

E-ISSN: 2278-4136

P-ISSN: 2349-8234

<https://www.phytojournal.com>

JPP 2024; 13(2): 215-219

Received: 07-01-2024

Accepted: 19-02-2024

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Isolation and characterization of mucilage from flower petals of *Bombax ceiba*

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DOI: <https://doi.org/10.22271/phyto.2024.v13.i2c.14881>

Abstract

The objective of present investigation was to extract the mucilage from flower petals of *Bombax Ceiba* and explore its use as a promising excipient for Pharmaceutical preparation. The natural polymers have always an exceptional property which makes them distinct from synthetic polymer and *Bombax Ceiba* flower mucilage is one such example which shows more valuable properties making it a useful excipient for wide range of applications. Development of new excipients is time consuming, involves tedious procedures and is highly expensive. Instead, identification of new uses for the existing substances is relatively inexpensive and less time consuming. Natural gums and mucilage's have been widely explored as pharmaceutical excipient. Mucilage's are polysaccharide complexes formed from sugar and Uronic acid units. Mucilage's form slimy masses in water are typically heterogeneous in composition. The flower petals of *Bombax Ceiba* contain a proportion of mucilage and it also being used for different therapeutic purposes. However there are no reports on isolation and characterization of mucilage of *Bombax Ceiba*. Hence the present study was designed for isolation and characterization of mucilage from the flower petals of *Bombax Ceiba* and explores its use as a pharmaceutical excipient. Various methods to isolate mucilage were tried and simple, mucilage and optimum method was developed. Physicochemical properties of *Bombax Ceiba* mucilage such as appearance, Odour, solubility, Bulk and Tapped Densities, Hausner's Ratio, swelling index, pH, and Angle of Repose were studied.

Keywords: *Bombax ceiba*, flower petals mucilage, polymer, eco-friendly, Natural excipient

Introduction

Formulation of an active pharmaceutical ingredient into desired dosage forms are rarely possible without the addition of excipients. They are vital part of medicinal compound, which may be also major portion in medicinal product. These are inert molecules that play a very important role in designing of dosage form ^[1]. Today, we have a number of plant-based pharmaceutical excipients, which may be selected and optimised based on the properties of drug, requirements of the dosage form and its site of action. Apart from its common functions like serving as inert vehicle for the administration of right volume of active pharmaceutical ingredient with consistency in weight, excipients also fulfil multifunctional roles such as release retardants, solubility enhancers, viscosity modifiers etc. In addition to this they offer significant advantages in ease of manufacturing, enhancement of patient compliance, improved bioavailability, reproducibility, targeted delivery etc. ^[2]. Mucilages are most commonly used adjuvant in pharmaceutical preparations. Plant mucilages are pharmaceutically important polysaccharide with wide range of applications such as thickening gelling agent, binding, disintegrating, suspending, Emulsifying, stabilizing and gelling agents. They have been also used as matrices for sustained and controlled release drugs ^[3]. Apart from its use in finished medicines, newer uses have been found in the preparation of cosmetics, textiles and paint paper. Hence the demand for these substances is increasing and new sources are getting tapped ^[4-5]. Vast application of plant mucilage and gums in various industries is because of low cost, ready availability and important properties which they confer on products. Demand for these substances is increasing and new sources are being developed. India, because of its geographical and environmental position, has traditionally been a good source for such products among the Asian countries. Naturally available mucilages are preferred to synthetic materials due to their non-toxicity, low cost, ease of availability, emollient and non-irritating nature & less regulatory problem ^[6].

The different species of *Bombax* are reported to possess various medicinal properties *viz* cholera, fractures, smallpox, coughs, urinary problems, influenza. The *Bombax Ceiba* is widely grown as an ornamental plant throughout the tropical and subtropical regions ^[7]. In the present study, an effort was made to extract the mucilage from *Bombax Ceiba* flower petals by

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the, microwave assisted method, Conventional method and Soxhlet extraction method. The three different methods were used to check the yield and efficiency of method of extraction. It was then evaluated to check the possibility of using this mucilage as binding/granulating agents in tablet formulation. The binders are the pharmaceutical excipient that is commonly employed in tablet formulation to improve the flow properties of the granules.

