

## ISOLATION AND ANALYTICAL STUDIES OF MUCILAGE OBTAINED FROM THE SEEDS OF DILLENIA INDICA (FAMILY DILLENACEAE) BY USE OF VARIOUS ANALYTICAL TECHNIQUES

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### ABSTRACT

The aim of the present study is to isolate the mucilage from the seeds of the *Dillenia indica* (Family Dilleniaceae) and then study the mucilage by using various analytical techniques like Fourier Transformation Infrared spectroscopy (FTIR), Thermogravimetric analysis (TGA) and X-ray diffraction techniques (XRD). The zeta potential of the mucilage was studied using Zeta potentiometer, Malvern instrument and was found to be -17.2 towards the increasing positive potential. The swelling properties and mucoadhesion properties of the mucilage were determined in-vitro and it was found that the mucilage had high swelling capacities along with appreciable mucoadhesive strength. Moreover from the analytical studies, it was found out that the mucilage is amorphous in nature and has one step mass loss event. Thus the mucilage obtained from *Dillenia indica* can be used as a natural mucoadhesive polymer for formulating various drug delivery systems.

**Keywords:** Mucilage, *Dillenia*, mucoadhesion, Zeta potential of mucilage, FTIR of mucilage

### INTRODUCTION

Mucilage and gums have been known since ancient times for their medicinal uses. In the modern times also they are widely used in the pharmaceutical industries as thickeners, water retention agents, emulsion stabilizers, suspending agents, binders and film formers due to their compatible nature and ecofriendliness<sup>1,2,3</sup>. The seeds of *Dillenia indica* (Syn. *P. lanigerum*; Family-Dilleniaceae)<sup>4</sup>, is used for the present study for obtaining mucilage and then analyzing the mucilage by use of Fourier Transformation Infrared spectroscopy (FTIR), Thermogravimetric analysis (TGA) and X-ray diffraction techniques (XRD), Zeta potentiometer. The swelling properties and mucoadhesion properties of the mucilage were determined in-vitro.

### MATERIALS AND METHODS

#### Chemicals and Reagents

Ethanol (Purchased from Jiangu Huaxi international Trade Co. Ltd, Made in China, AR-99.9%)

#### Plant material

The fruits of *D. indica* were collected from BIT, Mesra, Ranchi campus, especially during the month of October-November, 2009, and were authenticated by Botanical Survey of India, Howrah, Kolkata, where the specimen of the plant was deposited.

#### Extraction of the mucilage from the seeds of *D. indica*

Initially fresh ripe fruits of the plant were collected and the seeds were separated. These ripe seeds were then soaked in water to get a slimy mucilaginous mass and then this viscous solution was filtered through a thin and soft clean muslin cloth to remove any kinds of plant matter. This mucilaginous solution was then treated with 100% ethanol so that the mucilaginous matter totally precipitated out. The precipitated mucilage was then dried in a hot air oven at 40°C to remove all the traces of ethanol<sup>5</sup> and the dried mucilage named as "*Dillenia*". The dried mucilage was then powdered using a mortar pestle and again dried in oven to remove any remaining traces of ethanol.

#### Determination of swelling index of *Dillenia*

Swelling characteristics of the *Dillenia* was tested in distilled water. The Swelling index is the volume in ml occupied by 1g of the substance. The Swelling index of the mucilage powder was determined by according to British Pharmacopoeia method<sup>6</sup>. The test was performed by taking 1gm of the mucilage powder in a 50.0ml ground glass stoppered cylinder graduated over a height of 120 to 130mm in 0.5 divisions. To this 25ml of distilled water was added and this was shaken vigorously every 10min for 1hour and

then allowed to stand for 24hours. The volume occupied by the mucilage powder was measured.

#### Analytical Studies of mucilage *Dillenia*

##### FTIR studies

An FTIR-8400S spectrophotometer (Shimadzu, Japan) was used to obtain the infrared spectra of the mucilage. Samples were prepared in KBr (Potassium Bromide) disks of 2mg sample in 200mg of KBr. The Scanning range was 400-4000cm<sup>-1</sup> and the resolution was 2cm<sup>-1</sup>.

