5.2 Extraction and Characterization of *Aesculus indica* Mucilage

*A. indica* is an important tree of social forestry. It is popularly known as Pangar and Bankhor. It is a tree of about 40m height, found in deciduous forests of India, Sri Lanka, Pakistan, Thailand, China and Phillipines. Flowers are pinkish white in color. Flowers and fruits can be collected during the months from March to November. The cream colored wood is used to make pots and vessels. The fruits are given to cattles. Flour from seed is mixed with wheat flour during famine. Seed paste made with oil is applied in rheumatic pain. The flowers are useful in apiculture as bee forage [1].

*A. indica* is available locally in India in large quantities and has not been explored as a pharmaceutical excipient. The aim of this study was to extract mucilage from the bark of *A. indica* and to study the various pharmaceutical properties of the mucilage to assess its functionality as an excipient in pharmaceutical sustained-release formulations.

5.2.1 Collection of the Plant

*A. indica* bark was collected in the month of April from the Himalayan region of Garhwal, Uttarakhand (India). The tree was identified by Prof. R.D. Gaur, Department of Botany, HNB, Garhwal University. The voucher specimen (GUH-8812) is also entered in the Herbarium of the University.

5.2.2 Extraction of gum mucilage

The bark of *A. indica* was cut into small pieces with help of sharp knife. The small pieces were taken and washed with water to remove dirt and debris. The bark was soaked in water for 5–6 h, boiled for 30 min, and left to stand for 1 h to allow complete extraction of the mucilage into the water. The mucilage was extracted using an eight-layer muslin cloth bag to remove the marc from the solution. Acetone (three times the volume of filtrate) was added to precipitate the mucilage. The mucilage was separated, dried in an oven at a temperature of less than 50°C, collected, ground, passed through a 80 # sieve (nominal aperture size is 180 µm) and stored in desiccators at 30°C and 40% relative humidity before use [5]. The percent yield obtained from the above is tabulated in Table no.5.2.1.
Table 5.2.1: Total percent yield of mucilage from *Aesculus indica*

<table>
<thead>
<tr>
<th>Name of the Plant</th>
<th>Total Yield (%)</th>
<th>Color of the mucilage Powder</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aesculus indica</em> (Pangar)</td>
<td>15</td>
<td>Light brown</td>
</tr>
</tbody>
</table>

The mucilage was isolated from the bark of *A. indica*. The total yield was 11% light brown powder.

### 5.2.3 Physicochemical characterization of *A. indica* mucilage

The dried mucilage was studied for percentage yield, chemical test, particle size, weight loss on drying, solubility, viscosity, pH, swelling index, bulk and tapped density, angle of repose, compression properties, and microbial load.

#### Chemical test

The presence of mucilage in extracted material was confirmed using Molisch's test and by treatment with ruthenium red. Both tests were positive for the presence of mucilage.

#### Loss on drying

Weight loss on drying was determined for an appropriate quantity of mucilage at 105°C for 2h [6]. The results obtained are summarized in Table 5.2.2.

#### Particle size

The particle size of the dried-powder mucilage was determined by the microscopic method. All readings were taken in triplicate and are shown in Table 5.2.2.

#### pH of solution

The pH of the 1% solution was measured with a pH meter. The results obtained are summarized in Table 5.2.2.

#### Charring

Small amount of dried mucilage was placed in a melting-point apparatus. The temperature was taken and recorded when the material started to char and temperature is shown in Table 5.2.2.
Density

Granular density of each formulation was determined by using fluid displacement method and applying the equation

\[ P_g = \frac{W}{[(a+w) - b]} S_g \]

Where \( P_g \) = granular density in gms per cubic centimeter
\( W \) = granules weight in gram
\( S_g \) = specific gravity of liquid paraffin (0.802)
\( a \) = pycnometer + liquid paraffin weight in grams
\( b \) = pycnometer + liquid paraffin weight in grams + granule weight in grams

All the observations recorded are summarized in Table 5.2.2.

