RESEARCH ARTICLE

Extraction, Phytochemical Screening and HPTLC Analysis of Fenugreek Gum for Analysis of Different Phytoconstituents.

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ABSTRACT

Fenugreek gum is a natural polysaccharide that has been explored for various biomedical applications. The present study investigated the phytoconstituent profile of fenugreek gum, revealing it as a potent source of diverse bioactive compounds. The analysis encompassed steroids, fats and oils, alkaloids, proteins, saponins, tannins, phenolics, flavonoids, and carbohydrates. Extraction using methanol and aqueous solvents demonstrated the highest concentration of phytoconstituents, underscoring the efficacy of these solvents in isolating bioactive components from fenugreek. Further exploration involved the determination of Rf values and percentage area in chloroform, petroleum ether, and chloroform extracts, providing a comprehensive identification of the mixture's components. The results suggested a rich and varied chemical composition in fenugreek gum, offering potential applications in diverse fields owing to the presence of steroids for physiological effects, fats and oils for nutritional benefits, alkaloids with potential pharmacological properties, proteins for cellular functions, saponins for their soap-like properties, and tannins and phenolics as antioxidants. Additionally, the presence of flavonoids and carbohydrates adds to its nutritional and medicinal value. The study's findings contribute valuable insights into the phytochemical composition of fenugreek gum, shedding light on its potential applications in the realms of medicine, nutrition, and industry. The identification of optimal extraction methods and the characterization of phytoconstituents lay the foundation for future research and utilization of fenugreek gum as a versatile and valuable natural resource. Overall, this investigation underscores the significance of fenugreek gum as a rich repository of bioactive compounds with diverse potential applications across various domains.

Keywords: Fenugreek gum, HPTLC, Phytochemicals, Extraction, Herbal, Natural gum.

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INTRODUCTION

Fenugreek *(Trigonella foenum graecum)* is an annual plant classified under the Leguminosae or Fabaceae family. Its seeds are composed of a high amount of solublise fibers which is also known as galactomannans. This plant is very potential in terms of herbal remedy due to the presence of wider phytoconstituents.[1] The gums derived from this plant has potential benefits in the pharmaceutical industry. Galactomannans obtained from different sources have different physicho chemical properties. They may have different molecular weights and solubility profiles.[2] The distinctive feature of fenugreek gum lies in its equal galactose: mannose ratio of 1:1, showcasing the highest galactose concentration. This property results in increased solubility in water as compared to guar and locust bean gum. The greatest hydration and solubility of fenugreek gum are attributed to the homogeneous linking of its galactose and mannose moieties. Galactomannan physicochemical qualities are significantly influenced by the M/G ratio, with solubility exhibiting an inverse relationship with this ratio. This characteristic leads to enhanced aqueous solubility in comparison to the other gums. The uniform linkage of galactose and mannose in this gum is responsible for its higher solubilities and hydration properties. The M/G ratio influences the physicochemical properties of galactomannans, with solubility showing an inverse correlation to this ratio.[3]

The M/G ratio plays a pivotal role in influencing the physicochemical properties of galactomannans, exhibiting an inverse relationship with the gum's solubility.[4] By adhering to oil droplets, galactomannans help to stabilize the steric balance of emulsions by inhibiting flocculation and coalescence. These biopolymers also exhibit emulsification, interfacial, and surface activity. Fenugreek gum is favored above other natural hydrocolloids because of these unique qualities, which make it a great component for many culinary applications.[5]

Fenugreek gum imparts desirable characteristics such as texture, appeal, gelling, thickening, and emulsifying properties.

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The seeds of fenugreek contain wider phytoconstituents. The gum from this plant is derived from this widely cultivated plant, ensuring a sustainable supply. Fenugreek is a naturally occurring edible spice known for its notable anticancer activity. The applications of fenugreek gum extend to various drug delivery systems, including sustained drug delivery and gastro-retentive drug delivery, making it a subject of extensive exploration in the pharmaceutical field.[6,7]

The present study deals with the extraction of fenugreek gum in different extracts followed by phytochemical screening and HPTLC analysis to identify the phytoconstituents.

MATERIAL AND METHOD

Materials

The plant material was obtained from Vineet Analytical Research Laboratories Pvt Ltd Pune in powder form fenugreek gum. Petroleum ether, chloroform, and methanol were purchased from the Loba Chemie, Mumbai, India.

