Ind. J. Pharm. Edu. Res., 2024; 58(2):642-650.
https://www.ijper.org

Original Article

Isolation and Analysis of *Phanera variegata* Mucilage by Various Analytical Techniques

Keshav Bansal, Meenakshi Bajpai*

Department of Pharmaceutics, Institute of Pharmaceutical Research, GLA University, Mathura, Uttar Pradesh, INDIA.

ABSTRACT

Objectives: The objective of the present study was to isolate mucilage from the leaves of *Phanera variegata* L., to do the thermal, microscopic, elemental analysis, and physicochemical characterization of isolated mucilage. The various analytical methods were applied to mucilage and evaluated its nature, thermal stability, and structure. **Materials and Methods:** The mucilage was isolated and analyzed by various analytical methods like SEM, PXRD, particle size analysis, DSC, TGA, elemental analysis (CHNS), zeta potential, FTIR, and 1D (^{1}H and ^{13}C) NMR spectroscopy. **Results:** The SEM analysis indicated that mucilage particles had irregular shapes and sizes. The PSA indicated that particles of mucilage had a particle size of nanometers. The DSC analysis observed the mucilage's glass Transitional temperature (Tg) at 95.9ºC. The TGA analysis suggested the three-stage decomposition with the good thermal stability of mucilage. A complete amorphous nature of the mucilage was indicated by PXRD analysis. The specific content of CHNS was revealed by elemental analysis. FT-IR spectra identified the major functional groups include 3240 cm^{-1}(-OH), 2848 cm^{-1}(C-H), 1599 cm^{-1}(C-OH), 1419 cm^{-1}(-COO-), 1253 cm^{-1}(C-O). 1D Hydrogen-1 and Carbon-13 NMR confirmed the presence of polysaccharides that have many similar sugar residues. **Conclusion:** The *P. variegata* mucilage was found amorphous, thermally stable, and can be used as an excellent alternative natural pharmaceutical excipient for conventional pharmaceutical drug products and novel drug delivery systems in varying concentrations.

Keywords: *Phanera variegata*, Pharmaceutical excipients, Mucilage, Microscopic analysis, Elemental analysis, Structure analysis.

Correspondence:
Prof. Meenakshi Bajpai
Head of Department, Department of Pharmaceutics, Institute of Pharmaceutical Research, GLA University, Mathura-281406, Uttar Pradesh, INDIA.
Email: meenakshi.bajpai@gla.ac.in; mbajpai05@gmail.com
Orchid ID: 0000-0002-4440-4635

Received: 16-02-2023;
Revised: 18-07-2023;
Accepted: 16-01-2024.

INTRODUCTION

Pharmaceutical excipients are used in the conversion of Active Pharmaceutical Ingredients (APIs) into dosage form, excipients are a non-active ingredient, but they are essential in the conversion of raw into a dosage form. In recent years excipients (polymer) isolated from natural origins (plants) evoked great interest due to wide application in all types of pharmaceutical formulations such as binders, diluents, disintegrants in solid dosage form, viscosity enhancers, protective colloids in liquid dosage forms, as bases and gelling agents in semisolid dosage form, etc.[1] Natural excipient also have a wide role in cosmetics, paints, paper making, and food industries.[2,3] Natural excipients are those which can be or are obtained from natural sources. Choosing natural excipients is more appropriate than synthetic ones as they are biodegradable, non-toxic, and harmless 'What comes from nature degrades easily in nature.

Polymers obtained from plants have been researched and reported for their efficacy as they have vast applications in all types of dosage forms like film coating agents, nanoparticles, microspheres, controlled drug delivery systems, implants, buccal films thickeners in ophthalmic preparations, emulsifiers, solubilizers, suspending and thickening agents, bioadhesives, binders in dosage forms.[4-7] The mucilage in plants is a term used for materials that are practically soluble or they swell very noticeably in water as well as they get precipitate in granular mass or in amorphous form upon the addition of alcohol or ketone. They emanate as a part of cell content or as a wall part of that.

Natural polymeric materials like Almond gum from *Prunus dulcis*, Locust bean gum mucilage from seeds of *Ceratonia siliqua*, Khaya gum, Tara gum,[8] Gums from *Cassia Fistula* and *Tamarindus indica*,[9] Hibiscus mucilage from leaves of *Hibiscus rosasinensis*, Okra gum from pods of *Abelmoschus esculentus*,[10] Gum Sesbania from *Hibiscus esculenta*, gum copal, konjac gum, galactomannan from *Mimosa scrabella*.[11] Gums and mucilage have extremely wide applications in non-food as well as in food industries as they are completely or partly water-soluble polysaccharides, ready availability, and based on their distinctive important physicochemical properties; they are available at quite a low cost than synthetic polymers. Amongst the Asian countries,

DOI: 10.5530/ijper.58.2.72

India is been an important source of these types of products due to its environmental and geometrical positioning.[12]

Phanera variegata Linn. (Fabaceae) is a deciduous tree; it is of medium size having dark drown bark, nearly smooth brown-pubescent young shoots, large fragrant flowers mainly of a white or purplish color, and theleaves are obcordate (heart-shaped attached by the pointed end) shaped, rounded, broad and long, and having lobes at the apex and base. It is widely distributed in India mainly in sub-the Himalayan and outer Himalayan of Punjab, Rajasthan, and Sikkim, and extending from Burma to China, in South East Asia. It is also known as the camel's foot tree, mountain ebony, or orchid tree. *P. variegata* is having wide medicinal use (treatment of leprosy also cures diarrhea, dysentery, inflammations, diabetes, etc.) and also use as a food source (buds of Kachnar are used as edible in various parts of India).[13]

"Mucilage in plants" means those substances which are soluble or at least swell very perceptibly in water and which, upon the addition of alcohol, are precipitated in either amorphous or granular mass. Mucilage analysis has an important significance as they have wide applications in the pharmaceutical industries. Mucilages are used as pharmaceutical excipients for various roles in different dosage forms like in control release formulation, site-specific delivery, sustain release solid dosage form, binding agent, suspending agent, viscosity enhancers etc., therefore proper analysis of mucilages is required like swelling index, binding property, thermal stability, structural analysis and physicochemical analysis.

The objective of this research work was to determine the morphological, physicochemical, and elemental mucilage assessment isolated from *Phanera variegata* Linn. (Kachnar) leaves. The analysis was done by the following methods. (i) (SEM) Scanning Electron Microscopy, (ii) (DSC) Differential Scanning Calorimeter, (iii) (TGA) Thermo Gravimetry Analysis, (iv) Particle size analysis, (v) (PXRD) Powder X-ray Diffraction, (vi) elemental analysis {Carbon (C), Hydrogen (H), Nitrogen (N), Sulphur (S)}, (vii) Zeta potential, (viii) (FTIR) Fourier Transform Infrared Spectroscopy (ix) 1D (^{1}H and ^{13}C) (NMR) Nuclear Magnetic Resonance spectroscopy analysis.