Swelling ratio

Swelling characteristics of the mucilage powder was studied in different media such as 0.1N HCl, phosphate buffer (pH- 7.4) and in distilled water. The study was carried out using a 100-mL stoppered graduated cylinder. The initial bulk volume of 1 g of dried mucilage was recorded. Water was added in sufficient quantity to make up the volume upto 100 mL of the dispersion. The sediment volume of the swollen mass was measured after 24 h, stored at room temperature. The swelling ratio was calculated by taking the ratio of the swollen volume to the initial bulk volume [7]. The results obtained are summarized in Table 5.2.2.

Microbial count

The microbial count of the dried mucilage was performed as given in the Indian Pharmacopoeia for total aerobic microbial count of bacteria and fungi using the plate count method [10].
The results of other investigations (percentage yield, particle size, pH of solution, density, and charring) are shown in Table 5.2.2. The loss on drying was well within official limits and the weight loss on drying indicates the amount of moisture present in the material available to interact with other materials during processing. The result of microbial testing of the mucilage was within official limits {less than 100 colony-forming units (cfu)/g}. The swelling ratio of mucilage was determined in different media and ratio was found to be highest in distilled water i.e., 40. There was a significant change in swelling by the end of the study, which indicated that the mucilage had excellent swelling properties. Swelling characteristics studies revealed that the swelling was affected by pH of the medium.

**Determination of viscosity of the mucilage**

The viscosity of the mucilage was determined as per the method described earlier and results are shown in Table 5.2.3.
Table 5.2.3: Viscosity of gum mucilage and other gums at different time interval

<table>
<thead>
<tr>
<th>S.N</th>
<th>Days</th>
<th>Viscosity (cp) of solution*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>A. indica mucilage (10%)</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Starch (10%)</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1390</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1340</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1331</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>1102</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>1090</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>1075</td>
</tr>
<tr>
<td>% decrease</td>
<td>22.66</td>
<td>29</td>
</tr>
</tbody>
</table>

The viscosity of the extracted dried mucilage was compared with starch. The viscosity of the dried mucilage has viscosity comparable with starch.

**Evaluation of granules**

The granules were evaluated for flow properties, bulk density, tapped density, compressibility index, Angle of repose, Carr’s index, Hausner’s ratio and the results are summarized in Table 5.2.4.

**Bulk and tapped density**

A pre-weighed, pre-sieved quantity of dried mucilage was poured into a graduated cylinder, and the volume recorded. The cylinder was tapped until the powder-bed volume reached a minimum value, and the tapped volume was recorded. The bulk and tapped densities were calculated [8].

- Compressibility index = \([\text{tapped density} - \text{bulk density}] / \text{tapped density}\)

**Carr's index and Hausner ratio**

Carr's index and Hausner ratio were calculated from the bulk and tapped densities [9].

**Angle of repose**

The angle of repose was determined by the fixed-height funnel method and calculated using the following equation:

$$\text{Angle of repose} = \tan^{-1} \frac{h}{r} \quad [1]$$
in which \( h \) is the height of the powder heap and \( r \) is the radius of the powder heap. Comparison was made between dried mucilage and starch.

### Table 5.2.4: Evaluation of granules prepared using \( A. \) indica mucilage

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Bulk density (g/cc)</th>
<th>Tapped density (g/cc)</th>
<th>Compressibility Index (%)</th>
<th>Hausner's Ratio</th>
<th>Angle of Repose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>0.51</td>
<td>0.59</td>
<td>13.5</td>
<td>1.15</td>
<td>35°</td>
</tr>
</tbody>
</table>

The flow properties and compressibility of the dried mucilage, including bulk and tapped density, Carr's index, the Hausner ratio, and the angle of repose, were assessed. The compressibility index and angle of repose indicated that the powder have good flow with moderate compressibility.

