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Research

Isolation and Characterization of Fenugreek Seed Mucilage, A Natural Mucoadhesive Polymer.

Jooly Kurien*, Rakesh Kumar Jat, Boby Johns G

Department of Pharmacy, JJTU University, Vidyanagari, Churu Jhunjhunu Road, Chudela, Jhunjhunu, Rajasthan, India.

Author for correspondence: Jooly Kurien
Email: joolykurien@gmail.com

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Abstract

This study focuses on isolating and characterising fenugreek seed mucilage, a natural mucoadhesive polymer derived from Trigonella foenum-graecum. The extraction process of mucilage, which involved using deionized water followed by precipitation with acetone, demonstrated high efficiency, yielding 31.78% mucilage from the seeds. Characterization of the mucilage confirmed the presence of carbohydrates and mucilaginous properties, with an ash value indicating high purity. The mucilage displayed favourable micromeritic properties, such as good flowability and low moisture content, which are essential for pharmaceutical applications. Its pH and swelling index suggest compatibility with gastrointestinal environments. Comparative analysis revealed that fenugreek seed mucilage has mucoadhesive strength similar to Carbopol 934 P and significantly higher than sodium alginate, making it a promising candidate for use in drug delivery systems.

Keywords: Fenugreek seed mucilage, Trigonella foenum-graecum, natural mucoadhesive polymer, pharmaceutical excipient, drug delivery.

INTRODUCTION

For centuries, natural materials have played a crucial role in medicine and pharmaceuticals. Today, there is a growing global interest in natural drugs and excipients due to their non-toxic, biocompatible, cost-effective, biodegradable, and widely available properties. These materials can be customized for specific drug delivery systems, making them competitive with synthetic alternatives [1,2,4,5].

Plant mucilages have emerged as promising excipients for drug delivery systems in recent years. Mucilages, which are slimy aqueous dispersions produced by plants, animals, and microbes, primarily consist of water-soluble polysaccharides. Plant polysaccharides are complex carbohydrates that play essential roles in plant structure and metabolism, including energy storage and cell wall integrity. Mucilages are generally normal products of metabolism, formed within the cell or produced without plant injury. These mucilages are being explored as potential excipients in various pharmaceutical formulations, including tablets, capsules, nanoparticles,

microparticles, beads, spheroids, pellets, creams, gels, emulsions, suspensions, pastes, transdermal patches, and buccal patches [1,2,3,4,5,15].

A prominent example of a natural drug delivery excipient is fenugreek seed mucilage, extracted from Trigonella foenum-graecum seeds (Family Leguminosae). Fenugreek is one of the oldest cultivated medicinal plants and grows to a height of about 60 cm. This annual legume is cultivated in regions such as northern Africa, Canada, western Asia, and India [8,9,15].

Traditionally, fenugreek seeds, also known as Methi, are used both as a spice in culinary applications and for treating diabetes. In traditional Indian medicine, fenugreek seeds are valued for their hypoglycemic, antioxidant, laxative, gastric ulcer, and anti-inflammatory properties.[11] Fenugreek seed mucilage has demonstrated considerable potential as a drug delivery excipient. Its applications include functioning as an oral drug release retardant, mucoadhesive, emulsifier, suspending agent, gelling agent, binder, granulating agent, disintegrant, and matrix-former.[10] Additionally, it plays a role in the formulation of nanoparticles. The impressive functional and medicinal properties of fenugreek are linked to its diverse chemical composition. It contains approximately 20-25% protein, 45-50% dietary fiber, 20-25% mucilaginous soluble fiber, 6-8% fixed fatty acids and essential oils, and 2-5% steroidal saponins. In addition, fenugreek includes minor constituents such as alkaloids (including trigonelline, choline, gentianine, and carpaine), the uncommon amino acid 4-hydroxyisoleucine, and individual spirostanols and furostanols like diosgenin, gitogenin, and yamogenin, all of which contribute to its various biological effects [3.6,12,16].

Fenugreek seed mucilage is classified as a galactomannan and is composed of a (1 → 4) β-D-mannan backbone. This backbone is linked with α-D-galactopyranosyl groups at the O-6 position of the D-mannopyranosyl units, resulting in a ratio of D-galactose to D-mannose of either 1:1 or 1:1.2. Additionally, the mucilage contains minor quantities of sugars other than mannose and galactose[12,16] .

