

## CHARACTERIZATION OF MUCILAGE FROM *CASSIA FISTULA* AS PHARMACEUTICAL EXCIPIENT

Madhu Bala, \*Mr. Pravin Kumar, Vinay Pandit and M. S. Asawat

Department of Pharmaceutics, Laureate Institute of Pharmacy, H. P., India.

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### \*Corresponding Author

Mr. Pravin Kumar

Department of  
Pharmaceutics, Laureate  
Institute of Pharmacy, H. P.,  
India.

### ABSTRACT

Natural polymers remain appealing primarily because they are plant-based products that are readily available, inexpensive, and capable of a wide range of chemical modifications. The purpose of this study is to extract and characterize mucilage obtained from *cassia fistula* seeds for use as pharmaceutical excipients. Acetone was used in the mucilage extraction. The solubility of extracted mucilage in various solvents was investigated. Various parameters, including tests for carbohydrates, protein, starch, reducing and non-reducing sugars, alkaloids, and other parameters, as well as Micromeritic properties such as bulk and tapped density, Carr's consolidation index, hausner's

ratio, and swelling index, were discussed in order to characterize the extracted mucilage. *Cassia fistula* (Family: fabaceae). It has numerous medical and pharmacological applications. It has a high mucilage content, which can be used as an additive in pharmaceutical formulations. It is used in traditional medicine and has diuretic, expectorant, stomachic, ophthalmic, antipyretic, anti-inflammatory activity, antioxidant, demulcent, emollient, febrifuge, and haemostatic properties. This paper provides an overview of plant pharmacological and photochemical properties, as well as therapeutic benefits.

**KEYWORDS:** Antioxidant, Antibacterial, Antifungal, Flavonoids.

### INTRODUCTION

Excipients play an important role in the formulation and development of dosage forms; however, despite their importance, the development of excipients receives less attention than the development of active pharmaceutical ingredients. Excipients are generally thought to be inert substances.<sup>[1]</sup> According to the International Pharmaceutical Excipients Council (IPEC), a pharmaceutical excipients is any substance other than the active drug or pro-drug that has

been appropriately evaluated for safety and is included in a drug delivery system to either aid processing of the system during manufacture; to protect, support, or enhance stability, bioavailability, or patient acceptability; to aid in product identification; to improve any other aspect of the drug product's overall safety and effectiveness during storage or use.<sup>[1]</sup>

Natural polymers have sparked renewed research interest due to their benefits such as low cost, biocompatibility, biodegradability, non-toxicity, local availability, environmentally friendly processing, and improved patient tolerance and acceptance.<sup>[2-4]</sup> Natural polysaccharides of plant origin are among the most commonly used excipients in pharmaceutical formulations. A variety of plant-based polysaccharides, such as starch, agar, acacia, alginate, and celluloses, are used as diluents, binders, disintegrating agents, gelling agents, and drug release sustaining agents in pharmaceutical formulations.<sup>[2,3]</sup>

Mucilage's are normal physiological byproducts of plant metabolism that are formed and retained within the cell wall of plant cells.<sup>[4]</sup> They are polysaccharides or complex carbohydrates that contain one or more monosaccharide or their derivatives linked in various ways.<sup>[5]</sup>

*Cassia fistula* belonging to the family *fabaceae* is one of the most widespread in the forests of India, usually occurring in deciduous forests the whole plant possesses medicinal properties.<sup>[6,7]</sup> The golden shower plant is a medium-sized tree, growing to 10–20 m (33–66 ft) tall with fast growth.<sup>[8]</sup> The flowers are bright yellow in colour, widely spaced petals, about 2 inches wide with 10 stamens. The flowers are produced in pendulous racemes 20–40 cm (7.9–15.7 in) long, each flower 4–7 cm (1.6– 2.8 in) diameter with five yellow petals of equal size and shape. The leaves are deciduous, 15–60 cm (5.9– 23.6 in) long, and pinnate with three to eight pairs of leaflets, each leaflet 7–21 cm (2.8– 8.3 in) long and 4–9 cm (1.6– 3.5 in) broad. The fruit is a legume, 30–60 cm (12–24 in) long and 1.5–2.5 cm (0.59–0.98 in) broad, with a pungent odor and containing several seeds.<sup>[9]</sup>

## MATERIAL AND METHOD

**Plant Material Collected:** The seeds of *cassia fistula* were collected in Hamirpur region of Himachal Pradesh, India.