MATERIALS AND METHODS

Materials

Leaves of *Phanera variegata* Linn. were collected from the campus of GLA University, Mathura, U.P., India, and their authentication was done by Dr. Sunita Garg, CSIR - NISCAIR, New Delhi, India (Ref. No.-NISCAIR/RHMD/Consult/2018/3230-31). The leaves were separated, washed, and dried in ambient conditions under the shed. Mucilage was isolated from the leaves using maceration techniques and stored in airtight, desiccated jars. De-ionized water was used for all experiments. All other chemicals used were of analytical reagent grade.

Isolation of Mucilage

The fresh leaves of *Phanera variegata* Linn. were collected from the tree and washed properly with water to remove dirt and debris and then kept for drying. After drying they were crushed in a grinder to make a fine powder. Then the powder was soaked (homogenize) in water four times (x 4) for 2-3 hr (homogenization) after that slurry was boiled for 15-20 min. at 70°C and kept undisturbed for around 1 hr for the release of mucilage into water. Then the material was passed from eight folds muslin cloth for separation of the marc and the solution. Acetone in the ratio of 1:3 to the volume of the filtrate was added to the filtrate to precipitate the mucilage. The mucilage was filtered and dried in an oven at a temperature not exceeding 50°C. The dried material was then collected, crushed, and passed by mesh 80. The resultant fine powder was stored for further use in the desiccator. Deionized water was used to conduct all experiments. All chemicals were used as received analytical grades without further purification.[12] Further, the isolation process is explained in Figure 1.

Scanning electron microscopy

Scanning electron microscopy was carried out by mounting a dried sample (mucilage) on a specially designed aluminum stub along with double-sided adhesive tape; the stub with the sample was then coated with a thin layer of gold in a sputter, coater and observed under (JSM 6100 scanning microscope, JEOL, Japan) at an accelerating voltage of 10 kV. The study was done at SAIF, Panjab University, Chandigarh, India.

Powder X-ray Diffraction

Powder X-ray Diffraction (PXRD) analysis of isolated mucilage was done using (X'Pert-PRO, Panalytical, UK.) X-ray diffractometer equipped with a copper tube with Cu K-alpha-1 radiation. The required amount of sample was analyzed and scanning was performed between 2Θ=10 and 50° (specific length) with a scan step (time) of 29.84 s, operating at voltage 45Kv, current 40 mA, and temperature at 25°C which were kept constant. 2 g fine powder sample was taken on glass slide of size 3.5 cm x 2.5 cm and thickness 0.2 cm with uniform sample layer on one side. The analysis was done at SAIF, Panjab University, Chandigarh, India.

Particle size analysis

Particle size analysis of mucilage sample carried out by particle size analyzer (Zetasizer Nano ZS, Malvern, and Panalytical, UK). About 3.0 mL sample dispersion were added in sample holder. The dispersed sample was prepared in deionized water at a concentration of 1.0 mg/mL, the required amount of dispersed sample was placed in a cuvette, and particle size was recorded at

25°C, count rate of 225.4 (kcps). The determination of particle size is based on the principle of DLS (Dynamic Light Scattering), the distribution of the velocity of suspended particles in a dispersion medium helps to analyze particle size distribution and in the influence of an electric field, particles were analyzed in motion.[14] The analysis was done at GLA University, Mathura, India.

Differential scanning calorimetry

Differential scanning calorimetry analysis of mucilage sample carried out using a Differential scanning calorimeter (Mettler Toledo Star System, USA). The required weighed sample (6.182 mg) was placed in the Aluminum 40 µL pan with a pin-hole lid and measurements were done in a nitrogen atmosphere at a flow rate of 40 mL/min. The thermal cycles were performed with the heating rate of 10 K/min for the first heating cycle from 25-300°C and then a cooling cycle with the rate of -10 K/min from 300-25°C. DSC was done at Mettler Toledo India Pvt. Ltd., Mumbai, India.

Thermogravimetry analysis

Thermogravimetry Analysis (TGA) of mucilage samples was carried out using a Thermal analyzer (Mettler Toledo Star System, USA). Weighed amount (20.6 mg) of mucilage sample was placed on Alumina 70 µL pan w/o lid under nitrogen atmosphere (flow rate 60 mL/min). The sample was analyzed from 30-1000°C at a heating rate of 10 K/min. TGA was done at Mettler Toledo India Pvt. Ltd., Mumbai, India.

Elemental compositions (CHNS) analysis of mucilage

The analysis for elemental compositions (CHNS) of the mucilage was carried out by using a CHNS-O organic elemental analyzer (Flash 2000/Series, Thermo Scientific, USA). Weighed amount (2.718 mg) of mucilage sample was heated to 1800°C, from 0-12 min and electric potential between-2.61 to 76.28 mV, and all the elements were analyzed. The study was done at CIL, Panjab University, Chandigarh, India.

Zeta Potential (ζ)

The zeta potential (ζ) of isolated mucilage was done using (Zetasizer Nano ZS, Malvern, Panalytical, UK). The weighed amount of sample was dispersed in deionized water and 1.0 M NaCl separately (1.0 mg/mL) and the required amount of sample was filled in a zeta sizer cuvette (DTS1070). The analysis was done at 25°C, count rate of 111.5 (kcps). The analysis was done at GLA University, Mathura, India.

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) spectra of mucilage were analyzed on an FT-IR spectrometer (IRAffinity, Shimadzu, Japan). The mucilage was pulverized and blended with Kbr and transferred to the sample holder and spectra were obtained between 4000-400 1/cm. The analysis was done at GLA University, Mathura, India.

1D nuclear magnetic resonance (NMR ^{1}H liquid state and ^{13}C solid state)

NMR spectra of mucilage of ^{1}H liquid state was analyzed in the NMR spectrometer (Avance II 400, Bruker, Germany.). The mucilage in D_2O has been dissolved and the chemical shifts were analyzed in ppm relative to internal standard TSP. NMR spectra of mucilage of ^{13}C solid-state were analyzed in the NMR spectrometer (AV 500, Bruker,). ^{1}H NMR spectra were recorded at the base frequency of 400MHz and ^{13}C NMR spectra were recorded at the base frequency of 125 MHz. ^{1}H and ^{13}C NMR were done at SAIF, Panjab University, Chandigarh, India, and SIF, NMR research center, IISc, Bangalore, India respectively.