MATERIALS AND METHODS

Fenugreek seeds (Trigonella foenum-graecum L.) were procured from local markets in Kochi, Kerala, India. The sample was verified to match the characteristics of Trigonella Foenum-Graecum Linn, a member of the Leguminosae family and the Papilionatae subfamily, according to Bentham and Hooker's classification. The authentication was conducted by the Head of the Department of Botany and the Centre for Research at St. Teresa's College in Ernakulam, Kerala. All solvents, reagents, and chemicals used were of pharmacopoeial or analytical grad**e.**

Isolation of fenugreek seed mucilage

Fenugreek seeds were cleaned with deionized water, dried in an oven at 40°C for 30 minutes, and ground into a coarse powder. One hundred grams of the powder were soaked in 1 liter of deionized water for 10 hours, boiled for 1 hour, cooled, refrigerated overnight, and then decanted to remove residue. The remaining slurry was heated to one-fourth its original volume, filtered through muslin cloth, and mixed with acetone (1-part filtrate to 4 parts acetone) to precipitate the mucilage. The precipitate was collected, rinsed with acetone, air-dried for a day, finely ground, and stored in desiccators [2].

Fig 1: Crude fenugreek

Fig 2: Isolated fenugreek seed mucilage

Characterisation of isolated fenugreek seed mucilage

Organoleptic Evaluation

The fenugreek mucilage was assessed for organoleptic properties through olfactory evaluation of aroma, visual inspection for colour, taste testing, and texture analysis. Fracture behaviour was observed to understand its mechanical properties, aiding in its potential use as a pharmaceutical excipient[12,15].

Phytochemicals screening tests

Phytoconstituents screening tests were performed to confirm the identity of the isolated fenugreek mucilage[12]. The tests performed were listed below.

Molisch's Test: Carbohydrates in fenugreek mucilage were detected by adding Molisch's Reagent and sulfuric acid, resulting in a purple ring at the interface if positive.

Barfoed's Test: Monosaccharides were identified by mixing the mucilage with Barfoed's Reagent and boiling, which produced a red precipitate if present.

Ruthenium Red Test: The presence of mucilage was confirmed by observing a pink colour under a microscope after mixing the mucilage with ruthenium red.

Iodine Test: Starch was detected by adding iodine reagent to the mucilage solution and noting a bluish-black colour if starch was present.

Keller-Killiani Test: Glycosides were identified by adding acetic acid and ferric chloride to the mucilage, then sulfuric acid, which produced a brown ring at the interface.

Dragendorff's Test: Alkaloids were detected by adding Dragendorff's reagent to the mucilage, resulting in an orange or red precipitate if alkaloids were present.

Liebermann-Burchard Test: Steroids and sterols were identified by mixing the mucilage in chloroform with acetic anhydride and sulfuric acid, observing a colour change from red to bluish-green.

Ferric Chloride Test: Tannins were detected by adding ferric chloride to the mucilage solution, producing a bluish-black, green, or blue-green precipitate if present.

Ninhydrin Test: Proteins and amino acids were identified by adding ninhydrin reagent to the mucilage solution, resulting in a deep blue or purple colour if positive.

Shinoda Test: Flavonoids were detected by adding magnesium metal and hydrochloric acid to the mucilage solution, resulting in a reddish colour if flavonoids were present.

Determination of percentage yield [5]

The percentage yield measures the efficiency of mucilage extraction from fenugreek seeds by comparing the actual amount of isolated mucilage to the theoretical maximum. In this study, the yield was calculated by dividing the weight of the dried mucilage by the weight of the crude seeds and multiplying by 100.

Determination of Moisture content [9]

The moisture content percentage reflects the water content of the fenugreek mucilage relative to its total weight. To determine this, one gram of mucilage was dried in an oven at 105°C until a constant mass was achieved. The final mass was recorded, and the moisture content was calculated by subtracting the weight of the dried mucilage from the weight of the mucilage before drying, dividing this difference by the weight of the mucilage before drying, and then multiplying by 100.

pH of the Mucilage

The pH of the mucilage is a crucial factor in determining its suitability as a mucoadhesive polymer for use in drug delivery systems and gastrointestinal therapies. Understanding the pH of fenugreek mucilage ensures its compatibility with the body's natural pH environment, which is vital for effective mucoadhesion and functionality. In this study, the pH of a 1% w/v fenugreek seed mucilage solution in deionized water was measured using a digital pH meter (Labtronics LT-50)[5,9].

Determination of Solubility

Solubility testing is essential for determining the range of solvents in which dried fenugreek mucilage can dissolve. In this study, one part of the dried mucilage was assessed for solubility in several solvents, including cold and hot distilled water, ethanol, methanol, acetone, ether, benzene, chloroform, and dimethyl sulfoxide[2,12].

Determination of Viscosity

Viscosity measurements are crucial for evaluating the rheological behaviour of mucoadhesive polymers like fenugreek seed mucilage, as it influences their performance in drug delivery systems. The viscosity affects how the polymer swells, gels, and interacts within the gastrointestinal tract, impacting drug release, diffusion, and retention. Solution of fenugreek seed mucilage at concentrations of 1% was tested for viscosity using a Brookfield

Viscometer (Model-LV DD-E, spindle number 62) at 37±1°C and 100 rpm to determine their suitability as mucoadhesive agents[5,9].

