

Isolation, Characterization and Evaluation of *Mimosa pudica* Seed Mucilage as Pharmaceutical Additives

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Abstract

Aim: The aim of this present work was to isolate, characterized, and evaluate the natural plant-based polysaccharides from the seeds of *Mimosa pudica* plant which belongs to family Mimosaceae. In pharmaceutical industries large number of excipients is used which are obtained from natural sources, mucilage is one of them. Mucilage and their derivatives are a group of polymers extensively used in pharmaceutical dosage forms. **Materials and Methods:** The fresh *M. pudica* seeds were collected from Botanical Garden Greater Noida India. The plant was 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/2019/3437-38) All other chemicals used were of analytical grade, and distilled water was used throughout the experiments. *M. pudica* (family Mimosaceae), commonly known as sensitive plant and widely used as herbal medicine in India, is widely available and has very low cost. It is diffuse under shrub found widely in the tropical and subtropical parts of India. Seeds of *M. pudica* yield mucilage, which is composed of *d*-xylose and *d*-glucuronic acid. *Mimosa* seeds mucilage hydrates and swells rapidly on coming in contact with water. The present investigation involved isolation of mucilage from *M. pudica* seeds commonly known as chhui mui followed by phytochemical and physicochemical evaluation. **Results and Discussion:** The mucilage isolated from the seed of *M. pudica* will be useful as an excipient for oral 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 flowability, weakly acidic pH, swelling potential, and viscous in nature. The present study suggests that isolated mucilage from the seeds of *M. pudica* showed good flow properties which is suitable for a direct compression formulation and is non-irritating in nature to the mucosal membrane.

Key words: Characterization and evaluations of mucilage, extraction of mucilage, *Mimosa pudica* seed mucilage, natural excipients

INTRODUCTION

Herbal products appear to be more preferred over synthetic materials due to their accessibility, biocompatibility, low cost, and low toxicity potential. Mucilage's and gums are one of them which are obtained from natural sources and most widely used as pharmaceutical excipients due to their binding, diluents, and disintegrant properties in solid dosage forms, due to suspending and gelling properties in gels and due to thickening properties in oral liquids.^[1] Natural gums, mucilages, and their derivatives are widely employed in the pharmaceutical industries because of their non-toxic behavior and safe

for human and animal consumption.^[2] Mucilage's are the metabolized product which is produced within the cell or produced without injury to the plant. Mucilage is composed of polysaccharide uranides and proteins. Gums are pathological products formed by breakdown of cell following injury to the

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plant (extracellular formation known as gummosis). Hence, natural gums have its application in the pharmaceutical and food industries which are considered to be safe for human consumption, while mucilage is the physiological product which is the main difference between the gum and mucilage.^[3,4]

Natural gums and mucilage are either water-soluble or absorb water to form a viscous jell like solution. Natural gums are less economic and easily available. They have been widely used as tablet binders and thickeners in cosmetics and suspensions as film-forming agents and transitional colloids. Polysaccharides such as gums and mucilage are most commonly used additives in pharmaceutical preparations. They are widely used especially in the formulation of suspensions and emulsions. The usefulness of polysaccharides as emulsifying^[5,6] suspending,^[7] disintegrating,^[8] and binding agents^[9] has been well reported in various articles,^[10] chemically mucilage is polysaccharide complexes in nature which are formed from sugar and uronic acid units,^[11] the presence of hydroxyl and carboxyl group in the mucilage favors adhesion of mucilage to mucosal surface.^[12] They also form slimy masses when come in contact with water and are typically heterogeneous in composition. On hydrolysis mucilage gives arabinose, galactose, glucose, mannose, xylose, and various uronic acids components. Mucilage is obtained mainly from seeds or other parts of the plant. Some are also obtained from marine sources such as algae and selected microorganisms.^[13] Natural gums and mucilage are biocompatible, cheap and easily available materials and are preferred to semi-synthetic and synthetic excipients because of their various advantages over synthetic materials such as lack of toxicity, low cost, easily available, emollient, and nonirritating nature.^[14] Some advantages of natural polysaccharide over synthetic materials are as follows:

Advantages of natural plant-based excipients.^[15]

- Low cost
- Natural in nature
- Free from side effects
- Biocompatible and bio-acceptable
- Renewable source
- Environmental friendly processing
- Easily available
- Better patient tolerance as well as public acceptance
- They also improve the national economy by providing inexpensive materials for formulations to people, using locally available materials.