**Extraction Procedure:** *cassia fistula* (golden shower) was obtained from hamirpur, India. The collected seeds were carefully washed and dried in the shade for 24 hours before being

dried in an oven at 30-40°C. The size was reduced using a grinder. Powdered seeds were passed through sieve no. 22 before being used for further testing.

### **Mucilage extraction consists of two steps**

**Step 1: Extraction of Mucilage:** Mucilage was extracted using powdered seeds of *cassia fistula*. The powdered seeds are placed in a 1000 ml beaker with 500 ml distilled water and allowed it for sufficient mucilage release in water. The concentrated solution was then filtered through muslin cloth to separate marc from the filtrate and refrigerated (3-4°C) for cooling.<sup>[10]</sup>

**Step 2: Mucilage Isolation:** Acetone was added to the extract in a quantity three times the volume of filtrate in order for mucilage precipitation to occur. The precipitated mucilage was washed with acetone before being collected through muslin cloth filtration. Mucilage was further dried in a hot air oven at a temperature less than 40°C. The dried mucilage was ground and passed through sieve #80 before being stored in an airtight container.<sup>[11]</sup>

### **Physiochemical characterization of isolated mucilage**

#### **Organoleptic Characterization of Isolated Mucilage**

The extracted mucilage was characterized in terms of color, odor, taste, texture, and fracture.<sup>[12]</sup>

**Identification Tests:** The mucilage aqueous extracts were prepared and mixed with Molish's reagent before being treated with sulphuric acid. The presence of carbohydrates is indicated by the appearance of a violet color ring at the junction.<sup>[13]</sup>

**Determination of Purity of Mucilage:** The purity of the extracted mucilage was determined by testing for alkaloids, proteins, and tannins.<sup>[10-11]</sup>

**Swelling Index:** It was calculated by the volume in ml absorbed by 1 gm of the powder in specified conditions. The swelling index of extracted mucilage was carried out using a specific amount of isolated powder mucilage with a standard procedure. It was calculated after 24 hours, and the final result was calculated using the formula.<sup>[12]</sup>

$$\text{Swelling Index} = \frac{\text{Final volume} - \text{initial volume}}{\text{Initial volume}} \times 100 \quad (1)$$

**pH of Mucilage:** A digital pH meter was used to determine the pH of a 1 percent w/v solution in water.<sup>[12]</sup>

**Solubility of Mucilage:** The powdered mucilage's solubility was determined by shaking it in various solvents such as water, acetone, methanol, ethanol and benzene.<sup>[14]</sup>

### Micromeritic Properties

**Bulk Density and Bulkiness:** Fixed amounts of isolated mucilage were placed in a graduated measuring cylinder. The cylinder was placed on the bulk density apparatus, and the volume of mucilage covered was recorded. The powder was then tapped in a bulk density apparatus until it reached a constant volume.<sup>[15]</sup> The final bulk volume was recorded. The equations 2, 3, 4 were used to calculate bulk density, tapped density, and bulkiness.

$$\text{Bulk density} = \frac{\text{Weight of powder}}{\text{Weight of apparent volume}} \quad (2)$$

$$\text{Tapped density} = \frac{\text{Weight of powder}}{\text{Tapped volume}} \quad (3)$$

$$\text{Bulkiness} = \frac{1}{\text{Bulkiness}} \quad (4)$$

**Angle of Repose:** The fixed height funnel method was used to calculate the angle of repose. The height (h) of the formed heap was measured, as well as the radius (r) of the cone base, which was also observed and calculated.<sup>[16-17]</sup>

As previously stated, the angle of repose was calculated using the equation 5:

$$\tan \theta = h/r \quad (5)$$

$\theta$  = Angle of repose

h = Height of pile

r = Radius of pile

**Carr's Consolidation Index (Compressibility) and Hausner's Ratio:** Using a bulk density apparatus, finely powdered mucilage was transferred into a measuring cylinder and compressibility and Hausner's ratio were calculated.<sup>[18]</sup>

$$\text{Carr's Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \quad (6)$$

$$\text{Hausner's Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}} \quad (7)$$

**Particle Size Determination:** The optical microscope was used to determine the particle size of the powdered mucilage, which was then calculated using the equations.<sup>[8,9]</sup>

$$\text{Size of the particles} = \text{No. of particles in eye piece} \times \text{calibration Factor} \quad (8)$$

$$\text{Calibration Factor} = \frac{\text{Stage reading}}{\text{Ocular reading}} \times 0.01 \quad (9)$$

**Fourier Transform Infrared (FT-IR) Spectral Studies:** The functional groups in *cassia fistula* mucilage were identified using FTIR spectroscopy. Graph 3 depicts the FTIR spectra of mucilage derived from Hibiscus. The mucilage's FTIR spectrum revealed distinct peaks at 3267.3, 2917.1, 2319.3, 2112.4, 1598.3, 1404.5, 1315.1, 1240.6, and 1024.5cm.

