Extraction and Characterization of *Hibiscus rosasinensis* Mucilage as Pharmaceutical Adjuvant

Somya Gupta, Nayyar Parvez and Pramod Kumar Sharma

Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Greater Noida, U.P. India-203201

**Abstract:** The objective of the study was to characterize and evaluate the leaves of China rose (*Hibiscus rosasinensis* Linn) mucilage as pharmaceutical adjuvant. The mucilage was extracted using distilled water and isolated by acetone. Different parameters such as tests for carbohydrates, protein, fat, reducing and non-reducing sugars, alkaloids, tannins, phenolic compounds and other parameters like micromeritic properties, surface tension, swelling index and viscosity were evaluated for characterizing the extracted mucilage. The result shows that the extraction of mucilage with water has excellent flow properties. Various phytochemical tests show that carbohydrates and amino acids were only present. These tests indicate the purity of the mucilage. The pH and surface tension of 1% solution of mucilage was found to be 6.90 ± 0.011 and 81.62 ± 2.92, respectively. It has a good swelling index of 66.17 ± 0.462%. The total ash value was found to be 7.57 ± 0.233%. Extracted mucilage is insoluble in cold water and this property can be utilized for controlling drug delivery. The results of evaluated parameters showed that *Hibiscus rosasinensis* mucilage has satisfactory pH and physicochemical properties, which can be used as pharmaceutical adjuvant in the formulation of various dosage forms for sustaining the release of the drug.

**Key words:** *Hibiscus rosasinensis* · Mucilage · Isolation · Characterization · Natural Polysaccharides · Sustained Release

**INTRODUCTION**

Pharmaceutical excipients are the additives used to convert pharmacologically active substances into a dosage form suitable for administration to the patients [1]. The pharmaceutical excipients obtained from the natural sources are economic and used widely as compared to the synthetic pharmaceuticals. This is due to their advantageous properties such as low cost, relative abundance and biocompatibility as compared to their synthetic ones. These are used as gelling agent, lubricating agent, sweetening agent, binding agent, bulking agent, flavouring agent and suspending agent [2,3]. In sustained release dosage forms, release of drug is sustained due to the swelling property of these polymers by making a gel like thick layer which retard the release of drug. These polymers can be hydrophilic or hydrophobic in nature [4].

Natural Gums are naturally occurring polysaccharides found in plants to which multiple sugar units are linked together to form large molecules. These gums are pathological products formed by breakdown of cell following injury to the plant (extracellular formation: Gummosis). Hence, Natural gums have diverse application in the pharmaceutical and food industries which are considered to be safe for human consumption [5].

Mucilage is composed of polysaccharide uranides and proteins. Mucilage is the metabolized product, produced within the cell and/or produced without injury to the plant. The main difference between the gum and mucilage is that gums are pathological products, while mucilage is the physiological product [6]. Gum has the property of swelling in aqueous media forming a highly viscous solution while mucilage forms slimy mass in water. Both gum and mucilage are produced by plants during injury which are amorphous, translucent. Mucilage, resin, cellulose and gum are differentiated by the condensation of hexane and pentose [7-9].

Furthermore, natural gums can be used in food industry in which xanthan gum, flaxseed mucilage and
mixture have been used to prepare reduced fat mayonnaise and they can be helpful in reducing fat, sugar, cholesterol etc. as present time customers look upon anything natural, which is safe and free from side-effects [10].

This article emphasises on the extraction and characterization of mucilage from the leaves of *Hibiscus rosasinensis* Linn commonly known as China rose, belonging to the family Malvaceae and is a potent medicinal plant [11]. Mucilage of the leaf has anti-inflammatory activity [12] and reported to have various medicinal properties such as hypoglycaemic, antioxidant, antihypertensive activity [13]. Extracts of hibiscus also shows protective effect against cancer development [14].

**MATERIAL AND METHODS**

**Plant Material:** Leaves of *Hibiscus rosasinensis* were collected from Gr. Noida (Uttar Pradesh, India) in the month of October-November, 2014. The plant was identified by Biotechnology Department, Gautam Buddha University, Gr. Noida and voucher specimens were deposited in that Department.

**Extraction Procedure:** *Hibiscus rosasinensis* (China rose) was procured from the local area of Greater Noida, India. Collected leaves was carefully washed and dried under shade for 24 h and then further dried in oven at 30-40°C. Size was reduced with the help of grinder. Powdered leaves were passed through sieve no. #22 and then used for further evaluation.

Extraction of mucilage includes 3 steps.

**Step 1: Extraction of Mucilage:** Powdered leaves of *Hibiscus rosasinensis* were used for the extraction of mucilage. The powdered leaves are placed in 1000ml beaker containing 500ml of distilled water and allowed it to boil for at least 3-4 h with continuous stirring and heating at 60°C for sufficient release of mucilage in water. Concentrated solution was then filtered through muslin cloth in order to separate marc from the filtrate and refrigerated for cooling (3-4°C) [15].