Disadvantages of synthetic polymers used as excipients

- High cost
- Toxicity
- Non-biodegradability
- Environmental pollution caused by their synthesis.

Mimosa pudica (family Mimosaceae), commonly known as sensitive plant and widely used as herbal medicine in India, is widely available and has very low cost. It is diffuse under shrub found widely in the tropical and subtropical parts of India. Seeds of *M. pudica* yield mucilage, which is composed of *d*-xylose and *d*-glucuronic acid.^[16] *Mimosa* seeds mucilage hydrates and swells rapidly on coming in contact with water.^[17]

The present investigation involved isolation of mucilage from *M. pudica* seeds commonly known as chhui mui followed by phytochemical and physicochemical evaluation.

MATERIALS AND METHODS

Materials

The fresh *M. pudica* seeds were collected from Botanical Garden Greater Noida India. The plant was 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/2019/3437-38) All other chemicals used were of analytical grade, and distilled water was used throughout the experiments.

Description of seed (macroscopic characters)

- Shape: Compressed, oval-elliptic
- Color: Brownish yellow
- Size: 0.1–0.3-mm long, 2.5-mm broad, having a central ring on each surface
- Odor: None
- Taste: Mucilaginous [Figure 1].

Description of mucilage (microscopic characters)

- Color: Brownish-yellow, gray
- Size: Irregular
- Odor: None
- Taste: Mucilaginous
- Fracture: Smooth

Isolation of mucilage

Mucilage was isolated as per previously reported methods in various publications with little modifications. Accurate quantity (100 g) of seeds of *M. pudica* was weighed and processed for separating the brown peels from the kernel seed with the blander and plastic sieve were used to separate the seed. The seeds were then crushed lightly and soaked in double volume of water for 10 h, the hydrated mucilage along with seeds was spread in a thin layer on the stainless steel tray and dried in an oven at 50°C for 4–5 h. The dried mucilage was scraped from the tray by blade or knife and separated from the seeds by passing through a sieve 18 meshes. The mucilage was further purified by winnowing to separate seed husk. The dried mucilage powders were preserved in desiccators.^[18]

Characterization of mucilage

Isolated mucilage was characterized for their various properties by performing the following tests.

Organoleptic properties of isolated mucilage

Isolated mucilage was characterized for various parameters such as color, odor, taste, and texture.^[19]

Phytochemical properties of mucilage

Preliminary tests were performed to confirm the nature of isolated mucilage. The chemical tests that were conducted are as follows.^[19,20]

Test for carbohydrates: (Molisch's test)

To 1 ml of α -naphthol solution and concentrated sulfuric acid, 1 ml of isolated mucilage was added. The presence of carbohydrate was indicated by the appearance of purple or reddish violet color.

Test for tannins: (Ferric chloride test)

To 1 ml of isolated extract few drops of 5% w/v FeCl_3 solution were added. A green color indicates the presence of tannins.

Test for proteins: (Biuret test)

To 1 ml of 40% sodium hydroxide solution and two drops of 1% copper sulfate solution, 1 ml of isolated mucilage was added. The presence of proteins was indicated by formation of violet color.

Test for alkaloids: (Wagner's test)

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

Test for glycosides: (Legal test)

In pyridine and sodium nitroprusside solution, the mucilage was dissolved for making it alkaline. The presence of glycosides was exhibited by the formation of pink-red color.

Test for mucilage: (Ruthenium red test)

A drop of ruthenium red solution was added to a small quantity of mucilage placed on a glass slide and observed under microscope.

