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Extraction, Evaluation and A Comparative Study of *Mimosa Pudica* Seed Mucilage with *Trigonella Foenum Graecum* and Flax Seed Mucilage

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KEYWORDS

Natural polymers, Mucoadhesive, Comparative evaluation**,** Mimosa *pudica* seed mucilage, *Trigonella foenum graecum*, and Flax seed.

ABSTRACT:

Objectives: The aim of this research article is to provide a brief knowledge about the comparative study of the mucilage extracted from the seed of *Mimosa pudica* with the mucilage extracted from *Trigonella foenum graecum* (Fenugreek) and Flax seed. Nowadays, industrialist are likely to use natural polymers due to various problems associated with drug release and side effects with synthetic polymers. **Methods:** Mucilage from different seed were extracted and isolated by conventional method using ethyl alcohol and acetone as a precipitating solvent. The extracted mucilage were evaluated by means various organoleptic properties, phytochemical properties, swelling index, loss on drying and micromeritics. **Results:** The extracted mucilage were pale yellow to brown in color, % yield of all mucilage were in the range of 29.12%w/w to 33.51%. Phytochemical tests were done for all mucilage confirmed the absence of alkaloids and glycosides. The pH of 1- 5% w/v aqueous dispersion of all three samples of mucilage was found between5.5 to 7.1. The solubility and swelling index of all mucilage also found good. All the values of micromeritics properties were found within the range. **Conclusion:** The present study suggests that the extracted mucilage from all the seed will be useful as an additives for oral muco-adhesive drug delivery systems as per the results of phytochemical and physicochemical tests indicated the suitability of mucilage for tablet dosage form as well as a suspending agent for suspension due to its swell able properties, weakly acidic pH and viscous in nature.

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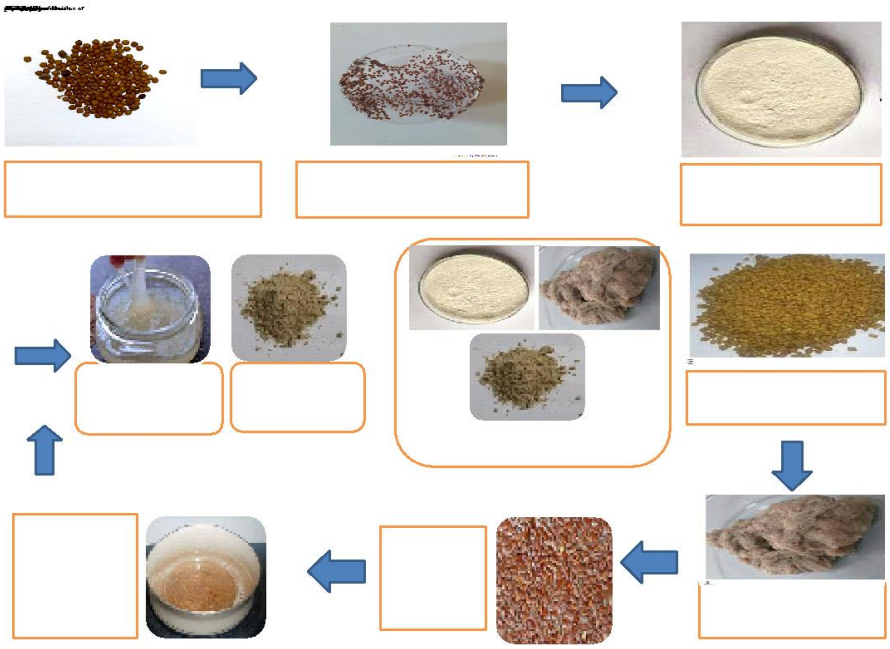

I. INTRODUCTION

Now a days, mucilage extracted from plant origin have evoked tremendous interest in pharmaceutical field because of their various applications such as binder, diluents [1] film coating and disintegrating agents[2] retarding the drug release[3] emulsification[4] protective colloids in suspension[5] bio adhesion, thickening agents in oral liquids, viscosity modifiers[6] gelling agents in gel formation, and bases in suppositories production.[7]