**Step 2: Isolation of Mucilage:** To the extract, acetone was added to the quantity, three times the volume of filtrate for precipitation of mucilage to occur. The precipitated mucilage was washed with acetone and then collected through filtration by muslin cloth. Mucilage was further dried in hot air oven at a temperature less than 40°C. The obtained dried mucilage was grinded and passed through sieve #80 and finally stored in air tight container [16].

**Physicochemical Characterization of Isolated Mucilage**

**Organoleptic Characterization of Isolated Mucilage:** The extracted mucilage was characterized for various parameters like color, odor, taste, texture and fracture [17].

**Identification Tests:** The Aqueous extracts of the mucilage was prepared and mixed with Molish’s reagent followed by addition of sulphuric acid. Appearance of violet color ring at junction shows the presence of carbohydrates [18].

**Determination of Purity of Mucilage:** Purity of extracted mucilage was measured by performing tests like alkaloids, glycosides, proteins, gum, fat, tannins and amino acids [15-16].

**Swelling Index:** It was calculated by weighing a butter paper of size 2X2 cm. then butter paper was dipped in a Petridish containing water and reweighed. After this 10 mg of the powdered sample was kept in a butter paper placing this on a Petridish containing 15 ml of water and the swelling index was calculated after 24 h and the final result was calculated using the formulae [17].

\[
\text{Initial Weight} = \text{Swelling Index} = \frac{\text{Initial Weight} - \text{Final weight}}{\text{Initial Weight}} \times 100 \quad (1)
\]

**pH of Mucilage:** The pH of 1% w/v solution in water was determined using digital pH meter [17].

**Solubility of Mucilage:** Solubility was determined by shaking the powdered mucilage in different solvent such as acetone, ethyl alcohol, benzene, chloroform and glycerine [19].

**Micromeritic Properties**

**Bulk Density and Bulkiness:** Fixed quantities of the isolated mucilage were transferred into a graduated measuring cylinder. The cylinder was placed on the bulk density apparatus and the volume covered by the mucilage was noted down. Then, the powder was tapped in a bulk density apparatus until a constant volume was obtained. The final bulk volume was noted [20]. Bulk density, tapped density and bulkiness were calculated using the equations 2, 3, 4.

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

\[
\frac{\text{Weight of powder}}{\text{Weight of apparent volume}} = \frac{\text{Initial Weight} - \text{Final weight}}{\text{Initial Weight}} \quad (3)
\]
Weight of powder
Tapped Density = ----------------------------------- (3)
                  Tapped volume

1
Bulkiness = ------------------------ (4)
            Bulkiness

Angle of Repose: Angle of repose was determined by fixed height funnel method. The height \( h \) of the heap formed was measured and the radius \( r \) of the cone base was also observed and calculated [21, 22].

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

\[
\tan \theta = \frac{h}{r}
\]

\( \theta \) = Angle of repose  
\( h \) = Height of pile 
\( r \) = Radius of pile

Carr’s Consolidation Index (Compressibility) and Hausner’s Ratio: Finely powdered mucilage (5gm) was transferred into a measuring cylinder and compressibility, Hausner’s ratio were calculated using bulk density apparatus [23].

\[
\text{Carr’s Index} = \frac{\text{Tapped density} - \text{Bulk density} \times 100}{\text{Tapped density}} (6)
\]

\[
\text{Hausner’s Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}} (7)
\]

Particle Size Determination: Particle size of the powdered mucilage was determined using optical microscope; and calculated using the equations (8), (9).

\[
\text{Size of the particles} = \frac{\text{No. of particles in eye piece}}{\text{calibration Factor}} \times X (8)
\]

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

Surface Tension: Surface tension of the powdered mucilage was measured using stalagmometer, using drop weight method [16]. Binding property of the polymer is related to surface tension.

Viscosity: Viscosity of 1% polymer solution was measured by Ostwald viscometer in which, the flow times of isolated polymer solution was compared with that of liquid whose viscosity is known [17].

Ash Value: 2 g of powdered mucilage was weighed accurately in a china dish and kept in muffle furnace (500°C) until the powdered sample is converted into ash and then reweighed [16]. Ash value was calculated using the equation (10).

\[
\text{Weight of ash} = \frac{\text{Total Ash value}}{\text{Weight of polymer}} \times 100 (10)
\]

RESULTS AND DISCUSSION

Isolated mucilage was subjected to various evaluation parameters. Various chemical tests were performed for confirmation of various phytoconstituents. Hibiscus rosasinensis mucilage gave positive test for carbohydrates and amino acids and negative test for alkaloid, tannins, protein, fat and oils. Thus, it confirms that the mucilage contains carbohydrates and amino acids. Other phytoconstituents as proteins, gum, fat, alkaloid and tannins were absent in isolated mucilage as depicted in table 1.

Isolated mucilage was evaluated for organoleptic properties. It has mucilaginous taste and has characteristic odour. Fracture and texture was found to be rough and irregular. The results are shown in table 2. The mucilage isolated from Hibiscus rosasinensis was soluble in warm water and slightly soluble in cold water and insoluble in benzene, ether, chloroform, n-butanol, ethanol, acetone, glycerine, paraffin.