Determination of Swelling Index

The swelling index is essential for mucoadhesive polymers, as it affects their water absorption, expansion, and adhesion to mucosal surfaces, crucial for effective drug release. For fenugreek mucilage, the swelling index was determined by mixing 1 gram of dried mucilage with 25 millilitres of distilled water in a 50 ml measuring cylinder, agitating for 1 hour, and allowing it to rest for 24 hours at room temperature. The volume of the swollen mucilage was then measured, with the average of three measurements calculated to ensure accuracy[14].

Estimation of Ash Value

The estimation of ash values is crucial for assessing the purity and quality of fenugreek seed mucilage.

Total Ash: Four grams of fenugreek mucilage powder were heated in a pre-weighed silica crucible until white, indicating complete combustion. After cooling and reweighing, the total ash content was calculated and expressed in milligrams per kilogram of air-dried mucilage.

Acid-Insoluble Ash: The total ash was treated with dilute hydrochloric acid to dissolve organic matter, leaving behind non-combustible impurities. The remaining insoluble ash was filtered, washed, and weighed to determine the percentage of acid-insoluble ash.

Water-Soluble Ash: The total ash was boiled with distilled water to dissolve soluble salts, leaving an insoluble residue. The weight of this residue was used to calculate the percentage of water-soluble ash, reflecting soluble mineral content and potential contaminants[5,9.]

Micromeritic Characteristics

Micromeritic properties are essential for evaluating the suitability of fenugreek seed mucilage as an excipient in pharmaceutical formulations [9.].Key parameters include:

Bulk Density and Tapped Density: Bulk density is determined by pouring the powder into a graduated cylinder, while tapped density is measured after tapping the cylinder until the volume stabilizes. The densities are calculated as:

Bulk Density = Mass of mucilage powder / Bulk Volume

Tapped Density = Mass of mucilage powder / Tapped Volume

Carr's Index: This index indicates compressibility and flow properties, calculated as:

Carr's Index = (Tapped Density - Bulk Density) / Tapped Density

Hausner Ratio: This ratio, derived from bulk and tapped densities, reflects flowability:

Hausner Ratio = Tapped Density / Bulk Density

Porosity: Reflects the void spaces within the powder and is calculated as:

Percentage Porosity = (1 - (Bulk Density / Tapped Density)) × 100

The angle of Repose: Determines flow characteristics based on the angle formed by the powder pile's surface, with values below 25° indicating excellent flow properties.

These parameters collectively assess the powder's packing, flow behaviour, and suitability for use in pharmaceutical formulations.

Determination of Mucoadhesion strength

Mucoadhesion tests are critical for evaluating the effectiveness of mucoadhesive mucilage in maintaining prolonged contact with mucosal surfaces, which enhances drug delivery. The mucoadhesive strength of fenugreek seed mucilage was assessed using an ex-vivo method, where mucilage was applied between two glass slides with goat intestinal mucosa, and weights were added until detachment occurred. This method, compared to Carbopol 934 P and sodium alginate, provides insights into the mucilage's potential for effective mucosal adhesion and sustained therapeutic benefits [14].

RESULTS AND DISCUSSION

The isolated mucilage exhibited a characteristic odour and a bitter taste, while its colour appeared brownish yellow. Its texture was noted as irregular and hard, with a rough fracture appearance.

Phytoconstituent screening of the isolated fenugreek seed mucilage identified the presence of carbohydrates and mucilage. Carbohydrates were confirmed using Molisch's Test, while the Ruthenium Red Test established the presence of mucilage. The absence of monosaccharides was indicated by a negative Barfoed's Test, and the Iodine Test showed no starch. Additionally, negative results in other tests confirmed the absence of alkaloids, glycosides, tannins, steroids, sterols, proteins, amino acids, and flavonoids. Results are given in table 1.

Table 1: Phytochemical screening report of fenugreek seed mucilage

Phytochemical Constituents	Name of test	Distilled water
Carbohydrates	Molisch's Test	Positive
Monosaccharides	Barfoed' s Test	Negative
Mucilage	Ruthenium Red Test	Positive
Starch	Iodine Test	Negative
Glycosides	Keller Killiani Test	Negative
Alkaloids	Dragendroff's Test	Negative
Sterols and Steroids	Liebermann Burchard	Negative
Tannins	Ferric Chloride Test	Negative
Amino acids &Proteins	Ninhydrin Test	Negative
Flavonoids	Shinoda Test	Negative

The extraction method yielded 31.78% ±2.435 mucilage from fenugreek seeds, based on three trials. This high percentage reflects the method's effectiveness and suitability for industrial applications, highlighting its efficiency in obtaining significant mucilage from the natural source.