These natural polymers like mucilage and gums are preferred over the synthetic polymer because of its biocompatibility, ease of availability and cheap in cost. They are also preferred over the synthetic and semi synthetic excipients because of soothing in nature, lack of toxicity, low cost, and non-irritant in nature. [8, 9] There are difference in natural gums and mucilage, like natural gums are the products which are obtained from pathological conditions brought about either by injury or by adverse conditions of growth in plant and usually formed by changes in existing cell wall on the other hand mucilage are generally normal products of metabolism that is grown within the cell and are produced without injury to the plant.[10]

Polymer are very important ingredients of any dosage form. They are used for the preparation of various dosage forms. They also provide thickness, consistency, volume, stability, proper targeting and control the drug release. Also improved biological compatibility, and patient compliance.[11] Plant polysaccharide in the form of polymers are hydrophilic in nature, enzymatically degradable, and are capable of enhancing the stability of protein molecule embedded in them and also increasing their therapeutic efficacy.[12]

The main aim of this research article is to compare different isolated mucilage and identify their unique properties as a pharmaceutical excipient. Mucilage are well known source of polysaccharide in most of the plants. We preferred the common conventional techniques for isolation of mucilage and the preliminary confirmatory test for mucilage are Molisch test and Ruthenium Red test. In this research work we compared various characteristics of extracted
mucilage such as phytochemical properties, organoleptic pro perties, micromeritics, percentage yield, pH, total ash percentage, swelling index etc. The mucilage obtained from each and every plant seed has a pharmaceutical use. Extracted

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mucilage of Mimosa *pudica* seed mucilage can also be utilized as anti-ulcer[13] anti-diabetic and anti-inflammatory[14] drugs.

II. MATERIALS AND METHODS

Materials

The fresh seed of *M*imosa *pudica*, *Trigonella foenum graecum*, and Flax seed were collected from the Botanical Garden Greater Noida India. All plants were authenticated at the Pharmacognosy Department by a scientist at National Institute of Science Communication and Information Resources (NISCAIR) Delhi. (Authentication voucher no. NISCAIR/ RHMD/Consult/2023/3480-18). All the chemicals and distilled water used were of analytical grade.

Extraction and isolation of mucilage from the seed of *Mimosa pudica*

Mucilage was isolated as per the methods reported in various publications with little modifications. A weighed quantity (100 g) of *Mimosa*. *Pudica* seed were taken and processed for separating the brown peels from the kernel by using the blander and plastic sieve were used to separate the seed. All the seed were then crushed and soaked in double volume of water for 10 h., a thin layer of the hydrated mucilage along with seed was then spread on the stainless steel tray and dried in an oven at 50°C for 4–5 h. The dried mucilage was collected from the tray by blade or knife and separated from the seed by passing it through a sieve no 80. The powdered mucilage was further purified by winnowing to separate seed husk. The dried mucilage powders were preserved in desiccators for further use.[15]

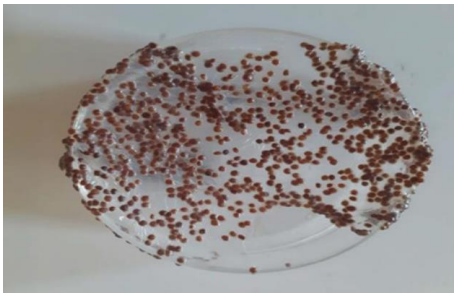

Fig. 1 Hydrated mucilage film obtained from the seed of *Mimosa pudica*

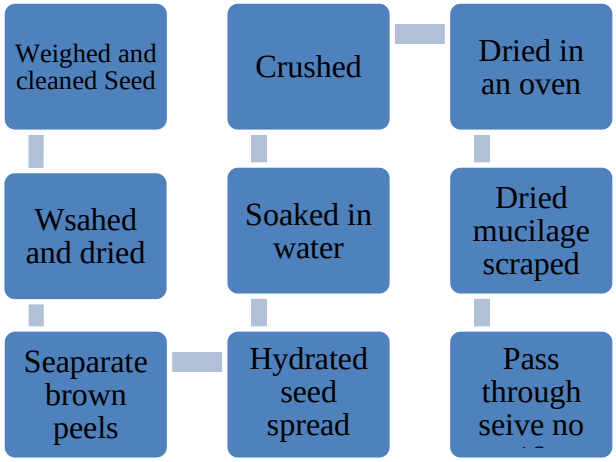

Fig. 2 Extraction method of mucilage from *Mimosa pudica* seed

Extraction and isolation of mucilage from the seed of *Trigonella foenum-graecum*