RESULTS

Scanning electron microscopy revealed irregular shapes and size

SEM analysis was carried out to analyze the surface morphology of mucilage from *P. variegata*. Microphotographs obtained are represented in Figure 2 in different magnifications. Microphotographs significant that the mucilage particles had an uneven irregular shape and are in the size of 20-100 microns. SEM results of the present study suggest that the hydration capacity of mucilage depends on the surface property it also suggested that it can be used for granulation technique.

Powder X-ray diffraction suggests amorphous nature

X-ray diffractogram pattern of *P. variegata* mucilage powder Figure 3 shows a broad peak with a maximum area at 2Θ=21.50, the average grain length estimated to d-spacing=4.129 Å and some halos having weak peaks. Isolated mucilage is amorphous in essence, this shows that the wet granulation technique for dosage formulation is suitable for the mucilage

Particle size analysis indicates good flowability

The size of *P. variegata* mucilage powder obtained by using a particle size analyzer was found to be 1989 nm and the average particle size (Z-average) obtained was 1923 nm. The dried powder mucilage has flow properties that could be suitable for the use of wet granulation technology. PSA also previews the degree of densification, which could occur during tableting which was further confirmed by particle size analysis.

Differential scanning calorimetry illustrated the amorphous nature

Figure 4 The Differential scanning calorimetry thermograms of *P. variegata* mucilage show the glass Transition temperature (Tg) at 95.9°C. The major intense peak recorded in the DSC thermograms is endothermic, followed by weaker exotherm(s). DSC compares the difference between the energy acquired or released by a sample and a suitable reference as a function of

Table 1: Elemental compositions CHNS) of *Phanera variegata* mucilage.

Sl. No.	Component	Retention time *	Element ^
1	Carbon	1.15	41.41
2	Hydrogen	3.77	5.80
3	Nitrogen	.80	3.46
4	Sulphur	8.08	0.27

Note: * Retention time was calculated in minutes ^Elements were calculated in (%).

Table 2: FTIR interpretation of *Phanera variegata* mucilage.

Sl. No.	Observed absorption peak (cm^{-1})	Functional groups and type of vibration
1	3500 cm^{-1}-3200 cm^{-1}	stretching vibration of (-OH).
2	3000-2840 cm^{-1}	aliphatic (-CH) stretching.
3	1599 cm^{-1}	(-CH-OH-) stretching.
4	1049-1072 cm^{-1}	Stretching vibration of C-OH from carbohydrates.
5	1253 cm^{-1}	(C-O) stretching of polysaccharides.
6	2848 cm^{-1}	($-CH_3$) methyl groups in cellulose.
7	1095 cm^{-1}	stretching of (C-O-C) group in saccharides.

temperature or time while the sample and reference are subjected to a controlled temperature rise.

Thermogravimetry analysis suggested thermal stability

The TGA curve of *P. variegata* mucilage is represented in Figure 5. The thermogram shows no major weight loss (approx. 13%) until 190°C, two main peaks result from the thermogram of mass loss. The secondary weight loss is occurring at 290°C (approx.). The details of thermal behavior stability data acc. to the primary thermograms and derivative thermograms for the gum. The weight loss onset represents the onset of oxidation or maybe the decomposition of polymer which suggests that mucilages have good thermal stability

Elemental compositions analysis of mucilage

The quantitative estimation of *P. variegata* mucilage is done. The result of elemental composition analysis shows that the % element of CHNS content to be 41.41, 5.80, 3.46, and 0.27 (w/w %) respectively and the % of elements are shown in Figure 6 and tabulated in Table 1. The presence of nitrogen may indicate the presence of protein in the sample. Presence of sulphur may indicate the presence of protein and amino acids, the formation of bond.

Zeta potential (ζ) indicated a poly-electric effect in pure water

Zeta potential (ζ) was measured to get information about the stability charge behavior of the polymer. The zeta potential (ζ) of *P. variegata* mucilage powder was recorded on zetasizer at 1.0 mg/mL concentration in an aqueous medium (deionized water) and was found to be-28.0 mV. The conductivity and dielectric constant often increase with thermal and electrical stress. These changes are indicative of the decomposition of material to yield a few smaller molecules. The concomitant shift in UV and IR Spectra. Taking account of earlier studies it can be ascertained that the ZP caused a huge impact on the properties of mucilage.

Fourier transform infrared spectroscopy

The main functions groups of *P. variegata* mucilage are interrelated by FTIR spectra which are represented in Figure 7, spectra show the typical bands and peaks that characterize mucilage. The major outcome of FTIR is tabulated in Table 2.

1D nuclear magnetic resonance (NMR 1H liquid state and 13C solid-state)

The NMR spectra of *P. variegata* mucilage are interpreted; a Hydrogen-1 liquid-state spectrum is represented in Figure 8 and a Carbon-13 solid-state spectrum is represented in Figure 9. NMR confirmed the presence of non-reducing sugars and the obtained results demonstrate that mucilage is a useful pharmaceutical aid and can be used for effectively controlling the release of drugs from the designed matrix systems. The major outcome of the ^{1}H liquid state and ^{13}C solid state is tabulated in Table 3.

DISCUSSION

Mucilage was isolated from the leaves of *Phanera variegata* Linn. The SEM analysis of the present study suggests that *P. variegata* mucilage is amorphous in nature similar results have been shown by mucilage obtained from *Diospyros melonoxylon* Roxb and some other mucilages. Scanning electron microscopy revealed irregular shapes and sizes. The water-holding capacity of the mucilage relies on the property of the surface. The differences in the structure surface or shape morphology can be affected by extraction and purification methods or product preparation.[15] X-ray diffractogram pattern of *P. variegata* mucilage powder suggests the amorphous nature of the *P. variegata* mucilage powder mucilage. Similar X-ray diffractogram pattern of natural

gums such as guar gum, Arabic gum, and gum karaya which reveal amorphous nature has been reported by other authors.[16-18] The PXRD is a very useful method for analyzing materials as it suggests the pattern of atoms and molecules in the material and the geometry of the polymer structure. Particle size analysis suggests that particles isolated from mucilage dried powder are fine in appearance and the mucilage has good flowability which is suitable for granulation technology and some liquid dosage forms.[19] In the DSC assessment, the heating cycle suggests the thermal history of polymer and crystallinity fundamentals, and the cooling cycle illustrates the enthalpy and crystallization temperature.[20] The DSC thermograms of *P. variegata* mucilage

Table 3: ^{1}H and ^{13}C NMR interpretation of *Phanera variegata* mucilage.