Test for flavonoid: (Shinoda test)

To 1 ml of magnesium and 1–3 drops of concentrated hydrochloric acid, 1 ml of the isolated mucilage was added. The presence of flavonoids was indicated by the formation of red color.^[19,20]

Physicochemical properties of mucilage

pH of mucilage

The mucilage was weighed and dissolved in water (1 g in 100 ml) to get a 1% w/v solution. The pH of solution is determined using digital pH meter. Again the process was repeated for 2% and 5%.^[19,20]

Bulk density

The bulk density of mucilage was measured by putting the accurately weighed (10 g) powder into a 100 ml graduated cylinder, and without disturbing the cylinder the volume of powder mucilage was read to give the bulk volume and was calculated using the following formula.^[21]

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

Tapped density

The tapped density was determined by three tap method. Weighed quantity (10 g) of powder mucilage was carefully introduced into a 100 ml graduated cylinder and dropped on hardwood surface on tiles 3 times from height of 2.5 cm. It was calculated using formula.^[21]

$$\text{Tapped density} = \frac{\text{Weight of powder}}{\text{Final volume after tapping}} \times 100$$

Carr's index determination

It was calculated from the value of bulk density and tapped density. Both of these properties were used for calculating the compressibility of the powder mucilage.^[22]

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

Hausner's ratio determination

It is a measure of flowability of the mucilage powder and was calculated by the following equation. The low value of Hausner's ratio means that the mucilage powder has high flowability. Hausner's ratio above 1.25 indicated poor flow.^[22]

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

Angle of repose

The angle of repose was determined by following the standard U.S.P 2010 method. For this mucilage powder 10 g was accurately weighed and carefully introduced into a funnel clamped to a stand with its tip 10 cm from a plane paper surface. The mucilage powder was allowed to flow freely onto the paper surface. After complete flow height of the cone, H

were and the radius of the cone, R were measured and used to calculate the angle of repose using the following equation.^[22]

$$\text{Angle of repose } \theta = \tan^{-1}(h/r)$$

Solubility behavior of mucilage

One part of dry mucilage powder was shaken with different solvents, and the solubility was determined.^[23]

Swelling index of isolated mucilage

Swelling index of the powdered mucilage was calculated by weighing a butter paper of size 2 cm × 2 cm, after this the butter paper was dipped in a Petri dish containing water and reweighed the wet butter paper again. After this, 10 mg of the powdered mucilage 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 min and the final result was calculated using the formula. The experiment was repeated using 0.1 N HCL and phosphate buffer solution (pH 6.8).^[24] The swelling index was measured using following equation.

$$\text{Swelling Index} = \frac{\text{Initial weight of the mucilage} - \text{Final weight of the mucilage}}{\text{Initial weight}} \times 100$$

Loss on drying

The moisture content of mucilage can be determined by loss on drying method. Accurately weighed 1 g sample is heated at 105°C to get a constant weight in a hot air oven and percent loss of moisture on drying is calculated using the following formula.^[25]

$$\text{LOD}\% = \frac{\text{Weight of water in sample}}{\text{Weight of dry sample}} \times 100$$

Total ash

About 5 g of mucilage was accurately weighed and taken in a silica crucible, which is previously ignited and weighed. The mucilage powder was spread as a fine, even layer on the bottom of the crucible. The crucible was 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 is calculated with reference to air-dried sample.^[26]

Water-soluble ash value

2 g of ash was boiled with 25 ml of water. The insoluble matter was filtered and collected on an ashless filter paper washed with hot water and ignited in a tarred crucible at a temperature not exceeding 450° for 4 h. The insoluble matter was cooled in desiccators, weighed, and subtracted from the total weight of 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 following formula.^[26]

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

Acid-insoluble ash value

Acid-insoluble ash value was determined by boiling the 2 g of ash for 5 min with 25 ml of 2 M HCl. The insoluble matter was filtered and collected on an ashless filter paper washed with hot water and ignited in a tarred crucible at a temperature not exceeding 450° for 4 h. The insoluble matter was cooled in desiccators and weighed. The percent of acid-insoluble ash was calculated with reference to the air-dried drug using the following formula.^[26]

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

RESULTS AND DISCUSSION

Isolation of mucilage from the seed of *M. pudica*, the percentage yield of mucilage was found to be 33.51%. The yield of mucilage was determined by weighing the dried isolated mucilage and applying the formula.