Materials and Methods

The flowers of *Bombax Ceiba* were collected from NRIASHRD Gwalior region of India and were authenticated at Botanical survey of India, Central regional centre, Allahabad, U.P. The authentication accession number is 103976 at 18 December 2018.

Isolation of mucilage from *Bombax ceiba* flower petals

Mucilage can be isolated by different methods. A number of methods were used to isolate the *Bombax Ceiba* petals mucilage. The mucilage isolated from the different methods was compared for the yield of the mucilage. The procedure giving maximum yield of mucilage was used for isolation. The various isolation methods used to isolate the *Bombax Ceiba* flower petals mucilage are given below.

Microwave assisted method

The fresh petals of *Bombax ceiba* flower were collected, washed with water to remove dirt and debris. The petals of flowers (150 g) were crushed and soaked in distilled water (500 ml) for 24 h. The soaked flowers were kept in a microwave oven along with a glass tube inside to prevent bumping and the process of microwave irradiation was start at 420 W intensity for 7 min. The beaker was detached from the oven and kept away for 2 hrs. for the discharge of mucilage into water. The material was filtered through a muslin bag and hot distilled water (25 ml) was added through the sides of the marc and squeezed well in order to remove the mucilage completely. Equal volume of Ethanol was added to the filtrate, thus precipitate of mucilage was shown and it was kept inside a refrigerator for one day for effective settling. It was filtered and dried totally in an incubator at 37 ± 2 °C, powdered and weighed. The amount of the mucilage acquired from microwave were calculated (Felkai-Haddache *et al.*, 2016) [8].

Conventional method

The completely dried *Bombax ceiba* petals of flowers were collected and 150g were powdered for 5 min in a mechanical blender and soaked in distilled water (500 ml) for 24 hrs in a Round bottom flask. It was then boiled in water for 1 hr under reflux with occasional stirring and kept aside for 2 hrs for the release of mucilage into water. The material was filtered through a muslin bag and hot distilled water (100 ml) was added through the sides of the marc and squeezed well in order to remove the mucilage completely. Equal volume of Ethanol was added to the filtrate to precipitate the mucilage and kept inside a refrigerator for one day for effective settling. It was filtered dried completely in an incubator at 37 °C, powdered and weighed. It was subjected to chemical tests to confirm its identity.

Soxhlet extraction method

The completely dried petals of flower of *Bombax ceiba* were collected, and dried then 150g were powdered for 5 min in a mechanical blender and packed in soxhlet apparatus for

deflating with petroleum ether, Benzene and Chloroform till completion of extraction and obtained extract was concentrated under reduced pressure using rotary evaporator to get extracts.

Then deflatted flowers were kept in boiled water for 1 hr under reflux with occasional stirring and kept aside for 2 hrs for the release of mucilage into water. The material was filtered through a muslin bag and hot distilled water (100ml) was added through the sides of the marc and squeezed well in order to remove the mucilage completely. Equal volume of Ethanol was added to the filtrate to precipitate the mucilage and kept inside a refrigerator for one day for effective settling. It was filtered dried completely in an incubator at 37 °C, powdered and weighed.

Characterization

Physical analysis of mucilage powder

The dried mucilage of *Bombax Ceiba* was studied for appearance, solubility, pH, swelling index, Loss on Drying, DSC Analysis, FT-IR Analysis, XRD Analysis, SEM Analysis and flow properties.

Solubility study of mucilage

The solubility of drug was determined in various solvents (Water, 0.1 N HCl, Ethanol, Chloroform). The excess amount of drug was added to 10 ml of medium and stirred constantly overnight at 37 ± 0.5 °C.

Loss on Drying

1 gm of mucilaginous powder was taken in a petridish. This was dried at 105 °C till constant weight. The value of loss on drying was calculated in percentage. The experiment was performed in triplicate.