Sl. No.	Signal (ppm)	Assignment
1	1.81 ppm, 1.03 ppm and 2.5 ppm	N-acetyl group, aliphatic alkyl and uronic acid.
2	5.8-5.0 ppm	Proton of α-anomeric carbon.
3	4.6-4.4 ppm	Proton of β-anomeric carbon.
4	4.0-3.27 ppm	OH and CH groups.
5	64-90 ppm	Rings carbons C-OH.
6	63 ppm	-CH OH
7	20-24 ppm	Methyl groups of Rhamnose.

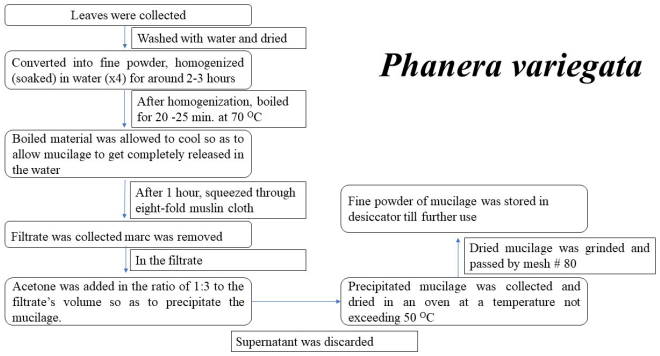

Figure 1: Isolation process of mucilage.

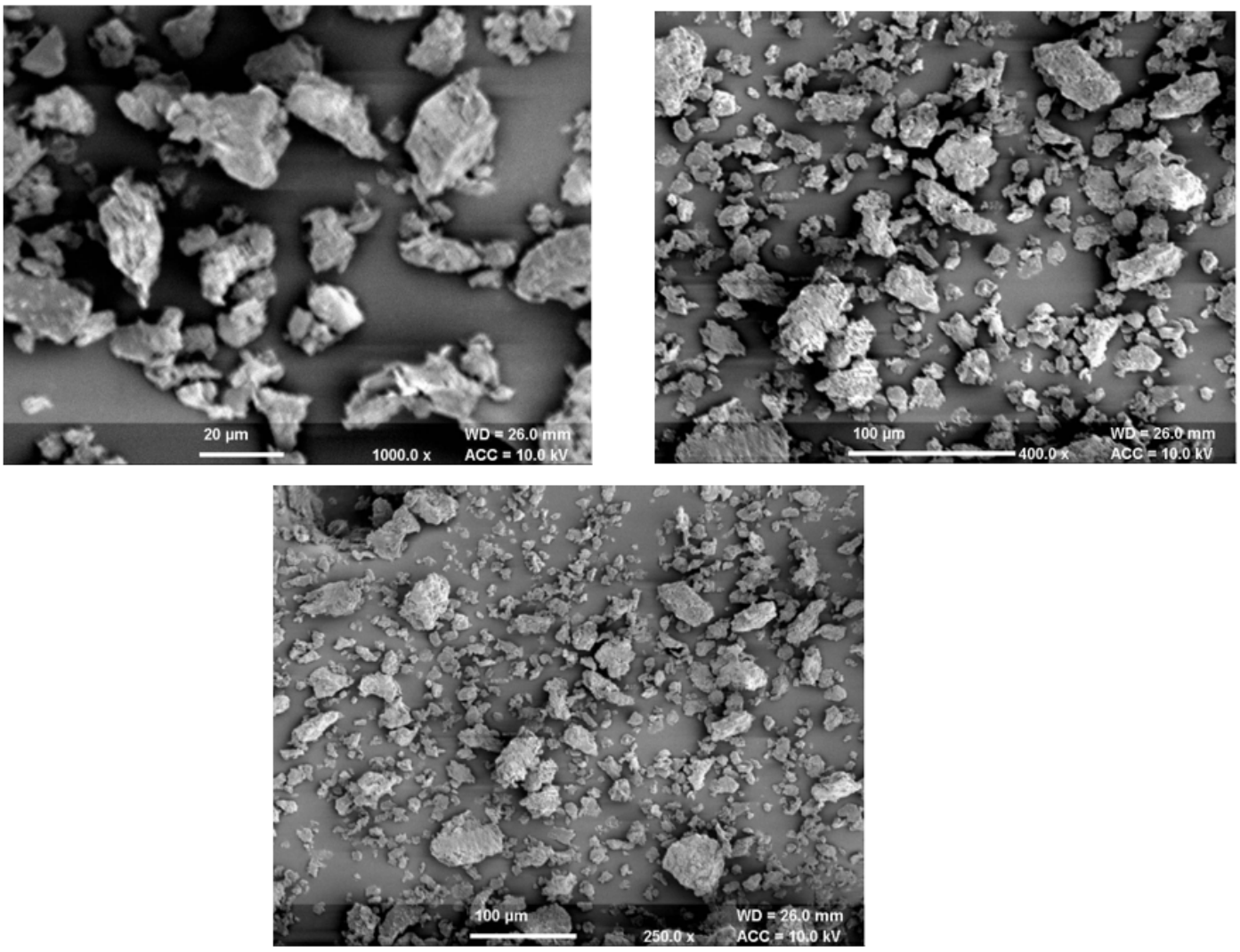

Figure 2: SEM images of mucilage at different magnifications.

show the glass Transition temperature (Tg) at 95.9°C. The intense peak recorded is an endothermic transition at 172.91°C. In the cooling cycle, no endothermic and exothermic peak has been found, this indicates the absence of crystallization and shows the same result as PXRD which indicated the amorphous nature of *P. variegata* mucilage. Therefore, the results of DSC show that the sample mucilage has shown thermal stability, dehydration, and depolymerization are also involved in the process.[21] In the TGA curve two main peaks result from the thermogram of mass loss. The primary minor weight loss is occurring at 80°C (approx.) which attribute to the weight loss of structural and absorbed biopolymers water,[22,23] or due to the release of moisture from the structure of saccharide. The secondary weight loss is occurring at 290°C (approx.) which attribute to the polysaccharide decomposition.[24] According to other authors, it is reported that the polysaccharide shows main degradation or decomposition between 210 and 320°C.[25] In addition, another weight loss is occurring at 590°C (approx.) which attribute to the carbonaceous residues.[26] The onset of weight loss represents the start of the decomposition/oxidation of polymer which indicates that mucilage has good thermic stability. Elemental analysis of the material (like chemical compounds, minerals, and soil) is

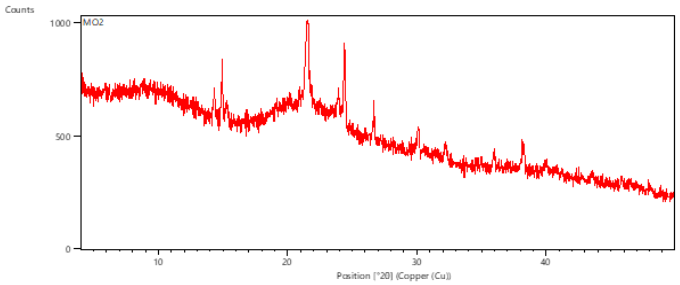

Figure 3: Powder X-ray diffractogram of *Phanera variegata* Linn. Mucilage.

