The Potential of *Trigonella foenum-graecum* L. Seed Mucilage as Suspending Agent

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ABSTRACT

The present study deals with the evaluation of mucilage isolated from fenugreek (*Trigonella foenum-graecum* L.) seeds (commonly named methi) as an innovative and cheap suspending agent. Zinc oxide suspensions (20 % w/v) were prepared using fenugreek seed mucilage as a suspending agent and it was evaluated to find its stability using the parameters like, sedimentation profile, degree of flocculation and redispersibility. The effect of the tested mucilage on suspension was compared with various commonly used suspending agents, like gum tragacanth, gum acacia, and bentonite at concentrations of 0.5, 1.0 and 2.0 % w/v. The results obtained indicated that the fenugreek seed mucilage could be used as a good suspending agent and was found to be superior than gum tragacanth, gum acacia, and bentonite in performance.

Keywords: suspension, suspending agent, mucilage, fenugreek seed

INTRODUCTION

Pharmaceutical excipients play an important role in various pharmaceutical formulations. Recent trends towards the use of natural origin excipients demand the replacement of synthetic excipients with the natural ones. Naturally derived excipients are attractive alternatives to synthetic excipients because of their easily availability, biocompatibility, biodegradability, low toxicity, environmental friendliness and low price compared to synthetic excipients[1, 2]. Furthermore, they can be modified to obtain tailor made materials for drug delivery systems and they can compete with the synthetic agents available in the market [3]. Ability to produce a wide range of excipients based on their properties and molecular weight, natural excipients became a thrust area in majority of investigations related with development of drug delivery systems.

A large number of plant-based pharmaceutical excipients are available today. Researchers and formulators have explored the usefulness of these excipients in the development of various formulations. The rationale for increase in importance of natural plant-based material is that plant resources are renewable and if cultivated or harvested in a sustainable manner, they can provide a constant supply of raw materials[4].

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Mucilages are naturally occurring, high molecular weight polyuronides consisting of sugars and uronic acid units[5, 6]. They are normal physiological metabolism products formed with in the cell or deposited on it in layers. Mucilages serve as food reserve, membrane thickener and in water storage and seed germination[6]. Plant mucilages are important polysaccharides with wide range of pharmaceutical applications such as thickeners, binding agents, water retention agents, emulsion stabilizers, suspending agents, disintegrating agents, gelling agents and film formers[1,2]. Acacia, tragacanth, gum ghatti, gum karaya etc are popular examples of plant mucilages, used in pharmacy. Thus, with the increase demand for these substances, it has been necessary to explore the newer sources to meet the industrial demands.

A pharmaceutical suspension, is thermodynamically unstable, thus, making it necessary to include in the dosage form, a suspending agent which reduces the rate of settling and permits easy redispersion of any settled particulate matter both by increasing the consistency of suspending medium and by protective colloidal action[7]. There are several reports about the successful use of various plant mulcilages, as innovative suspending agents like mucilages isolated from tamarind seed[8], *Malava sylvestris* and *Pedalium murex* fruits[7], *Hibiscus rosasinensis* L. leaves[9], *Hibiscus cannabinus* seeds[9], *Ocimum gratissimum* seeds[10], *Cassia tora* seeds[11], isapgol seeds[12], *Cassia roxburghii* seeds[13], *Chlorophytum borivilianum* tuber[14], *Abelmoschus esculentus* fruits[15], *Basella alba* L. leaves[16], *Spinacia oleracea* L. leaves[17], *etc*. The purpose of this investigation is to search for a chip and effective natural excipients that can be used as a new substitute suspending agent for the formulation of pharmaceutical suspensions.

Trigonella foenum-graecum L. (fenugreek) is known as methi and is also a commonly available material in nature[18]. Fenugreek seeds are used to flavour many foods, spice blends and pickles. Fenugreek seeds have several pharmacological effects such as hypoglycaemia[19] antioxidants[20, 21], laxative[22], etc. The ripe seeds have numerous applications in cosmetic and traditional medicine system of India. These seeds contain (dry basis): moisture (3.6 %), protein (25-30 %), insoluble fibres (20-25 %)[23]. Fenugreek seeds contain a high percentage of mucilage. The pharmaceutical utility of fenugreek seed mucilage is already established as gelling agent[24], granulating agent[25], binding agent[23], and disintegrating agent[26]. The objective of the present investigation was to isolate mucilage from fenugreek seeds, identify the isolated mucilage by different phytochemical tests, and investigate various pharmaceutical properties of the isolated mucilage to assess its suitability as suspending agent in pharmaceutical suspensions.

