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Extraction, purification and characterization of water soluble galactomannans from *Mimosa pudica* seeds

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Abstract

Water soluble galactomannans from seed endosperm of *Mimosa pudica* L. was extracted and characterized (**Fig. 1**). Nuclear magnetic resonance spectroscopy and Gas Chromatography results revealed the presence of 4-linked mannose backbone with galactose side chains linked at the C6 position. Scanning Electron Micrographs showed smooth, elongated and irregular granular structure of galactomannan. Structural analysis by Attenuated total reflection infrared spectroscopy presented the Mannose to Galactose ratio while the X-ray diffraction studies showed the presences of A-type crystalline pattern of the galactomannan. Thermo Gravitimetric Analysis showed the three steps weight loss event and determined the thermal stability. The results showed that the extracted polysaccharides are typically amorphous, thermally stable and have desirable properties for industrial applications.

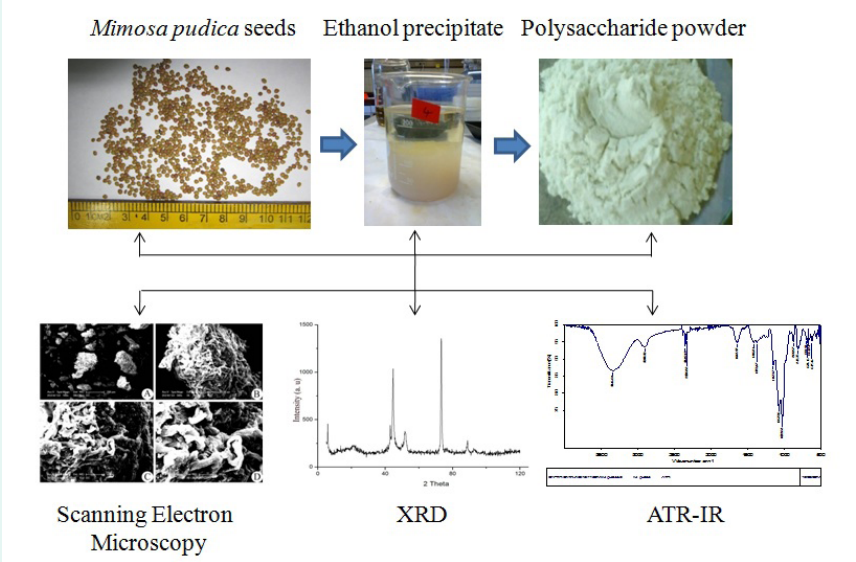

Figure 1. Graphic abstract of the polysaccharide extraction and characterization.

Introduction

Galactomannans are present in the endosperm of numerous plant seeds particularly in Leguminosae, also called as seed gums. These are comparatively cheap, non-toxic and eco-friendly and has a diverse range of industrial applications. The polysaccharides are built up of a b-(1-4)-D-mannan backbone with single D-galactose branches linked a-(1-6) and can be often used in different forms for human consumption (1). These galactomannans differ from each other by different Mannose/Galactose (M/G) ratios, which could be used as a marker in taxonomy (2,3). The different chemical properties of seed gums make them versatile materials for many different applications, like binding, emulsifying, gelling, holding capacity of H_2O, suspending, thickening, and formation of films. Featuring different physicochemical properties, galactomannans are also used as excellent stiffeners and stabilizers of emulsions (4), which allow their use in the pharmaceutical

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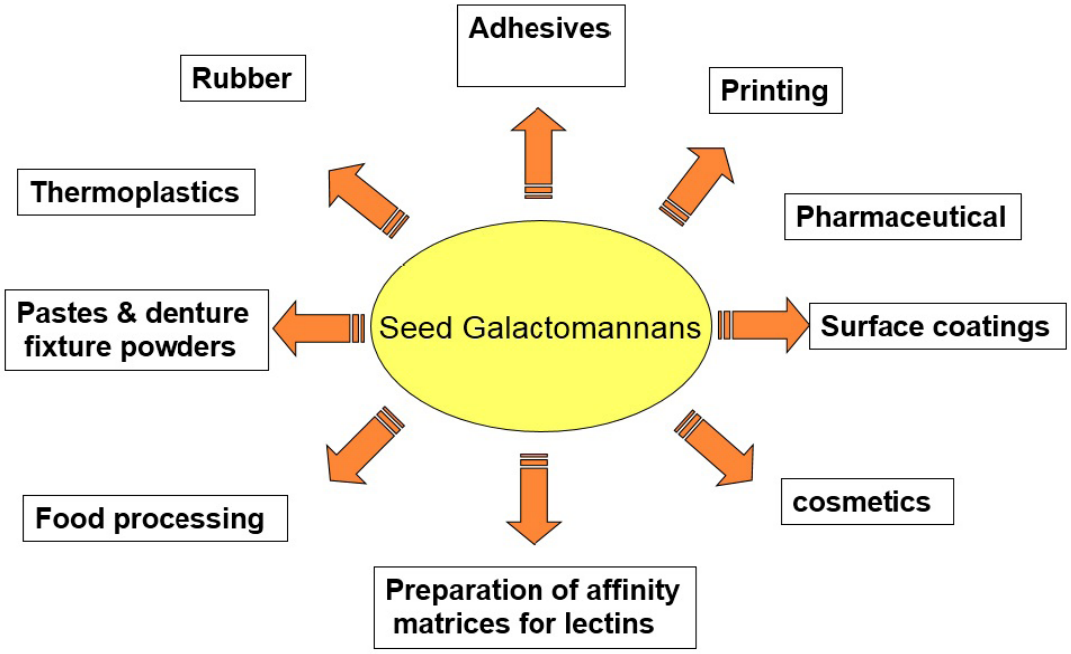

Figure 2. Various applications of galactomannas in different fields.