Accurately weighed seed of *Trigonella foenum-graecum L*. (100g) were soaked in 1000 ml of distilled water for 24 h. and boiled using water bath until a thick slurry was obtained. Slurry was allowed to cool and kept in the refrigerator overnight to settle out the undissolved materials. The supernatant was gradually poured and concentrated at 60°C using a water bath to one-third of its original volume. The solution was allowed to cool down and transferred into three times the volume of acetone with continuous stirring until a precipitate obtained. The precipitate was washed repeatedly with acetone and dried at room temperature for 24 h. The isolated mucilage was passed through sieve number 80 and stored in desiccators until further use.[16]

Fig. 3 Dried seed and precipitate of *Trigonella foenum-graecum*

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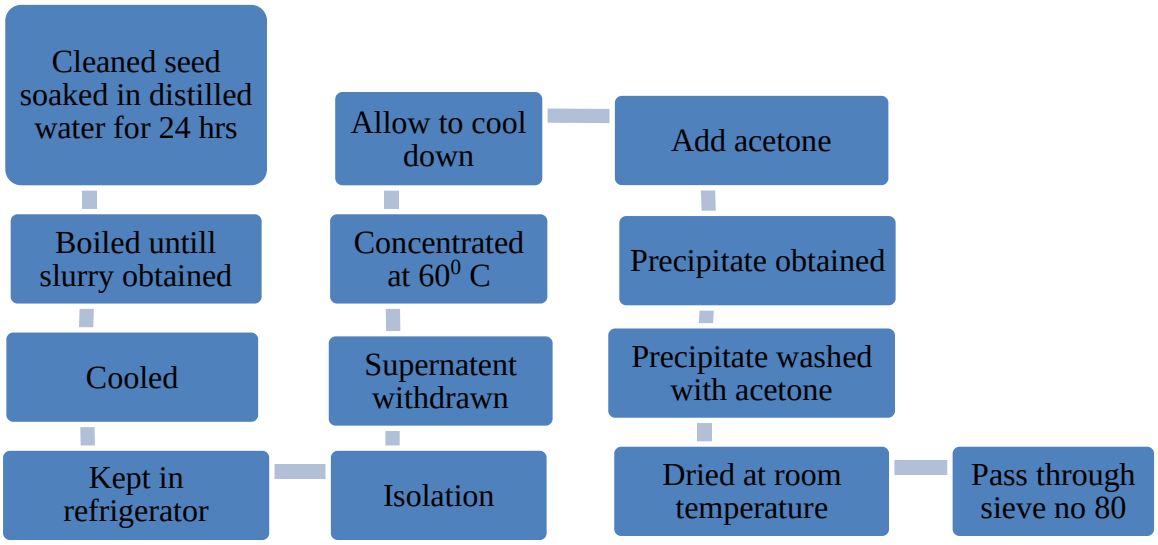

Fig. 4 Extraction and isolation of mucilage from *Trigonella foenum-graecum* seed

Extraction and Isolation of mucilage from Flax seed

Firstly dried seed were collected and cleaned. Then accurately weighed (300 gm) of seed were boiled with 2 liter of 0.1 M $NaHCO_3$ for 1 h. at 85°C. The boiled extract was cooled and filtered through a muslin cloth. Glacial acetic acid was added to neutralize the extract. Further the neutralized extract was added to ethyl alcohol. The amount of ethyl alcohol was 10-times greater than that of the filtrate. Extracted mucilage was precipitated immediately. The precipitated mucilage was separated and dried in the hot air oven at 55°C for 5 h. The yield of the dried mucilage was calculated and stored in desiccator until further use.[17]