MATERIALS AND METHODS

Materials

The materials used include zinc oxide (E. Merck, India); gum tragacanth, and gum acacia (Loba Chemie, India); bentonite, potassium dihydrogen phosphate, and sodium metabisulfite (Qualigens Fine Chemicals, India); benzoic acid (SD Fine Chemicals, India). Fenugreek (*Trigonella foenum-graecum* L.) seeds were collected from local market in the month of March, 2011. All solvents and chemicals used were analytical grade.

Isolation of mucilage fenugreek seeds

Fenugreek seeds (200 gm) were soaked in distilled water (1.5 litres) at room temperature for 12 hours and boiled using water bath until the preparation of slurry. After cooling, the slurry was cooled and kept in refrigerator overnight to settle out undissolved materials. The upper clear solution was decanted and concentrated at 60^0C by water bath to $1/3^{rd}$ of its original volume. The solution was cooled at room temperature and poured into thrice the volume of acetone with continuous stirring. The precipitate was washed repeatedly with acetone and dried at room temperature for 24 hours. The isolated material was passed though sieve no 80 and stored in desiccators until further use.

Identification tests

The isolated fenugreek seeds mucilage was subjected to some tests for identification of the obtained mucilage. The tests performed were to determine the presence of carbohydrates (Molisch's test), mucilage (Ruthenium red test), starch (Iodine test) alkaloids (Dragendroff's test), glycosides (Keller Killiani test), tannins (Ferric chloride test), steroids and sterols (Libermann-Burchard test), proteins and amino acids (Ninhydrin test)[27, 28].

Fourier transforms infrared (FTIR) spectroscopy

Isolated mucilage from fenugreek seeds was analyzed as KBr pellets by using a Fourier transform infrared (FTIR) spectroscope (Perkin Elmer Spectrum RX I, USA). The pellets were placed in the sample holder. The spectral scanning was taken in the wavelength region between 4000, and 400 cm^{-1} at a resolution of 4 cm-1 with scan speed of 1cm/sec.

Viscosity measurement of isolated mucilage

Viscosities of the aqueous solutions of isolated mucilage in different concentrations (0.5, 1 and 2 % w/v) were determined using a Brookfield digital viscometer Helipath (LVDV 2P230). The spindle was inserted into the test solutions and the viscometer was sheared at different speeds of 20, 50 and 100 r.p.m. at room temperature. Viscosity values were recorded for different speeds of rotation. Graphs of viscosity versus speed of rotation (in r.p.m.) were plotted. Distilled water was used to prepare aqueous solution of the isolated mucilage.

Preparation of zinc oxide suspension

20 % w/v zinc oxide suspension in water was prepared using fenugreek seed mucilage, and various conventional suspending agents (gum tragacanth, gum acacia, and bentonite) at concentrations of 0.5, 1.0 and 2.0 % w/v. Zinc oxide powder was levigated with glycerin (1:1). Then weighed amount of these suspending agents were added and triturated and 0.1% w/v benzoic acid was added as preservative. Then, the volume was made up with distilled water. All the suspensions were deflocculated. To determine the degree of flocculation, flocculated suspensions were made using potassium dihydrogen phosphate (0.004 M) as flocculating agent.

Evaluation of zinc oxide suspension

pH measurement

The pH of all formulated 20 % w/v zinc oxide suspensions using gum acacia, gum tragacanth, bentonite and isolated mucilage as suspending agents were measured using a digital pH meter (Systronic, 361-micro pH meter). The pH meter was calibrated before use.

Sedimentation volume

Sedimentation volume (F) was determined by allowing a measured volume of the suspension in a graduated cylinder at an undisturbed position for a definite period of time and noting the value of the ultimate height (H_u) of the sediment as a suspension settles and the initial height (H_o) of the total suspension ($F = H_u / H_o$)[17,18].

Degree of flocculation

The degree of flocculation (β) was determined from the following equation: $\beta = (V_u)_{floc} / (V_u)_{defloc}$, where $(V_u)_{floc}$ is the ultimate sedimentation volume in flocculated suspension and $(V_u)_{defloc}$ is the ultimate sedimentation volume in deflocculated suspension[16,17].

Redispersibility: 50 ml of various suspension was kept in calibrated measuring cylinders, which were then stored at room temperature at various time intervals (1, 5, 10, 15, 20, 30 and 45 days). At regular interval, one measuring cylinder was removed and shaken vigorously to redistribute the sediment and presence of deposit was recorded, if any[16,17].

Statistical analysis:

The data was analyzed with simple statistics. Statistical significance of degree of flocculation was analyzed by Tukey HSD test difference between means. The statistical analysis was conducted using using BioStat v2009 Statistical program (Analyst Soft Inc).