#### 5.2.4 Preparation and characterization of Tablets

All the concepts and the assumptions of biopharmaceutics, i.e., absorption, distribution, metabolism and excretion, are the important factors for mathematical design of the sustained release dosage forms. Pharmacokinetic studies showed that a dose of 25 mg of diclofenac sodium produces an effective blood level concentration of 0.7-1.5 \( \mu \)g/ml within 1.5-2.5 h with the half life of 1.1-4.0 h [11]. The preliminary formulation and dissolution studies showed that 100: 50 diclofenac sodium: gum ratio prolongs the drug release beyond 12 h. Therefore, in all cases, tablets were prepared using diclofenac sodium as a model drug and different ratios of dried mucilage powder (Table 5.2.5). Different batches of tablets were prepared (A₁ to A₄). Several batches of the Tablets with almost constant theoretical weight of 400 mg were prepared. In all the formulations, ingredients were passed through sieve #120. Then the ingredients were accurately weighed and granulated. Granules were allowed to dry at room temperature \((27\pm2 \, ^\circ \text{C})\). Dried granules after lubricating with magnesium stearate (2% w/w) were compressed on a motor-operated single-punch (9mm) tablet machine and were evaluated for hardness, friability, and uniformity of weight [12].
Table 5.2.5: Composition of diclofenac sodium Tablet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Drug mucilage ratio A₁ (1:0.5)</th>
<th>Drug mucilage ratio A₂ (1:1)</th>
<th>Drug mucilage ratio A₃ (1:1.5)</th>
<th>Drug mucilage ratio A₄ (1:2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac Sodium (mg)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Dried Mucilage (mg)</td>
<td>50</td>
<td>100</td>
<td>150</td>
<td>200</td>
</tr>
<tr>
<td>Dicalcium Phosphate (mg)</td>
<td>242</td>
<td>192</td>
<td>142</td>
<td>92</td>
</tr>
<tr>
<td>Magnesium stearate (mg)</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

The tablets, each containing 100 mg of diclofenac sodium, were prepared using dried mucilage of *A. indica* in various drug–mucilage ratios (1:0.5, 1:1, 1:1.5, and 1:2). A₁ – A₄ are various formulations of diclofenac sodium Tablets. Quantities of mucilage and dicalcium phosphate were varied and mixed with 8 mg of magnesium stearate to have each tablet with average weight of 400 mg.

5.2.5 Evaluation of Tablets

The diameter and thickness of tablet were measured by vernier calipers, and the hardness was determined by Monsanto hardness tester. The friability test was conducted using Roche friabilator. For each batch, 20 randomly drawn Tablets were checked for weight uniformity. The observations recorded for the above parameters are shown in Table 5.2.6.

Table 5.2.6: Evaluation of Diclofenac Tablets prepared using *A. indica* as binder

<table>
<thead>
<tr>
<th>Binding agent</th>
<th>Formulation code</th>
<th>Average Weight (mg)</th>
<th>Hardness (kg/cm²)</th>
<th>Friability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. indica</em></td>
<td>A₁</td>
<td>397.5</td>
<td>5.13±0.25</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>A₂</td>
<td>394.5</td>
<td>5.38±0.30</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>A₃</td>
<td>393.0</td>
<td>5.460.40</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>A₄</td>
<td>398</td>
<td>5.65±0.20</td>
<td>0.81</td>
</tr>
</tbody>
</table>

The physical tests like hardness test, friability, and weight variation were performed for all formulations. Mean hardness for all formulations was found between 5 to 6 kg/cm² and friability was less than 1%. The weight-variation results for the matrix tablets
complied with pharmacopoeial limits (Table-5.2.6). From the data it can be concluded that dried mucilage of *A. indica* possesses good tablet-forming properties.

### 5.2.6 Drug content determination [13]

For drug content, 20 tablets were weighed accurately and powdered. Powder equivalent to 50 mg of diclofenac sodium was shaken with 60 ml of methanol in 200 ml volumetric flask, and volume was further adjusted with methanol. Finally, 5 ml of this was diluted to 100 ml with methanol, and drug content was determined by UV-spectrophotometer (UV-1601, Shimadzu, Japan) at 276nm using calibration curve based on standard solutions.