Fig. 5 Extraction and isolation of mucilage from Flax seed

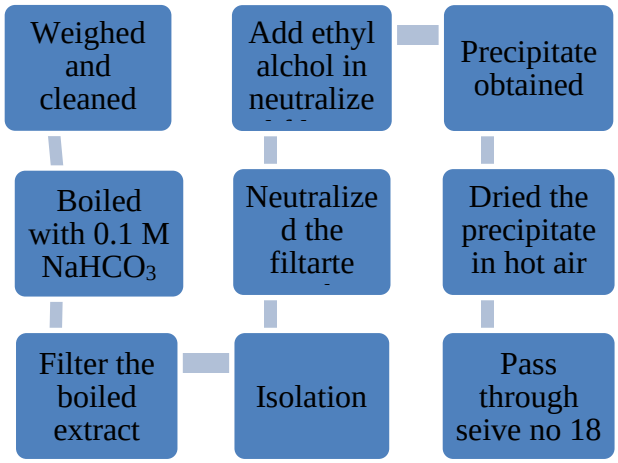

Fig.6 Extraction and isolation of mucilage from Flax seed

Evaluation of extracted mucilage

Extracted mucilage was evaluated further for their various properties by performing the following tests.

Percentage yield

The percentage yield of the extracted mucilage was determined by using following formula.

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$$\% \, Yield = \frac{Weight \ of \ isolated \ mucilage \ powder \ (gm)}{Weight \ of \ seed \ (gm)} \ X \ 100$$

Organoleptic properties of extracted mucilage

Isolated mucilage was evaluated for various tests such as colour, odour, taste, and texture.[18]

Phytochemical properties of mucilage

Preliminary tests were carried out to confirm the nature of the mucilage. The chemical tests that were conducted are as follows.[19]

Test for carbohydrates: (Molisch's test)

1 mg of powdered mucilage was added in 1 ml of α-naphthol solution then incorporated concentrated sulfuric acid. Purple or reddish violet colour was appeared indicated the presence of carbohydrate.[20]

Test for tannins: (Ferric chloride test)

Few drop of 5% w/v $FeCl_3$ solution were added to 1 ml of isolated extract. A green colour appeared indicated the presence of tannins.

Test for proteins: (Biuret test)

Add 2 drops of 1% copper sulphate solution to 1 ml of 40% sodium hydroxide solution. Presence of proteins was indicated by formation of violet colour.

Test for alkaloids: (Wagner's test)

1 ml of isolated mucilage was added to 2 ml of Wagner's reagent. The presence of alkaloids in the sample was indicated by the appearance of reddish-brown precipitate in test tube.

Test for glycosides: (Legal test)

Small amount of mucilage was dissolved in pyridine and sodium nitroprusside solution to make it alkaline. Pink red colour was appeared indicated the presence of glycosides in the sample.

Test for mucilage: (Ruthenium red test)

2-3 drops of ruthenium red solution was added to a small amount of dried mucilage placed in a beaker and observed under microscope.[21]

Test for flavonoid: (Shinoda test)

1 ml of extracted mucilage was added to 1 ml of magnesium and 1–2 drops of concentrated hydrochloric acid. The presence of flavonoids was indicated by the formation of red color.

Determination of mucilage pH

pH of mucilage

1 gm of accurately weighed mucilage was dissolved in 100 ml of water to get a solution of 1% w/v. The pH of solution was determined by using digital pH meter. The process was repeated for 2% and 5% solution.[22]

Micromeritics Evaluation of Isolated Mucilage

Bulk density

The bulk density of mucilage was calculated by placing the accurately weighed (10 g) mucilage powder into a 100 ml graduated cylinder, the volume of powder mucilage was noted down without disturbing the cylinder to determine. Bulk density was calculated by using the following formula.[23]

$$Bulk \ density \ = \frac{Weight \ of \ powder}{Volume \ occupied \ by \ powder} \ X \ 100$$

Tapped density

The tapped density was calculated by three tap method. Accurately weighed (10 g) of powder mucilage was introduced into a 100 ml graduated cylinder carefully and dropped on hardwood surface on tiles three times from height of 2.5 cm. It was calculated by using formula.[24]

$$Tapped \ density \ = \frac{Weight \ of \ powder}{Fainl \ olume \ after \ tappimg} \ X \ 100$$

Carr's index determination

Carr's index was calculated from the value of bulk density and tapped density. Both of these properties were used for the calculation of the Carr's index of the powder mucilage.[25]

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$$Carr's\ index = \frac{Tapped\ density - Bulk\ density}{Tapped\ density}\ X\ 100$$