##### TGA studies

Thermal analysis of the mucilage "*Dillenia*" was carried out using a Perkin Elmer, SII, Pyris Diamond, TG/DTA Instrument with sample weighing about 10mg and the programmed heating of the samples were done at a rate of 10°C/min with temperature starting from 40°C to 500°C and the various plots were recorded<sup>8</sup>.

##### XRD studies

The technique used currently for performing the X-ray diffraction of samples of mucilage powder *Dillenia* was the X-ray powder diffraction method. In this technique a small collimated beam of nearly monochromatic X-ray were directed onto a small specimen in the form of powder producing a pattern that is recorded with a counter-tube<sup>7</sup>. The conditions for diffraction are governed by Bragg's law and diffracted beams are often referred to as reflections. The X-ray diffractometer used for the present study was from SEIFERT & CO.D 2070, AHRENSBURG. The target material of the instrument was Copper (Cu) and Nickel was used as the filter and a voltage of 35kV and a current of 30mA was used. The diffraction was done at room temperature of 30°C.

##### Determination of Zeta potential

The Zeta Potential is a representative of particle charge. Zeta potential was measured by the Laser Doppler anemometry using a Zetamaster (Malvern, UK)<sup>9</sup>. Phosphate buffer of pH 6.8 was used as the medium where the powdered mucilage "*Dillenia*" was suspended by ultrasonication for 30min and the suspension was of concentration of 2%Wt. /Vol., and the cell was filled with the suspension and measured.

##### Determination of Mucoadhesivity mucilage

Bioadhesive strength of the dried mucilage powder "*Dillenia*" was measured on a modified physical balance using the method described by Gupta et al<sup>10</sup>. Carbopol 934P which was taken as

standard to compare the mucoadhesivity of mucilage. The mucilage was sandwiched between two mucosal surfaces of rat intestine. The intestine were placed on two oppositely placed platforms of two slides, one hanged to the left pan of balance and other placed on a water bath at the base. Then weights were placed to the right pan of the balance in ascending order starting from lower weights and after each addition of weights, allowing to stand for 1min, and then added the next weight and this process was continued till the two mucosal surfaces detached from one another on the left side of pan and thus the detachment force required to separate two glass slides was measured. This process was repeated for the Carbopol 934P also. The results are given in Table 2.

## RESULTS AND DISCUSSIONS

The method used for obtaining mucilage from the seeds of *D.indica* was economical method and the mucilage can be used as drug release and film forming polymer.

### Swelling index of the mucilage

The Swelling index of the mucilage was carried out in distilled water at room temperature of 37°C<sup>11, 6</sup>. These tests are done in triplicate and presented in Table 1.

Table 1: Swelling index of the mucilage powder (*Dillenia*)

Sl. No	Volume occupied by the mucilage prior to hydration (V <sub>1</sub> )	Volume occupied by the mucilage after hydration (V <sub>2</sub> )	Swelling Index = (V <sub>2</sub> /V <sub>1</sub> )	Mean Swelling Index ± S.D
1	10ml	30ml	3	
2	11ml	34ml	3.09	3.0022±0.08505
3	12ml	35ml	2.92	