The dried fenugreek seed mucilage had a moisture content of 5.41±1.72%, well within the Pharmacopoeial limit of 15%. This low moisture level reduces the risk of microbial contamination during storage and processing, indicating that moisture-proof packaging or desiccants should be used to preserve the mucilage's stability and quality.

The pH of a 1% w/v fenugreek seed mucilage solution was measured at 6.84 ± 1.28 using a digital pH meter (Labtronics LT-50). This near-neutral pH indicates that fenugreek seed mucilage is biocompatible and has a low potential for irritation, making it suitable for use as a mucosal adhesive polymer in gastrointestinal applications.

The solubility tests revealed that fenugreek seed mucilage is fully soluble in hot distilled water but dissolves slowly in cold distilled water, indicating temperature-sensitive solubility. It has minimal solubility in ethanol and methanol, while showing no solubility in acetone, ether, benzene, chloroform, and dimethyl sulfoxide (DMSO), underscoring its limited interaction with organic and non-aqueous solvents.

The study found that increasing the concentration of fenugreek seed mucilage from 1% to 3% w/v led to higher viscosity, consistent with the expected behaviour of polymer solutions where greater concentrations result in increased viscosity due to enhanced molecular interactions. This elevated viscosity can significantly impact drug delivery systems by extending drug release through enhanced swelling and gelling. Results are given in table 2.

Table 2: Viscosity data of fenugreek seed mucilage

Concentration of polymer solution	1% w/v	2% w/v	3% w/v
Viscosity (cP)	42±1.12	97±1.25	103±1.09

The swelling index of fenugreek mucilage, measured by the British Pharmacopeial method, was 18.56% ± 2.38%. This result underscores the mucilage's substantial swelling capacity in aqueous environments, a vital property for mucoadhesive polymers in drug delivery systems. Effective water absorption and swelling are key for optimal mucoadhesion, promoting prolonged interaction with mucosal surfaces and enhancing adhesion strength, which is crucial for sustained drug release and extended therapeutic action.

The total ash value of 11.46% ± 0.21% indicates a high level of purity in the fenugreek mucilage, with minimal organic or extraneous matter. The water-soluble ash, at 8.25% ± 0.19%, reveals the presence of soluble minerals and salts, contributing to the mucilage's mineral profile and potential benefits. The low acid-insoluble ash value of 1.04% ± 0.13% suggests minimal silica and calcium oxalate content, further affirming the mucilage's purity and quality.

The fenugreek seed mucilage exhibited a bulk density of 0.585 and a tapped density of 0.621, yielding a Hausner ratio of 1.06 and a Carr's Compressibility Index of 5.79%, which reflect excellent flow and compressibility. With an angle of repose of 32°, the mucilage demonstrates good flow properties, essential for uniform manufacturing. A porosity value of 6.2% further indicates its capacity for gastrointestinal fluid absorption and effective mucoadhesion, confirming its suitability for controlled drug release and mucoadhesive applications. Results are given in table 3.

Table 3: Report on micromeritic properties of fenugreek seed mucilage

Parameter	Value	Inference
Bulk Density	0.585	Fairly good
Tapped Density	0.621	Fairly good
Hausner ratio	1.06	Excellent flow and compressibility.
Carr's Index	5.79%	Excellent compressibility
Angle of Repose	32°	Good flow property
Percentage Porosity	6.2 %	Satisfactory for adequate swelling

This study evaluated the mucoadhesive properties of isolated fenugreek seed mucilage and compared its performance with Carbopol 934 P and sodium alginate using an ex-vivo method involving goat intestinal mucosa. The results demonstrated that fenugreek seed mucilage exhibited a mucoadhesive strength of 0.02214 N/m², which was similar to the strength of Carbopol 934 P at 0.02495 N/m². In contrast, sodium alginate showed a significantly lower mucoadhesive strength of 0.0015 N/m². These results highlight the potential of fenugreek seed mucilage as a mucoadhesive agent, showing comparable effectiveness to Carbopol 934 P.

CONCLUSION

The study successfully isolated and characterized mucilage from fenugreek seeds, demonstrating an effective and efficient extraction process with a notable yield. Characterization confirmed the presence of carbohydrates and mucilaginous properties, and ash value analysis validated the mucilage's identity and purity. Micromeritic properties indicated satisfactory flow characteristics for formulation use, while the pH level and swelling index highlighted its biocompatibility for gastrointestinal applications. The findings also revealed that fenugreek seed mucilage has mucoadhesive strength comparable to Carbopol 934 P and significantly higher than sodium alginate, underscoring its potential as an effective mucoadhesive agent.

CONSENT FOR PUBLICATION
Not applicable.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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