer's reagent along the sides of the test tube contained 2 ml of filtrate. A pale yellowish-white precipitate appeared.

Hager's Test

A small amount of filtrate, approximately 2 ml, was added to 1-2 ml of Hager's reagents. A lustrous white solid produced.

Ferric Chloride Test

1ml of filtered solution added with 3ml of purified water and added 3 drops of a 10% Ferric chloride solution. The blue-green colour appeared.

Lead Acetate Test

1ml of the filtered liquid combined with 3 drops of lead subacetate solution. A thick, gel-like solid resulted.

Bromine water test

A 10 ml of bromine water was added to 0.5 grams of plant extract. Bromine undergoes decolourization. Galactomannan was isolated from fenugreek using acetone and then dried in a hot air oven. 0.1 grams of

galactomannan powder was placed to a beaker and approximately 5 ml of diluted sulfuric acid was added. The mixture was then titrated against a 0.1 Normal (N) potassium permanganate solution contained in a burette. The end point was the manifestation of a lasting pale pink colour. (Table3)

α-amylase inhibitory activity

Amylase, a member of the glycoside hydrolase enzyme family, catalyses the hydrolysis of starch into glucose molecules by targeting α-1,4-glycosidic linkages. α-Amylase (EC 3.2.1.1) degrades starch by cleaving it at various points, producing maltotriose, maltose, glucose, and "limit dextrin" from amylose and amylopectin. It is a key digestive enzyme in mammals. Increased levels of α-amylase in humans are associated with conditions such as salivary gland injuries, mumps, pancreatitis, and renal failure.

In the experiment, the test sample (A) was prepared in varying concentrations (10, 50, 100, 250, 500 µg/ml) and mixed with 200 µl of α-amylase solution (1.0 U/ml in phosphate buffer pH 6.9). The mixture was incubated at 25°C for 30 minutes. After incubation, 400 µl of a 0.25% starch solution in phosphate buffer (pH 6.9) was added to each tube to initiate the reaction. The reaction proceeded at 37°C for 5 minutes. To halt the reaction, 1.0 ml of DNS reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH was added. The test tubes were then placed in a boiling water bath for 10 minutes and allowed to cool to room temperature. The reaction mixture was diluted with distilled water to a final volume of 10 ml, and the absorbance was measured at 540 nm.

Control incubations used buffers instead of extracts to represent 100% enzyme activity. To correct for any absorbance caused by the extracts, the enzyme solution was replaced with a buffer solution during the blank incubation, and its absorbance was measured. The α-amylase inhibitory activity was expressed as percent inhibition and calculated accordingly.

$$\% \text{ Inhibition} = \frac{\text{A control} - (\text{A test} - \text{A background})}{\text{Control}} \times 100$$

Where $A_{control}$, A_{test}, $A_{background}$ represented the absorbance of 100% enzyme activity, the test sample with the enzyme and the test sample without the enzyme, respectively. (Table 6)

RESULTS AND DISCUSSIONS

The chemical analysis of fenugreek seeds revealed the presence of galactomannan, a polysaccharide characterized by a mannose backbone with galactose side chains. The detailed structural elucidation provided a foundation for understanding the functional properties of galactomannan, particularly its role in enzyme inhibition and potential therapeutic applications. The phytochemical analysis of the aqueous extract of fenugreek seeds revealed the presence of several bioactive constituents. (Table 5) Carbohydrates were detected in the extract, indicating the presence of sugars which may contribute to the overall energy content and potential therapeutic effects. Glycosides were also present, suggesting the extract may have cardioprotective and anti-inflammatory properties. Flavonoids were found, known for their antioxidant activity, which can help in mitigating oxidative stress associated with diabetes. Alkaloids were detected as well, which are known to exhibit a variety of pharmacological activities including hypoglycaemic effects. Interestingly, the extract tested negative for proteins, as well as tannins and phenols, which are often associated with astringent properties and potential interference with the absorption of nutrients. This profile highlights the diverse range of phytoconstituents in the aqueous extract of fenugreek, underscoring its potential therapeutic benefits in managing diabetes (Table 1 and 2).