Hausner's ratio determination

It is a used to check the flow properties of the powder mucilage and was calculated by the given equation. The low value of Hausner's ratio indicates that the mucilage powder has high followability. Hausner's ratio above 1.25 indicates poor flow.[26]

$$Hausner's\ ratio\ = \frac{Tapped\ density}{Bulk\ density}$$

Angle of repose

The angle of repose was calciulated by following the standard U.S.P 2010 method. In this method accurately weighed mucilage powder (10 g) was carefully introduced into a funnel clamped to a stand with its tip 10 cm from the surface of a plane paper. The mucilage powder was allowed to flow freely onto the paper surface. After complete flow height of the cone, H and the radius of the cone, R were measured and used to calculate the angle of repose by using the following equation.[27]

Angle of repose $ø = ^{tan1\ (h/r)}$

Solubility behavior of isolated mucilage

Small amount of dry mucilage powder was dissolve and shaken with different solvents, and the solubility was determined.[28]

Swelling index of isolated mucilage

Swelling index of the powdered mucilage was calculated by weighing a butter paper of size 4 cm × 4 cm, after this the butter paper was dipped into a Petri dish containing distilled water and reweighed the wet butter paper again. After this, 10 mg of the mucilage powder was kept in a butter paper placing this on a Petri dish containing 15 ml of water and the swelling index was calculated at different intervals, i.e., 15, 30, 45, 60, 120, 240, and 360 minutes. The experiment was repeated using 0.1 N HCL and phosphate buffer solution (pH 6.8).The swelling index was measured using following equation.[29]

$$Swelling\ Index = \frac{Initial\ weight\ of\ mucilage - Final\ weight\ of\ mucilage}{Initial\ weight\ of\ mucilage} X100$$

Loss on drying

The moisture content of dried extracted mucilage can be determined by loss on drying method. Accurately weighed 1 g mucilage powder was heated at 105°C to get a constant weight in a hot air oven and percent loss of moisture was calculated using the following formula.[30]

$$LOD\% = \frac{Weight\ of\ water\ in\ sample}{Weight\ of\ dry\ sample} X100$$

Total ash

Accurately weighed powdered mucilage (5 g) was taken in a silica crucible, which is previously ignited and weighed. The mucilage powder was spread as a fine and even layer on the bottom of the crucible. The crucible was again incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to air-dried sample.[31]

$$Ash\ value = \frac{Total\ weight\ of\ ash\ formed}{Total\ weighy\ of\ dry\ mucilage}\ X\ 100$$

Water-soluble ash value

2 g of ash was boiled with 25 ml of water. The insoluble matter was filtered and collected on an ash less filter paper washed with hot water and ignited in a tarred crucible at a temperature of 45°C for 4 h. The insoluble matter was cooled in desiccators, weighed, and subtracted from the total weight of the ash. The difference in weight represented weight of water-soluble ash. The percent water-soluble ash was calculated with reference to the air-dried drug using the given formula.[31]

$$Water\ soluble\ ash\ (\%) = \frac{Wt\ of\ total\ ash - Wt\ of\ water\ insoluble\ ash}{Wt\ of\ crude\ drug\ taken}\ X\ 100$$

Acid-insoluble ash value

Acid-insoluble ash value was calculated by boiling 2 g of

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ash for 5-6 min with 25 ml of 2 M HCl. The insoluble matter was filtered and collected on an ash less filter paper washed with hot water and ignited in a tarred crucible at a temperature 45° C for 4 h. The insoluble matter was cooled in a desiccators and weighed. The percent of acid-insoluble ash was calculated with reference to the air-dried sample using the following formula.[31]

$$Acid\ insoluble\ ash\ (\%) = \frac{Weight\ of\ acid\ insoluble\ ash}{Weight\ of\ crude\ drug\ taken}\ X\ 100$$

III. RESULTS AND DISCUSSION

% Yield

The extraction of mucilage powder from the seed of *Mimosa pudica, Trigonella foenum graecum and* Flax seed was done by conventional method and percentage yield of mucilage was found to be 33.51% w/w, 16.22 % w/w, and 29.12%w/w. The yield of *Mimosa pudica* seed was found high as compared to *Flaxseed* and *Trigonella foenum graecum* seed by using conventional method. % yield of the mucilage was calculated by weighing the dried mucilage and applying the given formula. Results are shown in Table 3.

$$\%\ Yield = \frac{Weight\ of\ isolated\ mucilage\ powder\ (gm)}{Weight\ of\ seed\ (gm)}\ X\ 100$$

Organoleptic Properties

Extracted powdered mucilage was also evaluated for various organoleptic properties and the results obtained are summarized in Table 1. It was found that mucilage obtained from all seed was odorless, yellowish-brown in color and mucilaginous in taste (nutty in flaxseed). The texture was found to be smooth in *Mimosa pudica, Flaxseed* and rough in *Trigonella foenum graecum*. All these properties are shown in Table 1.