Preparation of the Extract

The solution technique was used to sequentially extract the dried aerial portions of fenugreek gum utilizing pet ether, chloroform, and methanol. At room temperature, water extraction was done concurrently using the maceration process. The resultant extracts were further evaporated at lower pressure after being concentrated in a water bath. Each extract's yields were calculated, and a basic phytochemical screening was carried out.[8]

Phytochemical Screening

For the initial phytochemical screening of steroids, alkaloids, tannins, saponins, phenolic compounds, flavonoids, proteins, amino acids, and carbohydrates, all three extracts were employed. The conventional protocols described in the literature were followed in order to accomplish the phytochemical screening.[9]

HPTLC Analysis of Various Different Extracts

Using an automated applicator (CAMAG Linomat IV), 10 µL of various extracts were applied to precoated Silica gel 60 F254 plates with a 0.2 mm thickness. After that, the plates were run in a CAMAG twin trough chamber with the appropriate solvent solution up to a distance of around 9 cm. Under UV light, the plates were visible at 254 and 366 nm. After applying the appropriate spray reagents, the plates were heated to 110°C for 10 minutes. The Desaga video documentation equipment was utilized to record and document the color and Rf values of the resolved bands on video. Using the CAMAG TLC scanner III, the plates were scanned densitometrically at an appropriate wavelength. After the peaks are integrated (mostly from valley to valley), the report includes the peak's height, retention duration, and percentage of the land under it. We can identify the chemicals using the retention time (RT), as the RT is particular to the drug under consideration. It is possible to compute the amount of the chemicals using the area and height.[10, 11]

RESULT AND DISCUSSIONS

Extracts (ethanol, methanol, petroleum ether and water) obtained through the soxhlation method were determined for the yield. The higher yield was obtained for all three extracts. The %yield of the extracts is presented in Table 1**.**

The yield ranged from 49.55 to 52.61%. The highest yield was observed in methanol due to lesser evaporation of the solvent. All four extracts were light yellow in color. The yield and color were found to be satisfactory.

Phytochemical Screening

All three extracts were subjected to phytochemical screening in order to determine qualitative phytoconstituents from each extract. The results of the phytoconstituents are summarized shown in Table 2.

In petroleum ether, only steroids, fats, and oils were detected other phytoconstituents were not observed in this extract. The reason may be the very less solubility of the phytoconstituents in petroleum ether. In chloroform extract Steroids, alkaloids, Fats, oils, and proteins were detected.

Table 1: Percentage yield of fenugreek extract

S.No.	*Extract solvent*	*Yield (%)*	*Color*
1	Ethanol	49.55	Light yellow
2	Methanol	52.61	Light yellow
3	Petroleum ether	51.11	Light yellow
4	Water	50.14	Light yellow

Table 2: Preliminary phytochemicals screening of fenugreek gum

S.No.	*Phytochemical*	*Test*	*Fenugreek gum*			
			PTE	*CHE*	*MET*	*AQE*
1	Steroids	Salkovaski	++	++	--	--
		Dragendroff'	--	++	--	--
2	Alkaloids	Hager's	--	++	--	--
		Mayer's	--	++	--	--
		Wagner's	--	++	--	--
3	Saponins	Foam	--	--	++	++
		Haemolysis	--	--	++	++
4	Fats and oils	Filter paper	++	++	--	--
5	Tannins and phenolic	Ferric chloride	--	--	++	++
		Lead acetate	--	--	++	++
		Pot. Dichromate	--	--	++	++
		Bromine water	--	--	++	++
6	Flavonoids	Shinoda	--	--	++	++
		Lead acetate	--	--	++	--
7	Carbohydrates	Molisch	--	--	++	++
		Fehling's	--	--	++	++
		Barfoed's	--	--	++	++
8	Proteins	Millon's	--	++	++	++
		Biuret	--	++	++	++

+ ve -- present; -- ve absent; PTE -- Petroleum ether extract; CHE -- Chloroform extract; MET -- Methanal extract; AQE -- Water extract

Table 3: Rf values of petroleum ether, chloroform and methanol extract

No of Spots	*Name of extracts*											
	Pet. ether		*Chloroform*		*Methanol*		*Rutin*		*Tannic acid*		*Saponin*	
	Rf	*% Area*	*Rf*	*% Area*	*Rf*	*% Area*	*Rf*	*% Area*	*Rf*	*% Area*	*Rf*	*% Area*
	0.03	2.19	0.07	3.09	0.07	0.44	0.42	100	0.12	100	0.46	100
	0.9	28.89	0.9	8.18	0.10	0.95	--	--	--	--	--	--
	0.25	47.15	0.25	22.07	0.12	0.99						
	0.40	4.00	0.40	21.05	0.23	0.75						
	0.49	5.95	0.45	32.06	0.40	15.05						
	0.59	0.85	0.50	26.40	0.50	35.42						
	0.64	1.25	0.63	8.54	0.54	26.40						
	0.88	8.14	0.67	3.23	0.58	4.54						
	0.90	1.59	0.07	3.09	0.67	12.23						

The fenugreek methanolic and aqueous extracts contained the highest concentration of phytoconstituents. In both the methanolic and aqueous extracts, steroids and alkaloids were not detected. Both extracts included the remaining phytoconstituents, which included proteins, carbohydrates, tannins and phenolics, lipids and oils, saponins, and flavonoids. According to the results of the phytochemical screening, fenugreek gum is rich in phytoconstituents and may be investigated as a possible natural treatment for a variety of illnesses.