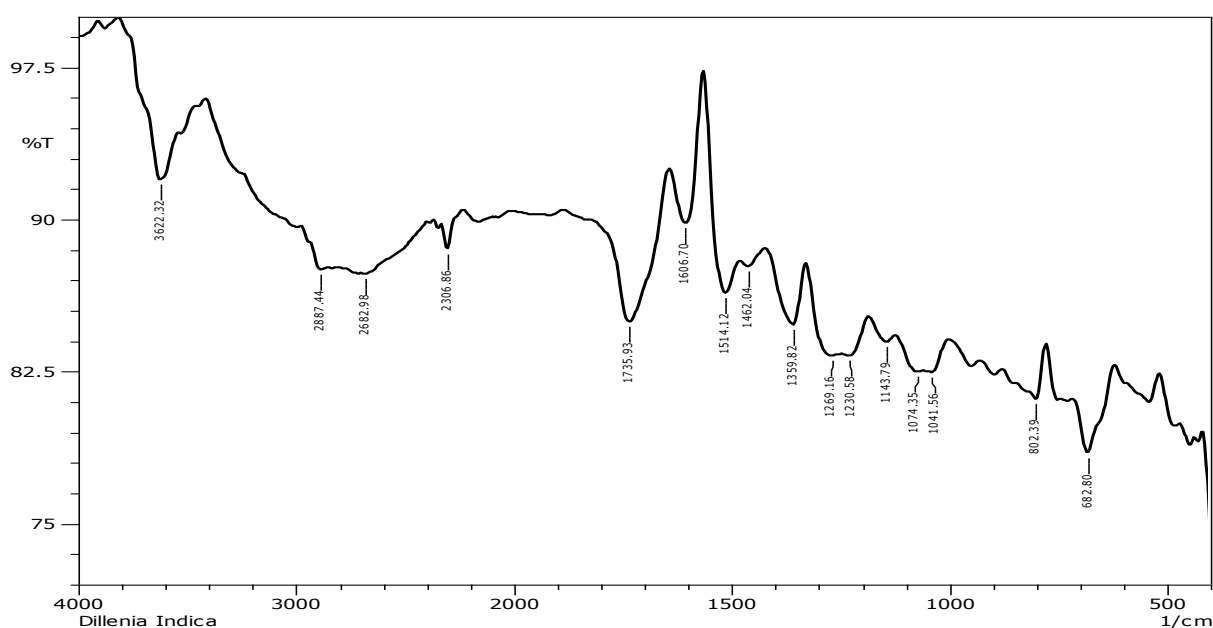


Figure 1: FTIR spectra of *Dillenia*

### Analytical Studies of the mucilage

#### FTIR studies

From the FTIR spectra obtained, it is seen that the mucilage has some functional groups as given in matches with that of the earlier findings<sup>12</sup>.

The above spectrum shows wave number of 3622.32cm<sup>-1</sup> indicating the presence of -OH group, 1735.93 cm<sup>-1</sup> indicating the presence of cyclic ketones, 1514.12 cm<sup>-1</sup> indicating aromatic -NO<sub>2</sub>. These results are in confirmation with earlier findings<sup>12</sup>.

#### TGA studies

The thermogram of mucilage powder "*Dillenia*" as shown in Figure 2, shows that "*Dillenia*" has one mass loss event between 210-500°C with %loss of 62.481%.

#### XRD studies

The XRD spectra of *Dillenia* shows that it is of amorphous nature as there is no sharp peaks present in the spectra as shown in Figure 3.

#### Zeta potential of *Dillenia*

The zeta potential of mucilage powder "*Dillenia*" was found to be -17.2, indicating that its value is towards the positive side. Thus it

also gives an indication that this mucilage can be a good mucoadhesive substance.

#### Mucoadhesivity of mucilage

As seen from Table 2, the mucoadhesive strength of "*Dillenia*" is found to be 0.02013N/m<sup>2</sup>. This result is compared with that of Carbopol 934P which found to be 0.0256041 N/m<sup>2</sup>. This comparison shows that the mucoadhesive strength of "*Dillenia*" is almost comparable with that of Carbopol934P. The results of zeta potential are in confirmation with that of mucoadhesion studies, proving that increase in zeta potential leads to increase of mucoadhesive strength.

Table 2: Mucoadhesive strength of *Dillenia*

Sl.No	Materials	Mucoadhesive strength(N/m <sup>2</sup> )
1.	<i>Dillenia</i>	0.02013N/m <sup>2</sup>
2.	Carbopol 934P	0.0256041 N/m <sup>2</sup>

## CONCLUSION

From the above studies, it can be concluded that mucilage powder "*Dillenia*" proves to be a good natural substance which can be used as polymer in formulating various delivery systems.