TABLE I: Organoleptic properties of extracted mucilage

Mucilage	Colour	Odor	Taste	Texture
Mimosa.pudica	Brown	Odorless	Mucilaginous	Smooth
Trigonella. foenum graecum	Yellow	Odorless	Bitter	Rough
Flax seed	Brown	Odorless	Nutty flavour	Smooth

Phytochemical screening

Phytochemical tests were carried out for all mucilage confirmed the absence of glycosides and alkaloids. When treated with ruthenium red, it showed red colour indicated the presence of mucilage in the sample. A violet ring formation at a junction of two liquids when reacted with Molisch's reagent showed the presence of carbohydrates, while tannins, alkaloids, proteins, glycosides, and flavonoids tests were found negative. Results of these all are shown in Table 2.

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TABLE II: Phytochemical properties of mucilage powder

Tests	**Observation for MP seed mucilage**	**Observation for TFG seed mucilage**	**Observation for Flax seed mucilage**
Test for carbohydrates (Molisch's test)	+	+	+
Test for tannins (Ferric chloride test)	–	–	–
Test for proteins (Biuret test)	–	–	–
Test for alkaloids (Wagner's test)	–	–	–
Test for glycosides **(**Legal's test)	–	–	–
Test for mucilage (Ruthenium red test)	+	+	+
Test for flavonoid (Shinoda test)	–	–	–

+: Present, −: Absent

pH, Solubility, Swelling Index, loss on drying, Ash Value, Particle Size of Isolated Mucilage

The pH of 1%, 2%, and 5% w/v aqueous solution of all three powdered mucilage was found between 5.5 to 7.1. The pH measurements indicated that the mucilage obtained from *Mimosa pudica* seed was at different concentrations was slightly acidic to neutral and non-irritating to mucous membrane. The acidic nature of mucilage indicated that the mucilage contains uronic acids in its structure. The mucilage obtained from *Trigonella foenum graecaum* and Flax seed was slightly alkaline in nature. Results are shown in Table 3.

The solubility of the extracted mucilage powder was determined in various solvents. Small amount of dry mucilage was dissolve and shaken with different solvents, and the solubility was determined. All mucilage was found soluble in warm water, sparingly soluble in cold water, and insoluble in organic solvents. The solubility of mucilage was also determined using the ash of isolated mucilage and it was found that the ash of the extract was in-soluble in organic solvent like ethanol, methanol, and acetone and petroleum ether. Results are shown in Table 3.

As per the previously reported literature, swelling factor is very important for mucilage and it is most widely accepted general mechanism of action for disintegration. Thus, it is observed that mucilage obtained from Mimosa *pudica* seed had good swelling index in warm water as well as 0.1 N HCL. It was swell able in an aqueous medium, and the swelling index of isolated mucilage was found to be 76.32% in warm water, 65.42% in HCL and 48.22% in phosphate buffer. The swelling index of mucilage obtained from *Trigonella foenum*

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graecum was found 69.34% in water, 58.34 % in HCL, and 48.22% in phosphate buffer. It has high swelling property, and this property can be used as suspending and super disintegrating agent in various pharmaceutical formulations. The swelling index of Flax seed was found 83.33% in warm water. 44.32% in acidic pH because these polymers are sensitive to pH. In 0.1 N HCL it has suffered shrinkage. This response may be associated with the presence of COOH groups in the polysaccharide structural network, which makes secondary bonds such as hydrogen and intramolecular bonding, and bonding between polysaccharide chains. Results are shown in Table 3.

TABLE III: Physicochemical properties of mucilage powder

The loss on drying of powder mucilage determines that both water and volatile matter were present in the crude material. The weight loss on drying indicated that some amount of moisture was also present in the powdered sample which was available to interact with other material at the time of formulation of different solid dosage forms. Ash values indicated the quality and purity of an extracted mucilage, especially in the powdered form. The objective of ash value of vegetable drugs was to remove all traces of organic matter, which may otherwise interfere in analytical determination. All these results are shown in Table 3.