HPTLC analysis

HPTLC serves as a valuable and efficient tool for the separation, identification, and quantification of phytochemicals in plant extracts, contributing to our understanding of the chemical composition of medicinal plants and other botanical materials.[12] The different Rf values observed through the HPTLC study are presented in Table 3.

Rutin, tannic acid and saponin showed Rf value of 0.42, 0.12 and 0.46 respectively in HPTLC analysis. HPTLC plates of extracts are presented in Figures 1A to 1E.

The retention factor (Rf) in HPTLC is pivotal for interpreting chromatographic results. Rf values, unique to specific compounds under defined conditions, aid in identifying phytochemicals by comparing sample components to standards. In quality control, Rf values ensure consistency and quality in herbal extracts.[13] Crucial in method development, Rf values guide optimization of separation conditions, influencing factors like stationary phase and mobile phase composition. For quantification, Rf values, measured as the distance a compound travels relative to the solvent front, estimate the compound quantity, particularly when using densitometry. Rf values contribute to method standardization, fostering result comparability across laboratories. In troubleshooting, deviations from expected Rf values signal issues like incorrect mobile phase composition. Lastly, Rf values are vital for accurate data reporting and documentation, providing a standardized reference for identified compounds. Overall, Rf values enhance HPTLC's reliability in phytochemical research and diverse applications, playing a crucial role in compound identification, quality assurance, method optimization, quantification, standardization, troubleshooting, and result reporting.[12] HPTLC densitometric chromatograms of different extracts are presented in Figure 2.

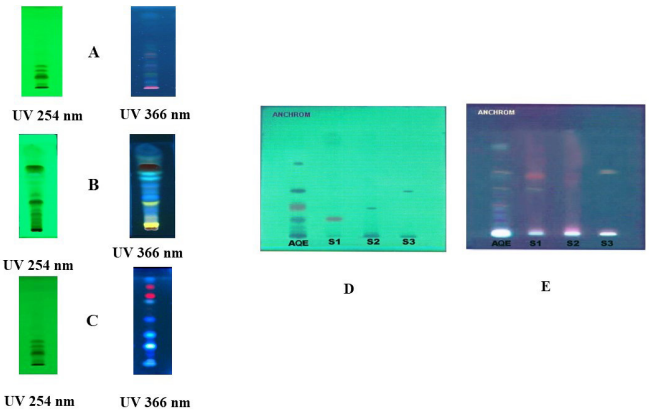

Figure 1: HPTLC profile of Fenugreek *gum at* 254 and 366 nm in A: pet ether extract; B: chloroform extract; C: Methanol extract; D: HPTLC Fingerprint images of water extract and standards @ 254 nm and E: @366 nm

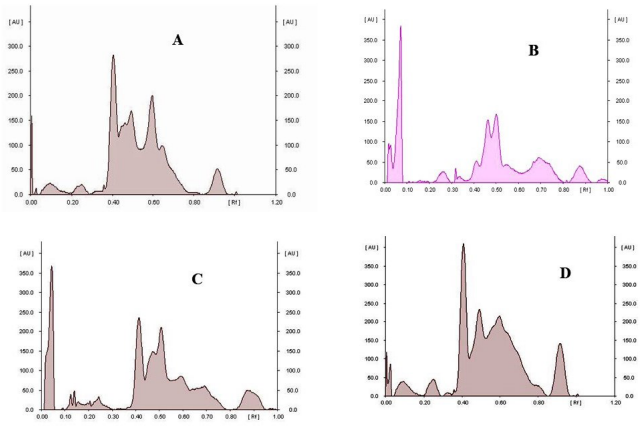

Figure 2: HPTLC densitometric chromatogram @ 366 nm of A: pet ether extract; B: chloroform extract; C: Methanol extract and D: Aqueous extract

CONCLUSION

The present study concludes that fenugreek gum is a rich source of phytoconstituents including steroids, fats and oils, alkaloids, proteins, saponins, tannins, and phenolic, flavonoids, and carbohydrates. The fenugreek methanolic and aqueous extracts contained the highest concentration of phytoconstituents. The components of a mixture are identified by the Rf value and area of % in chloroform, petroleum ether, and chloroform extracts.

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