RESULTS AND DISCUSSION

Isolation of mucilage and its phytochemical identification

The yield of mucilage isolated from fenugreek (*Trigonella foenum-graecum* L.) seeds was 17.36 % w/w. The phytochemical identification tests confirmed the absence of alkaloids, glycosides, tannins, steroids and sterols. On treatment of mucilage with ruthenium red, it showed red colour confirming the obtained as mucilage. A violet ring was formed at the junction of two liquids on reaction with Molisch's reagent indicating the presence of carbohydrates. Mucilage on treating with Ninhydrin reagent did not give purple coloration indicating the absence of proteins and amino acids. All these results of phytochemical identification tests on the isolated mucilage are summarized in Table 1.

Table 1: Phytochemical identification tests on isolated mucilage from fenugreek (*Trigonella foenum-graecum* L.) seeds.

Identification tests	Name of tests	Observations[$]
Test for carbohydrates	Molisch's test	+
Test for proteins and amino acids	Ninhydrin test	-
Test for mucilages	Ruthenium red test	+
Test for starches	Iodine test	-
Test for alkaloids	Dragendroff's test	-
Test for glycosides	Keller- Killiani test	-
Test for tannins	Ferric chloride test	-
Test for steroids and sterols	Libermann-Burchard test	-

[$] + indicates positive; - indicates negative

FTIR spectroscopy

The FTIR spectra of isolated fenugreek mucilage are shown in Figure 1. It showed characteristic peaks of –OH between 3511.6 and 3154.3 cm^{-1}, $–CH_3$ at 2923 cm^{-1}, –CH stretching between 2920.0 and 2852.4 cm^{-1}, ether linkage at 1450–1400 cm^{-1} and –CO stretching at 1017.7 cm^{-1}. These findings confirmed that the isolated mucilage was fenugreek seed mucilage[28].

Viscosity of isolated mucilage

The viscosities (in centipoises) of the aqueous solutions of isolated mucilage in different concentrations (0.5, 1 and 2 % w/v) were determined at different speed of rotation (in r.p.m.) in room temperature and the measured viscosities versus speed of rotation were plotted in Figure 2. From this plot, it was clearly observed that viscosity of the mucilage was increased with the increasing concentration.

Suspending activity evaluation

pH measurement

Fig. 1: FTIR spectra of isolated mucilage from fenugreek (*Trigonella foenum-graecum* L.) seeds

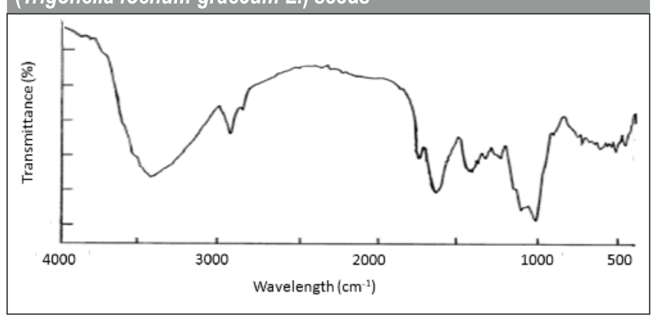
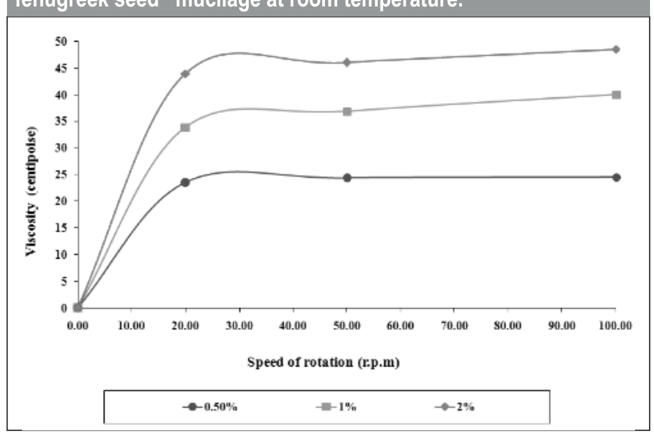


Fig. 2: The comparative viscosities of aqueous solutions of fenugreek seed mucilage at room temperature.



The pH of all formulated 20 % w/v zinc oxide suspensions using gum acacia, gum tragacanth, bentonite and isolated mucilage as suspending agents were measured and presented in Figure 3. This result showed comparable similar pH ($p < 0.05$) of all formulated suspensions investigated in this study.