Calculation

Loss on drying = $W2 - W1 / W \times 100$
W1 = Weight of empty weighing bottle
W2 = Final weight of weighing bottle
W = Weight of sample

Determination of viscosity

The viscosity study of 1.0% w/v solution for isolated mucilage at different temperatures was measured by Brook field viscometer. The sufficient quantity was filled in wide mouth jar separately and it should sufficiently allow dipping the spindle. The RPM of the spindle was adjusted to 2.5 RPM. The viscosities of the formulations were recorded and result indicated that viscosity decrease with increase of temperature.

Determination of swelling index

Swelling characteristics of the mucilage was tested in distilled water. The Swelling index is the volume in ml occupied by 1 g of the substance. The Swelling index of the mucilage powder was determined according to British Pharmacopoeia method. The test was performed by taking 1 g of the mucilage powder in a 50.0 ml ground glass stoppered cylinder graduated over a height of 120 to 130 mm in 0.5 divisions. To this 25 ml of distilled water was added and this was shaken vigorously every 10 minutes for 1 hour and then allowed to stand for 24 hours. The volume occupied by the mucilage powder was measured.

pH of solution

The pH of the 1% w/v aqueous mucilage solution was measured with a pH meter (Equip-Tronics, EQ-610).

Flow Properties

The flow properties of isolated mucilage powder were characterized in terms of Carr's index, hausner's ratio and angle of repose. The Carr's index ((IC)) and Hausner's ratio (HR) of powders were calculating according to following equation.

$$\text{Carr's Index (IC)} = \rho_{\text{Tapped}} - \rho_{\text{Bulk}} / \rho_{\text{Tapped}}$$

$$\text{Hausner's ratio (HR)} = \rho_{\text{Tapped}} / \rho_{\text{Bulk}}$$

The angle of repose (θ) was measured by fixed height method. This was calculated by following equation

$$\text{Angle of repose } (\theta) = \tan^{-1} 2 H / D$$

Where H is the surface area of the free standing height of the powder pile and D is diameter of pile that formed after powder flow from the glass funnel. The result is given in Table.

Chemical characterization

The polymer collected from flower petals was tested for chemical characteristics for identification, test for, test for tannins, test for alkaloids, Cardiac Glycoside, flavanoids, carbohydrates, triterpines, tannin, protein, and saponins. The results were tabulated.

Fourier transform analysis

Fourier Transform Analysis (FTIR) spectra of mucilage were recorded on a FT-IR spectrometer (Thermo Scientific). The dry powder was mixed with KBr and pressed into pellets under mechanical pressure. The FT-IR spectra were obtained by scanning between 4000 and 400/cm. (Figure 1). The mucilage showed the presence of spectrum lines at 1018, 1247, 1455, 1574, 1734, 2923 and 3280 cm⁻¹. The result showed the presence of lactone ring with -OH, -COOH, -CH₃ stretching, -C-O stretching in functional group structure.

Table 2: Physical Characterization of *Bombax Ceiba* flower petals mucilage

S. No.	Physical Property	Observation
01	Appearance	Dark brown colour
02	Odour	Characteristic
03	Solubility	Soluble in water & 0.1N HCL, Insoluble in ethanol & chloroform
04	Swelling ratio	30%
05	pH	7.13
06	Viscosity at 25°C	1338
07	Loss on Drying	1.34

Phytochemical tests carried out on *Bombax Ceiba* mucilage confirmed the presence of alkaloids, carbohydrates, tannin, saponin & protein. On treatment of mucilage with ruthenium red, it showed red colour confirming the obtained product as

Thermal Studies by DSC

Weighed amount of sample were placed in hermitically sealed aluminium pans and were heated at a speed of 20 °C/min over a temperature range of 50 °C to 450 °C in a differential scanning calorimetry (Perkin-elmer DSC-7) at a chart speed of 10 mm/min (Figure 2). The melting point of plant isolated mucilage particles was 287.54 °C.

Powder X-ray diffraction pattern

Powder X-ray diffraction (PXRD) patterns of mucilage were recorded using X-ray diffractometer (Goniometer, BI-200SM). The experiments were carried out at 25 °C: voltage and current were kept constant at 40 Kv, 30 m.A. respectively.