### Identification of Pathogenic Microorganisms

The total colony forming units in unit weight of the mucilage were used to calculate the microbial load. In a sterile conical flask, 150 mL of mucilage (1 g) was suspended in peptone water and incubated at 37°C for 60 minutes. Nutrient agar (NA) plates were sterilised.

Pipetting out the clear supernatant (0.1 mL) onto sterile nutrient agar plates In a laminar air flow cabinet, the sample was spread on these plates with a sterile autoclaved spreader. Petridishes were inverted and incubated for 24 to 48 hours at 37 °C for bacterial growth and 27 °C for fungal growth. Microbial growth was examined on plates, and the number of colonies was counted and expressed in colony forming units per gramme of substances (cfu/g).

**Table 1: Phytochemical tests of isolated mucilage.**

S. No.	Test	Present/absent
1.	Carbohydrates	+
2.	Mucilage	+
3.	Volatile oil	–
4.	Proteins	+
5.	Fats	–
6.	Tannins	–
7.	Alkaloids	–
8.	Reducing sugar	+

+ Present, - Absent

**Table 2: Solubility of isolated mucilage in different solvent.**

S. No.	Solvent system	Solubility
1.	Water	Slightly soluble
2.	Methanol	Insoluble
3.	Ethanol	Insoluble
4.	Acetone	Insoluble
5.	Benzene	Insoluble

**Table 3: Organoleptic characterization of *Cassia fistula*.**

S. No.	Organoleptic properties	Result
1.	Color	light brownish
2.	Odor	odorless
3.	Taste	Mucilaginous
4.	Texture	Rough
5.	Fracture	Irregular

**Table 4: Micromeritic study data of isolated mucilage.**

S. No.	Parameters	Result ( $\pm$ S.D)
1.	Swelling Index	69.17% $\pm$ 5.462
2.	pH	6
3.	Bulk Density (gm/ml)	0.63 $\pm$ 0.004
4.	Tapped Density (gm/ml)	0.88 $\pm$ 0.024
5.	Angle of repose ( $^{\circ}$ )	29.21 $\pm$ 0.12
6.	Carr's Index (%)	9.41 $\pm$ 5.98
7.	Hausner's Ratio (%)	1.08 $\pm$ 0.03
8.	Mean Particle Size (um)	214.38 $\pm$ 112.48

## RESULTS AND DISCUSSION

Various evaluation parameters were applied to isolated mucilage. Several chemical tests were carried out to confirm the presence of various phytoconstituents. *Cassia fistula* mucilage was found to be high in carbohydrates, but low in alkaloids, tannins, fat, and oils. As a result, it confirms the presence of carbohydrates and proteins in the mucilage. Table 1 shows that

other phytoconstituents such as volatile oil, fat, alkaloid, and tannins were absent in isolated mucilage.

The organoleptic properties of isolated mucilage were investigated. It has a mucilaginous taste and is odorless. The texture and fracture were discovered to be rough and irregular. Table 2 displays the results of *cassia fistula* mucilage was slightly soluble in water, and insoluble in methanol, benzene, ethanol and acetone.

For the mucilage, various micromeritic studies such as bulk density, tapped density, bulkiness Carr's index and angle of repose for flow behaviour were performed. The isolated mucilage's angle of repose was found to be  $29.21 \pm 0.12$  demonstrates that it has excellent flow properties.

The bulkiness and Carr's index value indicate that the powder is heavy and has excellent flow properties, as the Carr's index value is  $9.41 \pm 5.98$ . Table 4 displays the results.

The pH of a 1% solution was determined to be 6, which is non-irritating to the mucous membrane. This demonstrates the mucilage's compatibility. The isolated mucilage swelling index was found to be  $69.17 \% \pm 5.462 \%$  describing a high swelling property that can be used to delay drug release up to a desired time period and can be used in the formulation of controlled drug delivery.

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