Sedimentation volume

To evaluate the suspending properties of the isolated mucilage from fenugreek seeds, 20 % w/v zinc oxide suspensions were prepared with fixed concentration with varying concentration (0.5 %, 1 % and 2 % w/v) of the test mucilage as well as the commonly used suspending agents, like gum tragacanth, gum acacia, and bentonite. The sedimentation volume (F) of these formulated suspensions prepared with fenugreek seeds mucilage were compared with the suspensions prepared using various commonly used suspending agents and the sedimentation volume (F) profile of these suspensions are presented in and Figure 4 to 6. The dispersed particles were sediment at a faster rate in suspensions containing lower amount of suspending agents in comparison that of higher amount. The suspensions prepared with isolated fenugreek seed mucilage showed better suspending activity compared that of gum tragacanth, gum acacia, and bentonite.

Fig. 3: The comparative pH profile of the all formulated 20 % w/v ZnO suspensions

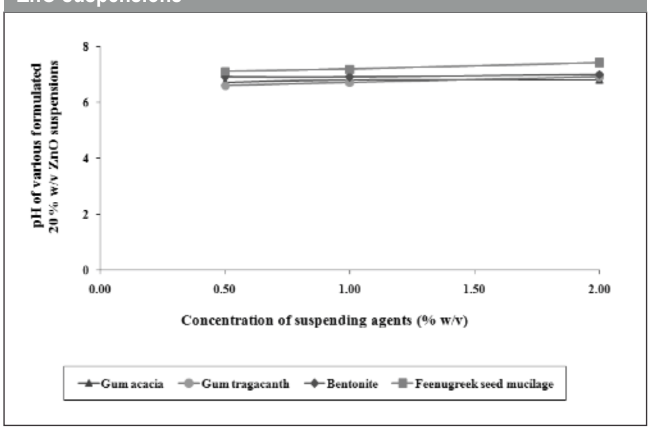


Fig. 4: The comparative sedimentation volume (F) profile of the suspensions with fenugreek seed mucilage and other traditional suspending agents (0.5 % w/v concentration)

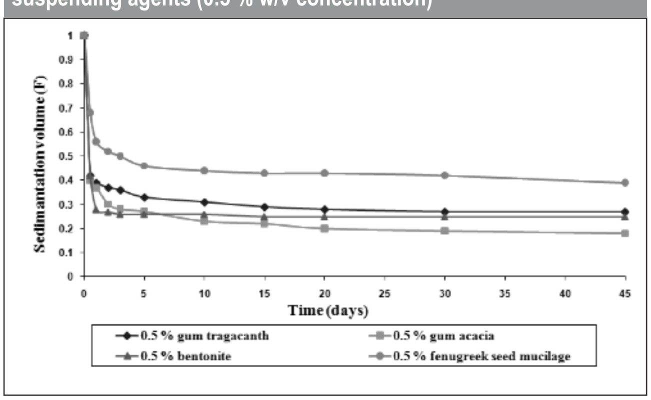


Fig. 5: The comparative sedimentation volume (F) profile of the suspensions with fenugreek seed mucilage and other traditional suspending agents (1 % w/v concentration)

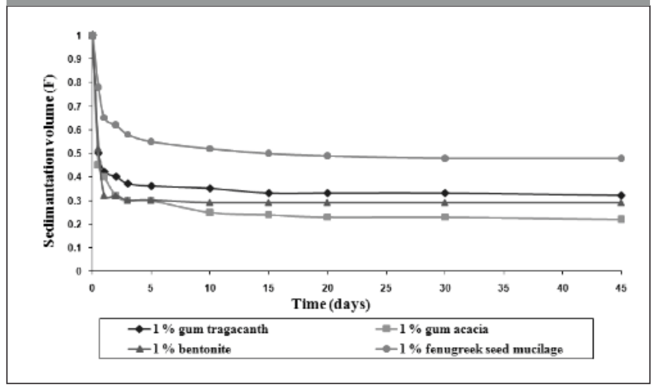
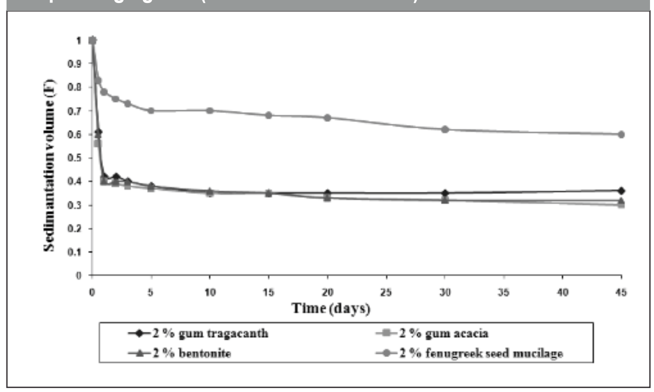