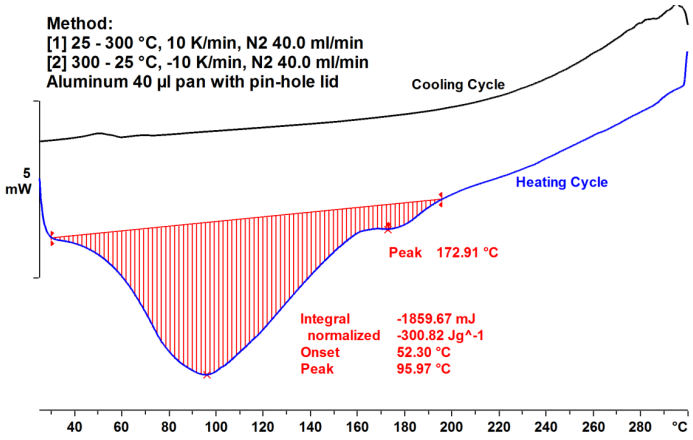

Figure 4: DSC Thermogram of *Phanera variegata* Linn. mucilage.

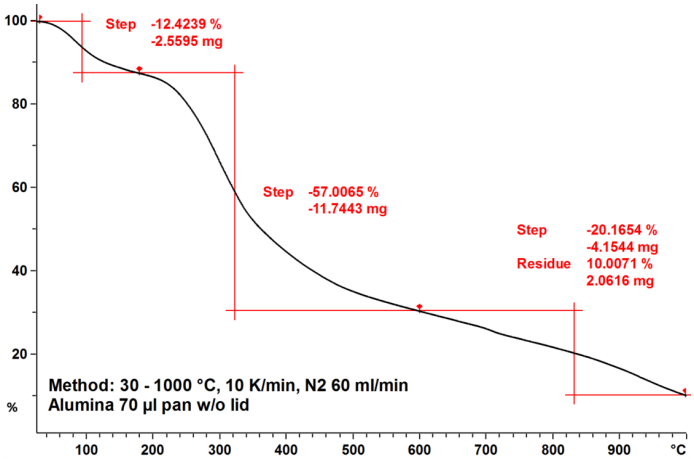

Figure 5: Thermogravimetry analysis thermogram of mucilage.

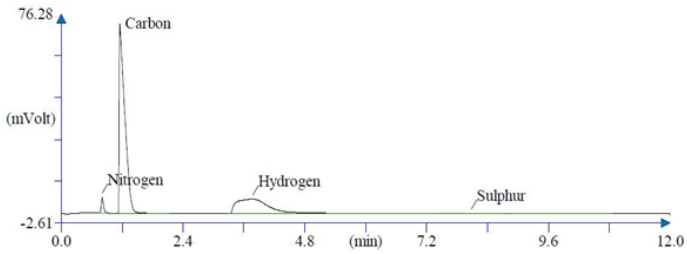

Figure 6: Elemental compositions (CHNS) graph of *Phanera variegata* mucilage.

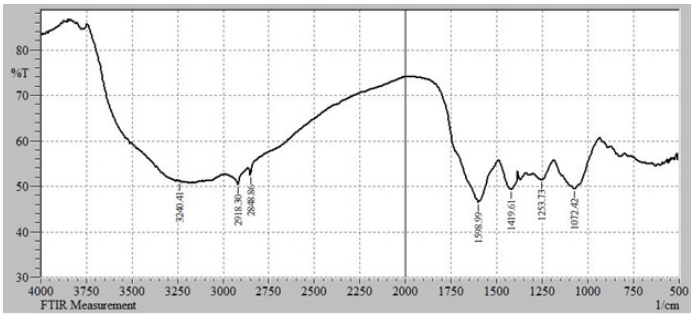

Figure 7: FTIR Spectra of mucilage obtained from *Phanera variegata* mucilage.

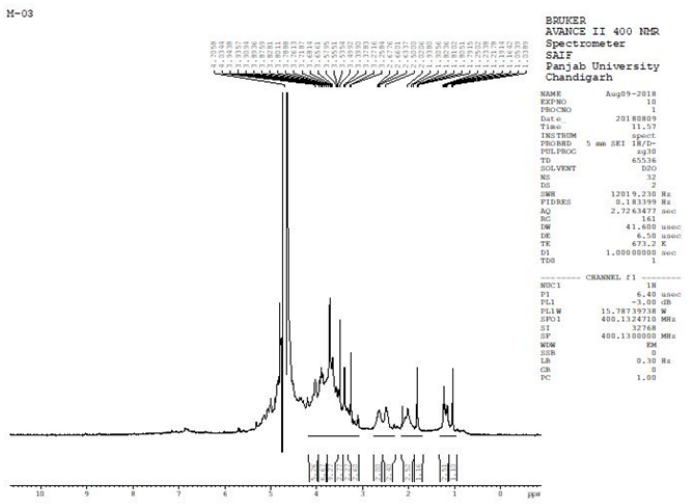

Figure 8: 1D Hydrogen-1 liquid state NMR spectrum.

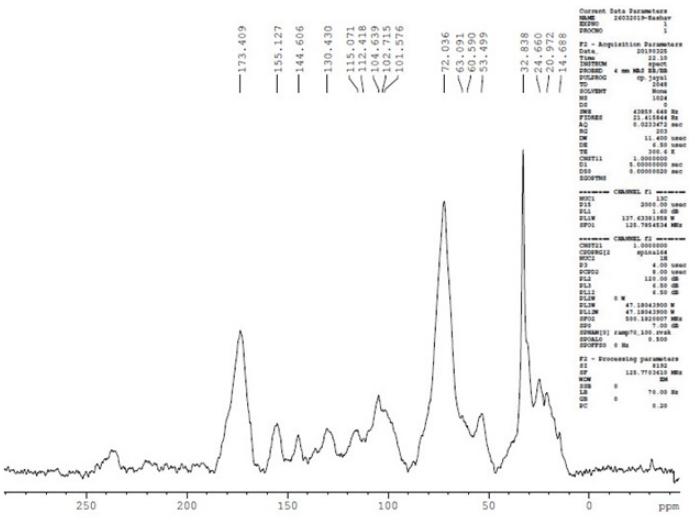

Figure 9: 1D Carbon-13 solid-state NMR spectrum.