Parameter	**Observation for MP *seed* mucilage**	**Observation for TFG seed mucilage**	**Observation for Flax seed mucilage**
% Yield	33.51%	16.22 % w/w	29.12%w/w
pH (1% w/v)	5.5±0.15	6.5±0.12	7.1±0.20
Solubility	Soluble in warm water, sparingly soluble in cold water, insoluble in organic solvents	Slowly soluble in cold water but quickly soluble in hot water forming viscous colloidal solution. Practically insoluble in ethanol, methanol acetone and ether	Soluble in water, sparingly soluble in cold water, insoluble in organic solvents
Swelling index (%v/v)	76.32 in water, 65.42 in HCL, 48.22 in phosphate buffer	69.34 in water, 58.34 in HCL, 48.22 in phosphate buffer	83.33 in water, 44.32 in HCL, in 65.18 phosphate buffer
Loss on drying (%)	5.3	11.5	8.3
Total ash (%)	9.15	7.82	5.35
Water-soluble ash (%)	6.51	6.63	5.65
Acid-insoluble ash (%)	11	0.58	4.25

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Micromeritics Properties of Isolated Mucilage:

Micromeritics evaluations were carried out for the powder mucilage as bulk density, tapped density, Carr's index, angle of repose, also for calculating the flow behavior of the mucilage. Result indicated that all the values were found within the range. The angle of repose of the isolated mucilage was found in the range of 31.34 ± 0.30 to 35.25±0.12 it indicated that it has good flow property. The Bulk density and tapped density of all the mucilage were found within the range. From this observation, it could be concluded that the mucilage possessed good flow properties which is suitable for a direct compression formulation. All these results are shown in Table 4.

TABLE IV: Micromeritics properties of mucilage powder

S.N.	Parameter	Observation for MP seed mucilage	Observation for TFG seed mucilage ***mucilage*mucilage**	Observation for Flax seed mucilage
1.	Bulk density(gm/ml)	0.182±0.02	0.116±0.34	0.24±0.15
2.	Tapped density(gm/ml)	0.216±0.12	0.221±0.18	0.151±0.18
3.	Carr's index (%)	15±0.01	7.46±0.11	5.23±0.51
4.	Hausner's ratio	1.18±0.02	1.35±0.05	1.49±0.07
5.	Angle of repose (°)	31.34±0.30	35.25±0.12	33.21±0.11

IV. CONCLUSION

In this present study, we had carried out a comparative study like phytochemical and physicochemical properties of the mucilage extracted from the dried seed of *Mimosa pudica, Trigonella foenum graecum and* Flax seed. The % yield and efficacy values obtained through the process used to extract the mucilage from the seed indicates that it is a sustainable extraction method and isolated mucilage can be used for further study.

The mucilage isolated from the seed of *Mimosa pudica, Trigonella foenum graecum* and *Flax seed* will be useful as an excipient for oral muco-adhesive drug delivery systems as the results of phytochemical and physicochemical tests indicated the suitability of mucilage for tablet dosage form as well as a suspending agent for suspension due to its swelling properties, weakly acidic pH and viscous in nature. The present study suggests that isolated mucilage from all these seed showed good flow properties which is suitable for a direct compression formulation and is non-irritating in nature to the mucosal membrane. All the studies hence showed that the mucilage obtained can act as a potentially good candidate for various pharmaceutical formulations for its high swell ability on coming in contact with water thus it can be used as a thickening agent, suspending agent or as a super dis-integrant in various pharmaceutical formulations.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

ABBREVIATIONS

MP: Mimosa pudica, TFG: Trigonella foenum graecum.

V. SUMMARY

In this current study we successfully extracted and evaluated natural polymer from three different seed of different plant by using conventional method. The method of extraction was very simple and easy. Percentage yield, swelling index and micromeritics study indicated that the mucilage extracted and isolated can be further use as a natural ingredients in various drug delivery system like binding agents, muco-adhesive agents and gelling agents in the preparation of different mucoadhsive, dosage form because nowadays, manufacturers are likely to use natural plant based polymers due to many issues associated with drug release and side effects with synthetic polymers.

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