### 5.2.7 Tablet swelling index

Tablets of equal weight were immersed in 50 mL of distilled water on a watch glass. At specific time intervals, tablets were carefully removed from the watch glass and blotted with filter paper to remove the water present on their surface and weighed accurately. The experiment was performed for 5 h. The swelling index was calculated using the following formula [14]:

\[
\text{Swelling index of tablet} = \frac{(\text{Wet weight} - \text{Dry weight})}{\text{Dry weight}} \quad [2]
\]

### 5.2.8 Radial and axial swelling of the Tablet

The initial diameter and height of the tablet were measured, and the tablet was stored in distilled water. The increase in diameter and height were measured at selected time intervals up to 5 h. The equilibrium degree of swelling (*Q*) was calculated from the radial and axial swelling ratio using the following equation:

\[
Q = \frac{V_t}{V_o} = \left(\frac{R_t}{R_o}\right)^2 \times \left(\frac{I_t}{I_o}\right) \quad [3]
\]

in which *V*\(_t\) and *V*\(_o\) are the Tablet volumes, *R*\(_t\) and *R*\(_o\) are the radii, and *I*\(_t\) and *I*\(_o\) are the heights at time *t* and zero, respectively [15]. The results are summarized in Table 5.2.7.
Table 5.2.7: Radial and axial swelling of Tablets in distilled water

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Drug-mucilage ratio</th>
<th>Diameter after swelling (mm)</th>
<th>Thickness after swelling (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>A₁ (1:0.5)</td>
<td>9.1</td>
<td>4.4</td>
</tr>
<tr>
<td>5</td>
<td>A₂ (1:1)</td>
<td>9.3</td>
<td>5.5</td>
</tr>
<tr>
<td>5</td>
<td>A₃ (1:1.5)</td>
<td>9.4</td>
<td>5.9</td>
</tr>
<tr>
<td>5</td>
<td>A₄ (1:2)</td>
<td>9.8</td>
<td>6.7</td>
</tr>
</tbody>
</table>

The study of dimensional changes in the tablets was carried out for 5 h in distilled water and the results are shown in Table 5.2.7. The radial and axial swellings of the tablets were found to be increasing with increase in the proportion of the dried mucilage. The swelling of the Tablet was found to be lowest for formulation A₁. As the ratio increased, the radial and axial swelling increased proportionally. The swelling of the tablet in the axial direction was found to be more as compared with the radial direction.

5.2.9 *In-Vitro* dissolution study

*In-vitro* dissolution studies of prepared tablets were performed using USP apparatus type-II at 50rpm in pH 7.8 phosphate buffer (900 ml) medium at the temperature 37±0.5°C. At specified intervals, 5ml of samples were withdrawn and filtered through Whatmann filter paper #41. After removal of each sample, the 5ml of fresh dissolution medium was added to the vessel to maintain the sink conditions. The samples were then analyzed at 249 nm by UV-Visible spectrophotometer (shimandzu-1700). The amount of drug released was determined by reference to a calibration curve constructed in same dissolution media [16]. Diclofenac sodium release profile of *A. indica* tablets is tabulated in Table 5.2.8 and is shown in Figure 5.2.1
Diclofenac sodium release profiles of the mucilage matrix tablets are shown in Figure-5.2.1. Decrease in drug release rate was observed when *A. indica* contents in the tablets were increased. This may be due to the reason that the mucilage in higher concentrations in the Tablets might have produced dense matrix around the drug particles, providing more barriers for them to escape and dissolve. Further, such dense matrix, specifically when it is hydrophobic in nature, may allow less penetration of the dissolution medium in the tablet. This may also be the auxiliary reason for obtaining slow drug release profiles through *A. indica* Tablets. Batches A1 and A2, at lower the ratios (1:0.5 and 1:1), released 34 and 29% of the drug in the first hour, and the remaining drug was released within 7 h. This could possibly attribute to the amount of mucilage present in the formulation. In batch A4, where the drug-mucilage ratio was 1:2, 22% of the drug was released in the first hour, and the remaining drug was released during 9 h. The rate of release was faster in batch A1 and slower in batch A4. These results showed that as the proportion of mucilage increases, the overall time of release of the drug from the Tablets increases.
Figure: 5.2.1: Effects of concentration of mucilage on the release of Diclofenac sodium tablet.
5.2.10 References