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

Isolated mucilage was also evaluated for organoleptic properties, and it was found that mucilage was odorless, yellowish-brown in color and mucilaginous in taste. The texture was found to be smooth. All these properties are shown in Table 1.

Phytochemical tests carried out on *M. pudica* seed mucilage confirmed the absence of alkaloids and glycosides. On treatment with ruthenium red, it showed red color indicated the presence

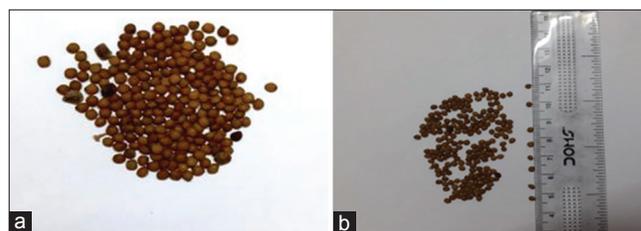


Figure 1: *Mimosa pudica*; a: normal seeds, b: seeds with measurement scale

Table 1: Organoleptic properties of mucilage

Colour	Odor	Taste	Fracture	Texture
Brownish-yellow	None	Mucilaginous	Smooth	Regular to gray

of mucilage. A violet ring formation at a junction of two liquids on reaction with Molisch's reagent showed the presence of carbohydrates, while tannins, alkaloids, proteins, glycosides, and flavonoids tests were found negative showed absence of these all as revealed in Table 2. The pH of 1%, 2%, and 5% w/v aqueous dispersion of the mucilage was found between 5.5 and 6. The pH measurements reveal that the mucilage at different concentrations was slightly acidic to neutral and nonirritating to mucous membrane. This acidic nature indicated that the mucilage contains uronic acids in its structure.

Micromeritic evaluations were done for the powder mucilage as bulk density, tapped density, Carr's index, angle of repose, for calculating the flow behavior of the mucilage, and all the values were found within the range. The angle of repose of the isolated mucilage was found to be 31.34 ± 0.30 it indicated that it has good flow property. The Bulk density and tapped density were found to be 0.182 ± 0.02 and 0.216 ± 0.02 g/ml, respectively. From this observation, it could be concluded that the mucilage possessed good micromeritic properties.

Table 2: Phytochemical properties of mucilage powder

Tests	Observation
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

Table 3: Physicochemical properties of mucilage powder

Parameters	Observation
pH (1% w/v)	5.5-6
Bulk density (gm/ml)	0.182 ± 0.02
Tapped density (gm/ml)	0.216 ± 0.02
Carr's index (%)	15 ± 0.01
Hausner's ratio	1.18 ± 0.02
Angle of repose (°)	31.34 ± 0.30
Solubility	Soluble in warm 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
Loss on drying (%)	5.3%
Total ash (%)	79.15%
Water-soluble ash (%)	63.5%
Acid-insoluble ash (%)	11%

The solubility of the isolated mucilage was studied in various solvents. One part of dry mucilage was shaken with different solvents, and the solubility was determined, mucilage was soluble in warm water, sparingly soluble in cold water, and insoluble in organic solvents.

As per the reported literature, swelling factor is very important and was the most widely accepted general mechanism of action for disintegration. Thus, from Table 3, it could be concluded that the *M. pudica* mucilage has good swelling index in water as well as 0.1 N HCL. It swells in an aqueous medium, and the swelling index of isolated mucilage was found to be 76.32% in water, 65.42% in HCL, and 48.22% in phosphate buffer which was similar to the value obtained using powdered seeds. It has high swelling property, and this property can be used as suspending and super disintegrating agent in various pharmaceutical formulations.

The loss on drying of powder mucilage determines both water and volatile matter present in the crude material. The weight loss on drying indicated that some amount of moisture was present in the material which is available to interact with other material at the time of formulation of different solid dosage forms.

Ash values indicated the quality and purity of a crude drug, especially in the powdered form. The objective of ash value of vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in analytical determination.

CONCLUSION

The mucilage isolated from the seed of *M. pudica* will be useful as an excipient for oral 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 flowability, weakly acidic pH, swelling potential, and viscous in nature. The present study suggests that isolated mucilage from the seeds of *M. pudica* 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 disintegrant in various formulations.

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