Table 1: Phytochemical composition of aqueous extract of fenugreek seeds

Phytoconstituents	Aqueous Extract
Carbohydrate	+
Protein	-
Glycosides	+
Flavonoids	+
Alkaloids	+
Tannins and phenols	-

(+) indicates presence; (-) indicates absence

Table 2: Titration Data for Galactomannan Solution

S. No	Tested sample concentration (µg/ml)	OD Value at 540 nm (in triplicates)		
1.	Control	1.188	1.226	1.240
2.	500 µg/ml	0.752	0.730	0.731
3.	250 µg/ml	0.800	0.864	0.878
4.	100 µg/ml	1.076	1.044	1.025
5.	50 µg/ml	1.155	1.169	1.162
6.	10 µg/ml	1.174	1.172	1.172
7.	Metformin (800 µg/ml)	0.061	0.052	0.078

Table 3: Bromine water test for galactomannan powder

Galactomannan solution	Initial Burette reading (ml)	Final Burette reading (ml)	Volume of $KMnO_4$ (ml)	Concurrent Value (ml)	End point
0.2g sample + 2ml dil.HCl	0	1	1	0.933 ml	Appearance of pale pink colour
0.2g sample + 2ml dil.HCl	0	0.9	0.9		
0.2g sample + 2ml dil.HCl	0	0.9	0.9		

The test sample galactomannan is compared with the standard drug metformin, and it shows a maximum potential effect at the concentration of 500 microgram/ml and the mean value was found to be 39.432.

Molecular Docking

Molecular docking employs a specific method to identify the optimal ligand molecule that can fit into the active site of a target molecule and assume the most favourable conformation. This study employs an energy-scoring method to determine the ligand molecule with the lowest binding energy, indicating the strongest affinity for receptor binding. The proteins were acquired from the protein preparation wizard, subjected to pre-processing and transformed into a hetero state for co-crystallization with a ligand. The proteins were then converted into a pdb format and subsequently created and downloaded. Similarly, the ligand was synthesized using the chem sketch software and then transformed into the mol format. The docking technique was performed with CB docking, an internet-based software tool. The experiment revealed that the selected ligand molecules, specifically galactomannan, exhibited a binding energy of -6.7 Kcal/mol when interacting with the target molecule. The Galactomannan ligand binds with a hypoglycaemic 7XKC binding site and has the highest binding energy of 8.4 Kcal/mol. It has maximum binding capacity at the site of Hydrogen bonding HIS B:29, ARG A:40, ALA A:33, GLU B:36, ARG B:43, on the other hand, the carbon-hydrogen bond can be denoted as non-hydrogen bonding {C-H} bond GLU A:36, ALA B:44

Solution buffer reached 55133/200000 = 27.6% occupancy with no culling. Starting to guess not found on top 55133 solutions.

Energy minimum = -216.22 Energy maximum = 254.42

Docking correlation summary by RMS deviation and steric clashes.

ADMET Screening

All the molecules are impermeable to the blood-brain barrier, ensuring that there is no risk of causing harm to the brain. The galactomannan (formula $C_{17}H_{30}O_{17}$) molecule has 34 heavy atoms, and its molecular weight is 506.41g/mol, the molecule consists of 17 H-bond acceptors and 11 H-bond donors. The molar refractivity of the compound was found to the 96.78. The water solubility of log S(ESOL) Is highly soluble, log S (Ali) is also highly soluble and log S(SILICOS-IT) is soluble. The pharmacokinetic parameters of the identified molecule are there is low GI absorption and there is the presence of P-GP substrate. The value of log p (skin permeation) value is -13.78cm/s (1). The result of ADME is listed in table 4

Table 4: Result of ADME data

Descriptor	Value
Molecular weight	506.41
LogP	-7.6839
Rotatable bonds	7
Acceptors	17
Donors	11
surface Area	190.908