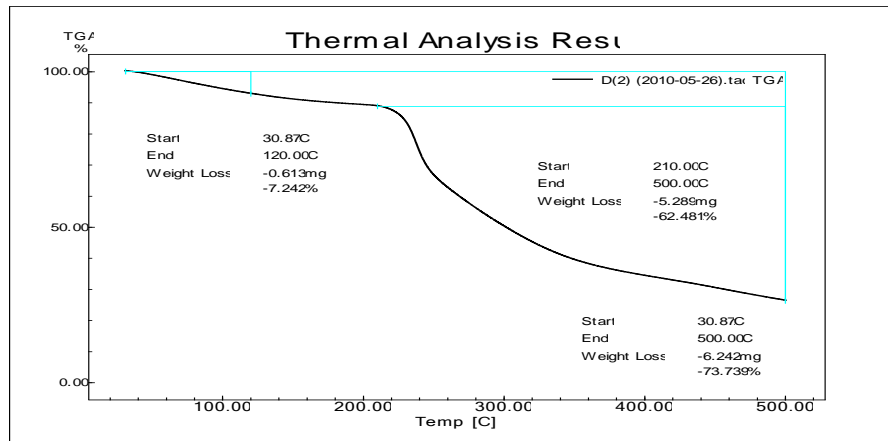


Figure 2: TGA Thermogram of Dillenia.

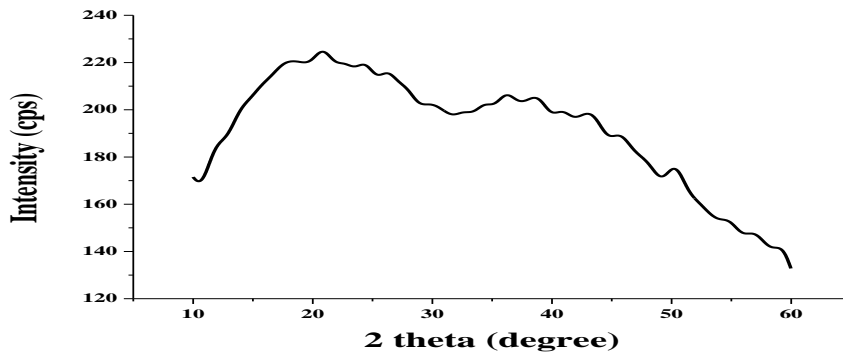


Figure 3: XRD spectra of "Dillenia".

**Zeta Potential Report**  
v2.2



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**Sample Details**

Sample Name: DILL 1  
SOP Name: mansettings.nano  
General Notes:

File Name: POLYMER SAMPLES.dts      Dispersant Name: Water  
Record Number: 7      Dispersant RI: 1.330  
Date and Time: Friday, February 11, 2011 3:35:4...      Viscosity (cP): 0.8872  
Dispersant Dielectric Constant: 78.5

**System**

Temperature (°C): 25.0      Zeta Runs: 20  
Count Rate (kcps): 70.5      Measurement Position (mm): 2.00  
Cell Description: Clear disposable zeta cell      Attenuator: 10

**Results**

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -17.2	Peak 1: -38.7	75.8	7.46
Zeta Deviation (mV): 43.5	Peak 2: 76.8	16.7	2.53
Conductivity (mS/cm): 0.0189	Peak 3: -8.10	7.6	4.67

Result quality : See result quality report

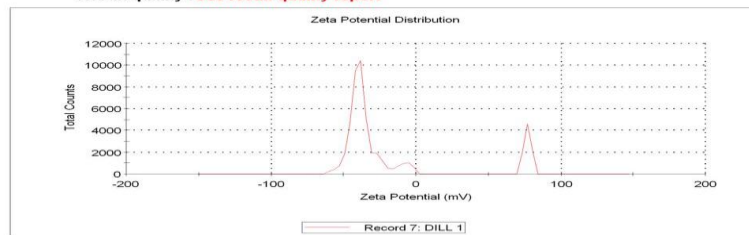


Figure 4: Zeta potential data of Dillenia.

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