Results and Discussion

Bombax Ceiba flower petals mucilage is isolated by Microwave assisted method. This method is selected because it gives highest total yield of mucilage as compared to other isolation methods and the details were shown in table 1. The average yield of dried mucilage obtained from *Bombax Ceiba* flower petals was 10g/kg.

Table 1: Average % yield of dried mucilage through different methods

S. No.	Method of Isolation	% Yield
1	Microwave Assisted method	7.76±0.007
2	Conventional method	5.84±0.004
3	Soxhlet extraction method	4.23±0.001

The mucilage obtained was subjected to physicochemical characteristics and the results were summarized in table 2. The mucilage of flower petals is soluble in water and in 0.1N HCL. Carr's index was 13.79±0.031%, Hausner ratio was 1.12±0.022 and angle of repose was found to be 24.8±0.121 for flower petals mucilage.

mucilage. A violet ring was formed at the junction of two liquids on reaction with Molisch's reagent indicating the presence of carbohydrates. The results of phytochemical screening of mucilage are summarized in table 3.

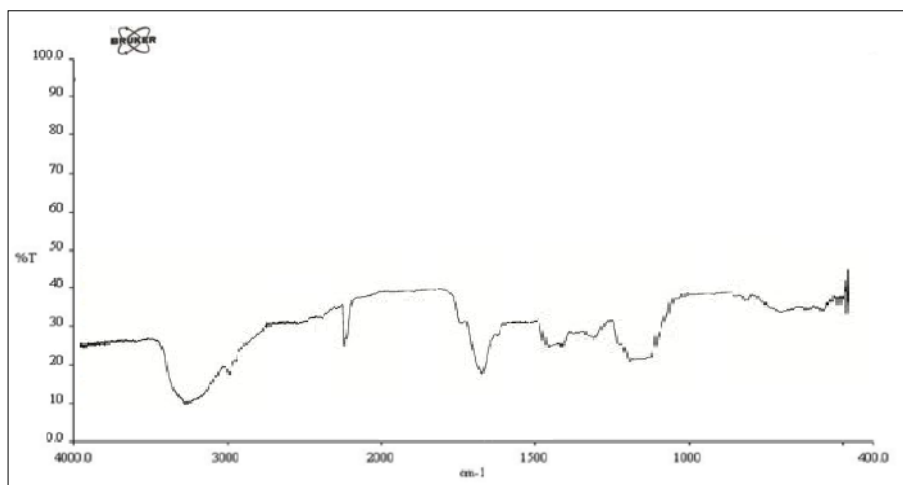
Table 3: Phytochemical analysis of *Bombax ceiba* flower extracts

S. No.	Chemical class	Chemical test	Microwave Assisted method	Conventional method	Soxhlet extraction method
1	Alkaloids	Dragendorff's test	+	+	+
2	Cardiac glycoside	Keller-killani test	-	-	-
3	Flavanoid	Shinoda Test	-	-	+
4	Carbohydrate	Molish test	+	+	+
5	Triterpenes	Vanillin-sulphuric acid test	-	-	+
6	Tannin	Ferric chloride test	+	+	+
7	Proteins	Biuret test	+	+	-
8	Saponins	Lead acetate test	+	+	+

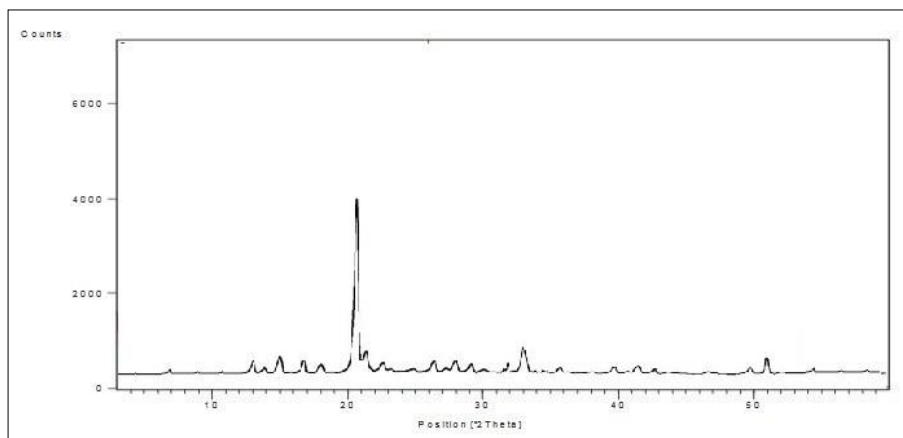
Table 4: Micromeritic properties of *Bombax Ceiba* flower petals mucilage (n=3)