Fig. 6: The comparative sedimentation volume (F) profile of the suspensions with fenugreek seed mucilage and other traditional suspending agents (1 % w/v concentration)



Degree of flocculation

The sedimentation volume provides only a qualitative account of flocculation of a suspension [29]. But, the degree of flocculation (β) is also more useful parameter, which is the ratio of ultimate sedimentation volume in the flocculated and deflocculated system. The degrees of flocculation (β) were determined for all the formulated suspensions using different concentration of isolated fenugreek seed mucilage and various commonly used suspending agents used in this investigation. The values of degrees of flocculation for all formulated suspensions were presented in Table 2. The degree of flocculation of the formulated suspensions using 0.5, 1 and 2 % w/v fenugreek seed mucilage as suspending agent showed significantly ($p < 0.05$) higher values with that of the suspensions using 1 % w/v gum acacia, gum tragacanth, and bentonite as suspending agents. Again, the degree of flocculation of suspensions using 2 % w/v fenugreek seed mucilage exhibited significantly ($p < 0.05$) higher value with that of the suspensions using 2 % w/v gum acacia, gum tragacanth, and bentonite as suspending agents. Among, suspensions using fenugreek seed mucilage as suspending agent, 2 % w/v fenugreek seed mucilage showed significantly ($p < 0.05$) higher value with that of the suspensions using 0.5 and 1 % w/v mucilage. These observations showed that the test mucilage (mucilage isolated from fenugreek seeds) was better suspending agent than gum tragacanth, gum acacia and bentonite.

Redispersibility

Since the suspensions produce sediment on storage, it must be readily dispersible so as to ensure a more uniform dosage administration of the medicament after shaking. If sediment remains even after shaking vigorously for specified time, the system is described as caked[11]. The redispersibility of all formulated suspensions was studied and compared with each other. All these suspensions were found to be easily redispersible after maximum 10 times shaking after 45 days (Table 3). But, the redispersibility of the suspensions formulated with the isolated fenugreek seed mucilage was found to be after more shaking compared to the suspensions formulated with gum tragacanth, gum acacia and bentonite as suspending agents. The redispersibility of the suspensions with lower concentration of suspending agents was quicker than that of higher concentration. This observation may be attributed to the higher viscosity of these formulations with higher concentration of suspending agents.

Table 2: Degree of flocculation (β) of various suspending agents (Mean ± S.D.; n = 3)*

Suspending agents	Concentrations (% w/v)	β^2
Gum tragacanth	0.5	-
	1	2.43 ± 0.08 [a,b,c]
	2	3.10 ± 0.10 [c]
Gum acacia	0.5	-
	1	2.33 ± 0.09 [a,b,c]
	2	3.07 ± 0.09 [c]
Bentonite	0.5	-
	1	2.32 ± 0.10 [a,b,c]
	2	3.12 ± 0.10 [c]
Fenugreek seed mucilage	0.5	2.85 ± 0.09 [c]
	1	3.13 ± 0.12 [c]
	2	4.07 ± 0.14 [a,b]

Data was recorded after 45 days keeping at room temperature.
[a]Statistical significance ($p < 0.05$) with 0.5 % w/v fenugreek seed mucilage; [b]Statistical significance ($p < 0.05$) with 1 % w/v fenugreek seed mucilage; [c]Statistical significance ($p < 0.05$) with 2 % w/v fenugreek seed mucilage; [*]S.D. = standard deviation.
Statistical significance was analyzed by Tukey HSD test difference between means using BioStat v2009 Statistical program (Analyst Soft. Inc.).

CONCLUSION

It can be concluded that the isolated mucilage from fenugreek (*Trigonella foenum-graecum* L.) seed has potential as a new suspending agent even as in low concentration. The present investigation is a primary platform to indicate the suitability of fenugreek seed mucilage as suspending agent. The work can be further extended for evaluation of its suitability in other pharmaceutical applications considering the easy availability and cost-effectiveness of it.

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Table 3: Redesapersibility behavior of formulated suspensions using various suspending agents

Days	Number of shaking for complete redispersion											
	Gum tragacanth			Gum acacia			Bentonite			Fenugreek seed mucilage		
	0.5	1	2	0.5	1	2	0.5	1	2	0.5	1	2
1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	2	1	1	1	1	1	1	1	2	2
15	1	1	2	1	2	2	1	1	2	1	2	4
20	1	3	3	2	3	4	1	1	2	2	3	6
30	2	3	5	3	3	4	1	2	3	3	5	7
45	3	5	6	3	5	7	2	4	5	5	8	10

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