a process in which samples analyze for elemental components (carbon, nitrogen, hydrogen, sulphur, and oxygen). Estimation of elements can be both qualitative (to determine whether the elements are present or not) and quantitative as well. CHNS composition of *P. variegata* mucilage was found to be almost equal to gum Arabic,[27] lower than *Diospyros melonoxylon* Roxb.,[28] and slightly higher than gum kondagogu.[29] Further, the presence of sulphur and nitrogen suggests the presence of S-containing protein as suggested by other authors.[30] The electro kinetic studies depend upon the potential between an electrolyte solution and surface, so it helps to determine the magnitude of the electrical double-layer repulsion. The results suggest that mucilage has moderate stability as colloids show moderate stability between +_ 25 to +_40 and isolated mucilage powder could help to check the rapid coagulation of substances in the liquid dosage form. No zeta potential could be recorded for the mucilage in the presence of 1.0M NaCl. FTIR spectroscopy is one of the main analysis parameters for the structural characterization of any compound. FTIR is widely used in the characterization of the polymer as it results in the identification of functional groups and their arrangement with the polymer backbone.[31,32] According to other authors, the so-called spectrum fingerprint region between 1400 cm^{-1}-1000 cm^{-1} may attribute to the polysaccharides.[33] The *P. variegata* mucilage showed absorption bands with stretching vibration of the hydroxyl (-OH) group in the region 3500-3200 cm^{-1}, aliphatic (-CH) stretching in the region 3000-2840 cm^{-1} and (-CH-OH-) stretching at 1599cm^{-1}.[32,34] The absorption bands appear near 1049, 1072 cm^{-1} concurred with stretching vibrations of C-OH from carbohydrates, the fingerprint region for carbohydrates attribute between wave numbers 1200-800 cm^{-1}.[9] According to other authors, the band at 1253 cm^{-1} is of (C-O) stretching in polysaccharides (complex), and the band at 1095 cm^{-1} refers to the stretching of the (C-O-C) group in saccharides.[35] The peak at 1419 cm^{-1} refers to the carboxylate group of galacturonic acid residues.[36] The peaks obtained at 2918 and 2848 were due to the stretching of (C-H) bonds of (-CH_3) methyl groups in cellulose and hemicellulose components.[33] There are generally fractions of sugar-acid units in natural gums that show weak anionic character to the macromolecule of gums.[37] According to another author broad band at 3280 cm^{-1} is of intra and intermolecular H_2 bonding of -OH groups relate to the structure of carbohydrates in *P. variegata* mucilage spectra is the broadband occurs at the wave number 3240 cm^{-1}.[38] The result of *P. variegata* mucilage spectra suggests that the mucilage is mainly composed of polysaccharides. To study the chain configuration and microstructure of polymers NMR is a key analysis in both the solid as well as liquid state of the material. Hydrogen-1 liquid state spectra of *P. variegata* mucilage shows multiple signal narrow region between 3-5 ppm typically like polysaccharides which confirm that many similar sugar residues are present in the mucilage.[39] The signal at 1.81 ppm, 1.03 ppm, and 2.5 ppm are attributed to the N-acetyl group, aliphatic alkyl groups, and uronic acid respectively. Other authors also reported the presence of acid sugars as D-galacturonic acid agreeing with the uronic acid. The signals between regions of 5.8-5.0 were attributed to the proton of α-anomeric carbon and 4.6-4.4 was attributed to the proton of β-anomeric carbon. The signals between 4.0-3.27 ppm can attribute to OH and CH groups. The regions around α- and β-anomeric carbon can be attributed to the galactose, rhamnose, xylose, and arabinose as neutral sugars,[40] Carbon-13 solid-state spectra of *P. variegata* mucilages show width lines that interpret the presence of typical amorphous polymer with band signals between 64-90 ppm due to the bulk of rings carbons C-OH. The signal at 63 ppm is a low-intensity signal which attributes to the -CH-OH belongs to the glucose. Signal high frequency at near 173 ppm attributed to -COOH group and shows exist of sugar acid. The anomeric carbons generally give signals at 90-110 ppm and the band shape indicates it consists of multiple signals. Signals at approx. between 20-24 ppm may be attributed to methyl groups of rhamnose, both signals indicate similar components.[41] The spectrum shows the presence of significant amounts of aliphatic C (10-50 ppm), as other authors also have reported.[42]

CONCLUSION

Phanera variegata Linn. isolated mucilage is a novel polysaccharide gum with little to almost no published characterization data. The mucilage is natural, non-toxic, and biodegradable. It is widely available as well as requires low production cost and time. The total percentage yield of isolated mucilage from *Phanera variegata* Linn. is found to be 10.2% which indicates that it can be used as alternative pharmaceutical excipients. The result obtained from the study indicated that the isolated mucilage has innate properties which can be used in the manufacturing of various dosage forms. SEM results of the present study suggest that, the hydration capacity of mucilage depends on the surface property it also suggested that it can be used for granulation technique. Some researchers reported that particle size influenced hydration kinetics. The mucilage is found to be

thermally stable which is confirmed by the results of DSC and TGA. Dehydration, decomposition, and depolymerization are involved in DSC and TGA at high-temperature stages resulting in the formation of H_2O, CO, and CH_4, because of the difference in the functional groups and structures, either the degradation routes or the resulting fragments will be different. Usually, the polysaccharides are comprised of carboxylate or carboxylic acid functional groups Significant element compositions are present in mucilage. The PXRD and DSC confirm that isolated mucilage is amorphous in essence, these shows that the wet granulation technique for dosage formulation is suitable for the mucilage. Particle size analysis showed that particles are fine in size which is good for the granulation technique. Zeta potential confirms the moderate stability of the mucilage. FTIR spectroscopy and 1D ^{1}H and ^{13}C NMR studies confirm the presence of non-reducing sugar. 13C NMR spectra of mucilages gave line widths which are typical of an amorphous natural polymer. The results obtained from the mentioned studies show that the mucilage isolated is a very useful pharmaceutical excipient as it can be used for modified drug delivery systems, sustained, prolonged, and control drug delivery systems as an alternative natural binder as well as a useful excipient in liquid dosage forms. Further research of the isolated mucilage from the leaves of *P. variegata* can be done on the various potential of the isolated mucilage in the formulation of different dosage form, its acceptability as pharmaceutical excipient for other dosage forms.