S. No.	Property	Values
01	Car's index	13.79±0.031
02	Hausner's ratio	1.12±0.022
03	Angle of repose	24.8±0.121

The FT-IR spectra of mucilage showed the presence of spectrum lines at 1018, 1247, 1455, 1574, 1734, 2923 and 3280 cm⁻¹. The result of FTIR study showed the presence of lactone ring with -OH, -COOH, -CH₃ stretching, -C-O stretching in functional group structure (Figure 1).

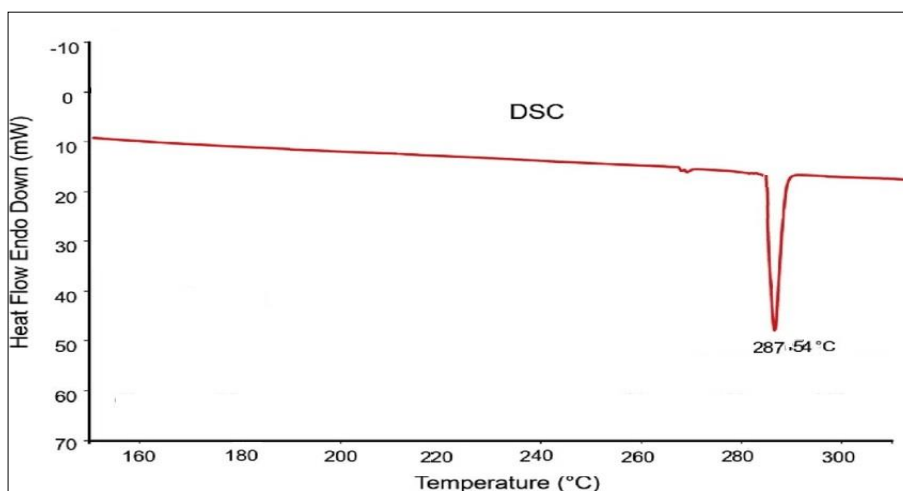
**Fig 1:** FTIR spectra of plant mucilage of Bombax ceiba Flower

The X-ray diffraction at an angle of 2θ with a scan step time of 10.33 sec for a specific length of 10 mm. XRD thermograms were shown in figure 2.

**Fig 2:** XRD spectra of plant mucilage of Bombax ceiba Flower

DSC is a very useful tool in the investigation of thermal properties of a compound. DSC thermogram of mucilage

powder shows sharp peak at 287.54°C which is corresponds to its melting point. DSC thermograms were shown in figure 3.

**Fig 3:** DSC thermogram of plant mucilage of Bombax ceiba Flower

Conclusion

It can be seen that the yield obtained from microwave method for extraction of mucilage is much better as compared to the conventional method because microwave method works on cellular level for extraction of the mucilage whereas conventional method is less efficient because it only uses heat externally for the extraction of mucilage. Hence we can conclude that microwave assisted method is more suitable, fast economic and simple for extraction of mucilage as compared to the conventional method. The *Bombex ceiba* flower mucilage exhibit good binding properties for uncoated tablets. The increased concentration of mucilage showed small retardation in drug release from tablet. There was increase in the disintegration time and hardness of tablet and decrease in the friability of the tablet with increasing the concentration of mucilage. Dissolution profile shows the steady release of drug from formulation within half an hour and also with increase in binder concentration release of drug decreases slowly. So, it can be concluded that mucilage isolated from *Bombex ceiba* flower can be used as a potential natural binding agent for formulating tablets.

Acknowledgement

The authors wish to thank Dr. Navneet Garud & Dr. Mukul Tailing, School of studies in Pharmaceutical sciences, for their skilful technical assistance.

Conflict of Interest

The authors declared no conflicts of interest.

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