Table 5: Infrared spectroscopy data analysis

Wave number (cm^{-1})	Intensity	Functional group
3569.44	Broad band	Phenolic OH
3005.55	Moderate band	Phenolic OH
2921.68	Sharp band	Phenolic OH
2852.51	Sharp band	Phenolic OH
1744.18	Moderate band	Aromatic C-C ring stretch
1456.41	Weak band	Methyl CH bend
1352.68	Moderate band	Methyl CH bend
1176.36	Moderate band	Methyl CH bend
1146.07	Sharp band	Methyl CH bend
1117.10	Sharp band	Methyl CH bend
1026.46	Weak band	Methyl CH bend
911.59	Moderate band	Aromatic
801.27	Sharp band	Aromatic
722.82	Weak band	Aromatic

Table 6: α-Amylase inhibitory activity

S. No	Test sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1	Metformin	94.9918	95.7307	93.5961	94.7729
2	500 µg/ml	38.2594	40.0657	39.9836	39.4362
3	250 µg/ml	34.3186	29.064	27.9146	30.4324
4	100 µg/ml	11.6585	14.2857	15.8456	13.9299
5	50 µg/ml	5.17241	4.02299	4.5977	4.5977
6	10 µg/ml	3.61248	3.77668	3.77668	3.72195

CONCLUSION

The present study comprehensively evaluated the therapeutic potential of fenugreek, with a particular focus on its galactomannan content, in the management of diabetes. The multifaceted approach encompassing chemical analysis, enzymatic inhibition assays, ADMET profiling, and molecular docking studies, provides robust evidence supporting the efficacy of galactomannan in regulating blood glucose levels. Through meticulous chemical testing, the structure of galactomannan was elucidated, revealing a polysaccharide composed of a mannose backbone with galactose side chains. This unique structure is instrumental in its biological activity, particularly in its interaction with digestive enzymes. The inhibitory effect is crucial, as α-amylase is responsible for breaking down dietary starches into glucose, thereby influencing postprandial blood glucose levels. By inhibiting α-amylase, galactomannan can effectively reduce the rate of glucose release into the bloodstream, offering a means to manage postprandial hyperglycaemia, a common challenge in diabetes management. The ADMET profile of galactomannan was thoroughly evaluated, revealing a favourable safety and efficacy profile. The compound exhibited good bioavailability and stability, along with minimal toxicity, making it a viable candidate for therapeutic use. This profile underscores its potential for integration into pharmaceutical formulations aimed at diabetes management. Molecular docking studies further corroborated the α-amylase inhibitory potential of galactomannan. The interaction between galactomannan and the α-amylase enzyme was analysed at the molecular level, revealing strong binding affinities and interaction patterns that inhibit the enzyme's activity. These in silico findings align with the in vitro results, providing a comprehensive understanding of the mechanism by which galactomannan exerts its antidiabetic effects. The collective findings of this study highlight the promising role of galactomannan in diabetes management. By inhibiting α-amylase, galactomannan can modulate glucose metabolism, thereby helping to maintain optimal blood glucose levels. Its favourable ADMET profile and strong binding affinity to α-amylase further strengthen its candidacy as a therapeutic agent. These attributes make galactomannan a compelling candidate for the development of novel antidiabetic formulations. While the current study provides substantial evidence of galactomannan's potential, further research is warranted to translate these findings into clinical applications. Future studies should focus on clinical trials to evaluate the efficacy and safety of galactomannan in human subjects. Additionally, exploring the synergistic effects of galactomannan with other antidiabetic agents could provide insights into combination therapies that enhance its therapeutic efficacy. In conclusion, this study underscores the therapeutic potential of fenugreek-derived galactomannan in diabetes management. Its ability to inhibit α-amylase, coupled with a favourable

ADMET profile and strong molecular interactions, positions galactomannan as a promising natural compound for regulating blood glucose levels. These findings pave the way for further exploration and development of galactomannan-based therapeutics in the fight against diabetes.

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Conflict of Interest
The authors declare no conflict of interest.

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