LIMITATIONS

Mucilages are plant-derived polymers and hence their availability is based on environmental and seasonal conditions, these variations may affect the quality, yield, and production of mucilages. The extraction and isolation processes of mucilage are quite complicated. Mucilage yield and consistency also depend upon physical damage to the part of the plant from which mucilage will be extracted, posing a serious challenge to associated costs and the potential for mass production. The moisture content of mucilages may lead them to the risk of microbial contamination during any stage of their processing if not stored properly. The storage period is also a key factor in the contamination of mucilage as many researchers have reported that variation in storage leads to changes in the quality of mucilage as this requires control of the handling methods used at various stages. Further, some modifications can lead to control of the disadvantages of mucilages.

ACKNOWLEDGEMENT

The authors are thankful to GLA University, Mathura, Uttar Pradesh, India for the financial assistantship and for providing the necessary facilities to carry out this research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SEM: Scanning electron microscopy; **DSC:** Differential scanning calorimeter; **TGA:** Thermogravimetry analysis; **PXRD:** Powder X-ray diffraction; **FTIR:** Fourier transform infrared spectroscopy; **NMR:** Nuclear magnetic resonance spectroscopy.

SUMMARY

Mucilage was isolated from the leaves of *Phanera variegata* Linn. which is a novel polysaccharide gum with little to almost no published data on characterization as well as on its formulation. In the present study, the mucilage from the leaves of *Phanera variegata* plants was isolated and analyzed by various analytical techniques like SEM, PXRD, particle size analysis, DSC, TGA, elemental analysis (CHNS), zeta potential, FTIR, and 1D (^{1}H and ^{13}C) NMR spectroscopy. Finally, the obtained results showed that *P. variegata* mucilage was found to be amorphous, thermally stable, and can be used as an excellent alternative natural pharmaceutical excipient for conventional pharmaceutical drug products and novel drug delivery systems in varying concentrations.

REFERENCES

1. Zatz JL, Kushla GP, Reiger MM. Pharmaceutical dosage forms: disperse system. New York: Marcel Dekker Inc; 1989; 2: 508.
2. Jani GK, Shah DP, Prajapati VD, Jain VC. Gums and mucilage's: versatile excipients for pharmaceutical formulations. Asian J Pharm Sci. 2009; 4(5): 309-23.
3. Pinto Cotrim MA, Mottin Caron A, Ayres E. Preparation and characterization of okra mucilage (*A. esculentus*) Edible films. Macromol Symp. 2016;367(1):90-100. doi: 10.1002/masy.201600019.
4. Pandey R, Khuller GK. Polymer based drug delivery system for mycobacterial infectious. Curr Drug Deliv. 2004; 1(3): 195-201. doi: 10.2174/1567201043334669, PMID 16305383.
5. Chamarthy SP, Pinal R. Plasticizer concentration and the performance of diffusion-controlled polymeric drug delivery system. Colloidals Surf Physicochem Eng Aspect. 2008; 331(1-2): 25-30. doi: 10.1016/j.colsurfa.2008.05.047.
6. Alonso-Sande M, Teijeiro-Osorio D, Remuñán-López C, Alonso MJ. Glucomannan, a promising polysaccharide for biopharmaceutical purposes. Eur J Pharm Biopharm. 2009; 72(2): 453-62. doi: 10.1016/j.ejpb.2008.02.005, PMID 18511246.
7. Guo J, Skinner GW, Harcum WW, Barnum PE. Pharmaceutical application of naturally occurring water soluble polymers. Pharm Sci Technol Today. 1998; 1(6): 254-61. doi: 10.1016/S1461-5347(98)00072-8.
8. Sharma DR, Sharma A, Kundal A, Rai PK. Herbal gums and mucilage as excipients for pharmaceutical purpose. Res J Pharmacogn Phytochem. 2016; 8(3): 145-52.
9. Huanbutta K, Sittikijyothin W. Use of seed gums from *T. indica* and *C. fistula* as controlled-release agents. Asian J Pharm Res. 2018; 13(5): 398-408. doi: 10.1016/j.ajps.2018.02.006.
10. Kulkarni PK, Reddy S. Evaluation of sericin as a binder in tablet formulation. Ind J Pharm Educ Res. 2009; 43(4): 370-4.
11. Efentakis M, Koutlis A. Release of furosemide from multiple unit and single unit preparations containing different viscosity grade of sodium aliginate. Pharm Dev Technol. 2001; 6(1): 91-8. doi: 10.1081/PDT-100000048, PMID 11247279.
12. Ameena K, Dilip C, Saraswathi R, Krishnan PN, Sankar C, Simi SP. Isolation of the mucilages from *Hibiscus rosa sinensis* Linn. Asian Pac J Trop Med. 2010; 3(7): 539-43. doi: 10.1016/S1995-7645(10)60130-7.
13. Sharma S, Kumar A. Tribal uses of medicinal plants of Rajasthan: kachnar. Int J Life Sci Pharm Res. 2012; 2(4): 69-76.
14. Sudarshan S, Sangeeta S, Sheth NR, Roshan P, Ushir YU, Gendle R. Colon specific drug delivery system of mesalamine for eradication of ulcerative colitis. Res J Pharm Technol. 2009; 2(4): 819-23.
15. Qian J, Chen W, Zhang W, Zhang H. Adulteration identification of some fungal polysaccharides with SEM, XRD, IR and optical rotation: a primary approach. Carbohydr Polym. 2009; 78(3): 620-5. doi: 10.1016/j.carbpol.2009.05.025.