Various micromeritic studies was done for the mucilage as carr’s index, angle of repose, bulk density, true density, bulkiness for flow behaviour. The angle of repose of the isolated mucilage was found to be 7.968 ± 0.755. It shows that it has excellent flow property.

The bulkiness and Carr’s index value indicate that powder is heavy in nature and shows excellent flow properties as value of Carr’s index is 7.33 ± 1.98. The results are shown in table 3.

pH of 1% solution was found to be 6.90 ± 0.011 which is non-irritating to the mucous membrane. This shows good compatibility of the mucilage. Swelling index of isolated mucilage was found to be 66.17% ± 0.462%
**Table 1: Phytochemical tests of isolated mucilage**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test</th>
<th>Present/absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Hexose sugar</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Monosaccharides</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Proteins</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Fats and Oils</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Tannins and Phenolic compounds</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Amino acids</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Present, - Absent

**Table 2: Organoleptic characterization of Hibiscus rosasinensis**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Organoleptic properties</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Color</td>
<td>Green</td>
</tr>
<tr>
<td>2.</td>
<td>Odor</td>
<td>Characteristics</td>
</tr>
<tr>
<td>3.</td>
<td>Taste</td>
<td>Mucilaginous</td>
</tr>
<tr>
<td>4.</td>
<td>Texture</td>
<td>Irregular</td>
</tr>
<tr>
<td>5.</td>
<td>Fracture</td>
<td>Rough</td>
</tr>
</tbody>
</table>

**Table 3: Micromeritic study data of isolated mucilage**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Result (±S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Swelling Index</td>
<td>66.17±0.462</td>
</tr>
<tr>
<td>2.</td>
<td>pH</td>
<td>6.90±0.011</td>
</tr>
<tr>
<td>3.</td>
<td>Surface tension (dyne/cm)</td>
<td>81.62±2.92</td>
</tr>
<tr>
<td>4.</td>
<td>Bulk Density (gm/ml)</td>
<td>0.508±0.006</td>
</tr>
<tr>
<td>5.</td>
<td>Bulkiness (ml/g)</td>
<td>1.968±0.027</td>
</tr>
<tr>
<td>6.</td>
<td>Tapped Density (gm/ml)</td>
<td>0.55±0.008</td>
</tr>
<tr>
<td>7.</td>
<td>Angle of repose (°)</td>
<td>7.968±0.755</td>
</tr>
<tr>
<td>8.</td>
<td>Carr’s Index (%)</td>
<td>7.33±1.98</td>
</tr>
<tr>
<td>9.</td>
<td>Hausner’s Ratio (%)</td>
<td>1.08±0.03</td>
</tr>
<tr>
<td>10.</td>
<td>Mean Particle Size (µm)</td>
<td>165.38±15.48</td>
</tr>
<tr>
<td>11.</td>
<td>Viscosity (poise)</td>
<td>12.23±0.070</td>
</tr>
<tr>
<td>12.</td>
<td>Ash Value (%)</td>
<td>7.57±0.233</td>
</tr>
</tbody>
</table>

**Table 4: Functional groups and peaks in IR spectra of Hibiscus rosasinensis mucilage**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Functional Group</th>
<th>Peak (Frequency) (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>C-H bend out of plane</td>
<td>1038.56</td>
</tr>
<tr>
<td>2.</td>
<td>C-O bend ethers, aromatics</td>
<td>1250.22</td>
</tr>
<tr>
<td>3.</td>
<td>C-H rock</td>
<td>1369.37</td>
</tr>
<tr>
<td>4.</td>
<td>C-H bend</td>
<td>1420.56</td>
</tr>
<tr>
<td>5.</td>
<td>C-H bend</td>
<td>1455.60</td>
</tr>
<tr>
<td>6.</td>
<td>Amide</td>
<td>1540.36</td>
</tr>
<tr>
<td>7.</td>
<td>Alkyne</td>
<td>1678.48</td>
</tr>
<tr>
<td>8.</td>
<td>Ketone</td>
<td>1723.68</td>
</tr>
<tr>
<td>9.</td>
<td>Carboxylic acid</td>
<td>1740.00</td>
</tr>
</tbody>
</table>

**CONCLUSION**

From the whole study, it can be concluded that mucilage isolated from the leaves of *Hibiscus rosasinensis* can be used as pharmaceutical adjuvant for drug delivery.
The isolated polymer has pH value 6.9, so non-irritant in nature and has good biocompatibility. Various physicochemical studies showed that it was acceptable, suitable and has potential to be used as formulation additive in novel drug delivery systems for controlled drug delivery.

Conflict of Interest: The authors declare no conflict of interest on the manuscript.

ACKNOWLEDGEMENT

The authors are very thankful to the Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Greater Noida and NISCAIR (National Institute of Science Communication and Information Resources), New Delhi, India for providing facilities in the completion of this manuscript.

REFERENCES