16. Reddy TT, Tammishetti S. Free radical degradation of Guar gum. Polym Degrad Stab. 2004; 86(3): 455-9. doi: 10.1016/j.polymdegradstab.2004.05.017.
17. Mallik H, Gupta N, Sarkar A. Anisotropic electrical conduction in gum arabic-A biopolymer. Mater Sci Eng C. 2002; 20(1-2): 215-8. doi: 10.1016/S0928-4931(02)00036-X.
18. Murali Mohan Babu GV, Prasad ChD, Ramana Murthy KV. KVEvaluation of modified gum karaya as carrier for the dissolution enhancement of poorly water-soluble drug nimodipine. Int J Pharm. 2002; 234(1-2): 1-17. doi: 10.1016/s0378-5173(01)00925-5, PMID 11839433.
19. Shah K, Shrivastava SK, Mishra P. Formulation and evaluation of suspensions: mefenamic acid prodrugs. Pak J Pharm Sci. 2014; 27(4): 917-23. PMID 25015460.
20. Komath S, Garg A, Wahajuddin M. Development and evaluation of chrysin-phospholipid complex loaded solid lipid nanoparticles-storage stability and *in vitro* anticancer activity. J Microencapsul. 2018; 35(6): 600-17. doi: 10.1080/02652048.2018.1559369, PMID 30557060.
21. Zohuriaan MJ, Shokrolahi F. Thermal studies on natural and modified gums. Polym Test. 2004; 23(5): 575-9. doi: 10.1016/j.polymertesting.2003.11.001.
22. Vendruscolo CW, Ferrero C, Pineda EAG, Silveira JLM, Freitas RA, Jiménez-Castellanos MR, *et al.* Physicochemical and mechanical characterization of galactomannan from *Mimosa scabrella*: effect of drying method. Carbohydr Polym. 2009; 76(1): 86-93. doi: 10.1016/j.carbpol.2008.09.028.
23. Kittur FS, Harish Prashanth KV, Udaya Sankar K, Tharanathan RN. Characterization of chitin, chitosan and their carboxymethyl derivatives by differential scanning calorimetry. Carbohydr Polym. 2002; 49(2): 185-93. doi: 10.1016/S0144-8617(01)00320-4.
24. Varma AJ, Kokane SP, Pathak G, Pradhan SD. Thermal behavior of galactomannan guar gum and its periodate oxidation products. Carbohydr Polym. 1997; 32(2): 111-4. doi: 10.1016/S0144-8617(96)00155-5.
25. Li M, Fan Y, Xu F, Sun R. Structure and thermal stability of polysaccharide fractions extracted from the ultrasonic irradiated and cold alkali pretreated bamboo. J Appl Polym Sci. 2011; 121(1): 176-85. doi: 10.1002/app.33491.
26. Archana G, Sabina K, Babuskin S, Radhakrishnan K, Fayidh MA, Babu PA, *et al.* Preparation and characterization of mucilage polysaccharide for biomedical applications. Carbohydr Polym. 2013; 98(1): 89-94. doi: 10.1016/j.carbpol.2013.04.062, PMID 23987320.
27. Abuarra A, Bauk S, Hashim R, Kandaiya S, Tousi ET, Aldroobi K. Microstructure examination, elemental composition analysis of gum arabic bonded *Rhizophora* spp. particleboards and their potential as tissue equivalent material. Int J Chem Environ Biol Sci. 2014; 2(1): 71-5.
28. Singh S, Bothara SB. Physico-chemical and structural characterization of mucilage isolated from seeds of *D. melonoxylon* Roxb. Braz J Pharm Sci. 2014; 50(4): 713-25. doi: 10.1590/S1984-82502014000400006.
29. Vinod VTP, Sashidhar RB, Suresh KI, Rama Rao B, Vijaya Saradhi UVR, Prabhakar Rao TP. Morphological, physico-chemical and structural characterization of gum kondagogu (*Cochlospermum gossypium*): A tree gum from India. Food Hydrocoll. 2008; 22(5): 899-915. doi: 10.1016/j.foodhyd.2007.05.006.
30. Janaki B, Sashidhar RB. Physico-chemical analysis of gum kondagogu (*Cochlospermum gossypium*): A potential food additive. Food Chem. 1998; 61(1-2): 231-6. doi: 10.1016/S0308-8146(97)00089-7.
31. Baxter A, Dillon M, Taylor KDA, Roberts GA. Improved method for IR determination of the degree of N-acetylation of chitosan. Int J Biol Macromol. 1992; 14(3): 166-9. doi: 10.1016/s0141-8130(05)80007-8, PMID 1390449.
32. Garg A, Hassan MZ. Inclusion complex of thymol and hydroxypropyl-B-cyclodextrin (HP-B-CD) in polymeric hydrogel for tropical applications: physicochemical characterization, molecular docking, and stability evaluation. J Drug Deliv Sci Technol. 2021; 64: 102609. doi: 10.1016/j.jdst.2021.102609.
33. Dimopoulou M, Ritzoulis C, Panayiotou C. Surface characterization of okra hydrocolloid extract by inverse gas chromatography (IGC). Colloids Surf A Physicochem Eng Asp. 2015; 475(1): 37-43. doi: 10.1016/j.colsurfa.2014.09.042.
34. Nayak AK, Pal D. Development of pH-sensitive tamarind seed polysaccharide-alginate composite beads for controlled diclofenac sodium delivery using response surface methodology. Int J Biol Macromol. 2011; 49(4): 784-93. doi: 10.1016/j.ijbiomac.2011.07.013, PMID 21816168.
35. Freitas TKFS, Oliveira VM, de Souza MTF, Geraldino HCl, Almeida VC, Fávaro SL, *et al.* Optimization of coagulation-flocculation process for treatment of industrial textile wastewater using okra (*A. esculentus*) mucilage as natural coagulant. Ind Crops Prod. 2015; 76: 538-44. doi: 10.1016/j.indcrop.2015.06.027.
36. Okafor LS, Chukwu A, Udeala K. Some physicochemical properties of Grewia gum. Niger J Polym. 2001;2(1):161-7.
37. Emeje M, Isimi C, Byrn S, Fortunak J, Kunle O, Ofoefule S. Extraction and physicochemical characterization of a new polysaccharide obtained from the fresh fruits of *Abelmoschus esculentus*. Iran J Pharm Res. 2011; 10(2): 237-46. doi: 10.22037/ijpr.2011.971, PMID 24250349.
38. Wang Q, Ellis PR, Ross MSB. Dissolution kinetics of guar gum powders II Effects of concentration and molecular weight. Carbohdr Polym. 2003; 53(1): 75-83. doi: 10.1016/S0144-8617(03)00009-2.
39. Ma X, Pawlik M. Intrinsic viscosities and Huggins constants of guar gum in alkali metal chloride solutions. Carbohydr Polym. 2007; 70(1): 15-24. doi: 10.1016/j.carbpol.2007.02.024.
40. Madera-Santana TJ, Vargas-Rodríguez L, Núñez-Colín CA, González-García G, Peña-Caballero V, Núñez-Gastélum JA, *et al.* Mucilage from cladodes of *Opuntia spinulifera* Salm-Dyck: chemical, morphological, structural and thermal characterization. CyTA J Food. 2018; 16(1): 650-7. doi: 10.1080/19476337.2018.1454988.
41. Elijah IN, Barbara RC. Characterization of Grewia gum, a potential Pharmaceutical excipients. J Excip Food Chem. 2010; 1(1): 30-40.
42. Watanabe T, Misawa S, Hiradate S, Osaki M. Characterization of root mucilage from *Melastoma malabathricum*, with emphasis on its roles in aluminum accumulation. New Phytol. 2008; 178(3): 581-9. doi: 10.1111/j.1469-8137.2008.02397.x, PMID 18373518.

Cite this article: Bansal K, Bajpai M. Isolation and Analysis of *Phanera variegata* Mucilage by Various Analytical Techniques. Indian J of Pharmaceutical Education and Research. 2024;58(2):642-50.