(5-7) biomedical, textile (8-10) cosmetics and food industries (11-13) (**Fig. 2**). Presently, the galactomannans are widely used in commercial and nontraditional purposes such as films and coatings production (14). Now-a-days galactomannans play a vital role in international market where commercial gums, namely guar gum (*Cyamopsis tetragonolobo*, M/G ratio: 2:1), tara gum (*Caesalphinia spinosa*, M/G ratio: 3:1) and locust bean gum (*Ceratonia siliqua*, M/G ratio: 3.5:1) (15) dominate the market. Recently, due to the demand of galactomannans for industrial applications in the international market, the need for alternative source of galactomannans arises (16). Therefore, it is necessary to study the isolation, physicochemical and structural properties of galactomannans for its application in various fields.

This study aims to isolate the galactomannans from the seeds of *Mimosa pudica* and characterize using SEM, DSC, XRD, FTIR and surface chemistry.

Materials and Methods

Materials

The seeds of *Mimosa pudica* were collected from Warangal, Telangana State during 2012- 2013. The seeds were manually separated from the pods and kept in a cool, dry place. *Mimosa pudica* is a legume plant popularly known as touch-me-not, native to South America and Central America. It is one of the important plants in Biological Nitrogen fixation (17).

Polysaccharide extraction and purification

Whole seed dry weight was measured using electronic balance. The seeds were soaked in water overnight at room temperature. The seed coat and germ was separated manually and left to air dry and the percentage of whole seed weight was calculated. A known weight of endosperm was dispersed in water (1:5 ratio) blended in kitchen blender and autoclaved followed by centrifugation at 8000 rpm for 40 min. The supernatant was collected and filtered with different filters G1, G2, G3, G4 & 5 micron nylon membrane filters and precipitated with 99% ethanol. The final precipitate was purified using ethanol by gradient purification method. The purified sample was collected and air dried. Thereafter, the air-dried product was further drying at 45°C in an oven for 24 hours. The dried polysaccharide was weighed and stored in air-tight container for characterization (18,19).

SEM (Preparation for Scanning Electron Microscopy)

The powder was mounted on to the double stick carbon tape and sputter-coated with gold approximately 30 nm thickness, in vacuum coating apparatus. External morphology of powder was examined with Philips/ESEM XL-30 scanning electron microscope (Amsterdam, Netherlands). Photographs were taken at various magnifications for each sample with voltage of 1 kV.

NMR (Nuclear Magnetic Resonance Spectroscopy)

Proton (^{1}H) spectra of galactomannans were recorded in an NMR at 300 MHz and room temperature using a Bruker AV300 NMR spectrometer (Bruker, Bremen, Germany). Polysaccharide samples (10 mg) were dissolved in 1 mL of deuterated water (D_2O). Subsequently, the samples were freeze-dried and re-dissolved in D_2O for three times to remove exchangeable protons. The ^{1}H NMR spectrum was recorded at a base frequency of 300 MHz, at 30°C. The chemical shifts were reported in ppm (20).

Methylation and GC-MS analyses

The Extracted polysaccharides were activated with Dry NaOH and methylated with 200 µl methyl iodide (CH_3I) (21). 3-5 mg of

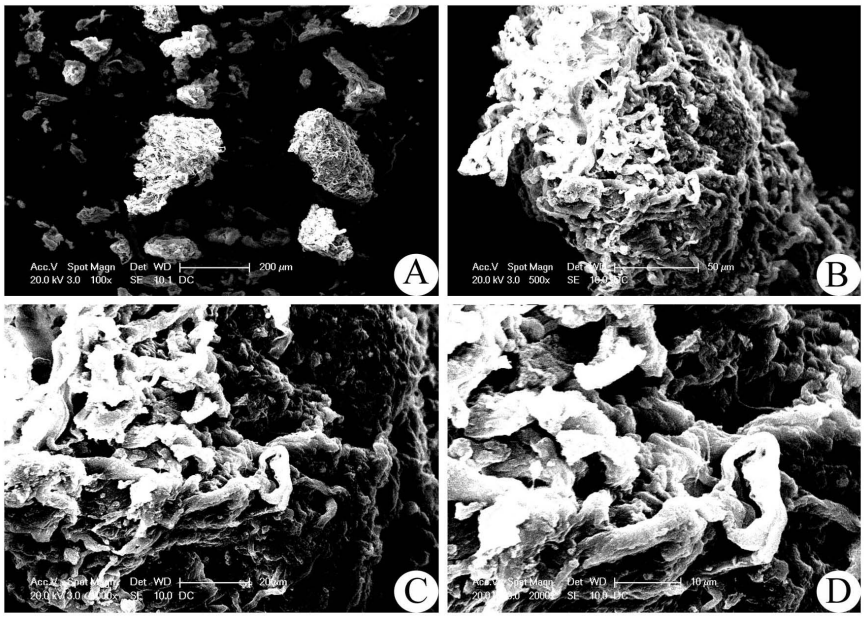

Figure 3. SEM photographs of *Mimosa pudica* polysaccharide powder (A) x100, (scale 200 µm); (B) x500 (scale 50 µm) and picture of individual granules; (C) x1000 (scale 20 µm) & (D) x2000 (scale 10 µm) magnifications.

powdered sample was dispersed in 2 ml of dried DMSO. NaOH (100mg) powder was added to the solution and sonicated for 90 min. The material mixture were dissolved in 3 ml of water and further extracted with 4 ml of chloroform. The organic layer was washed with 3 ml of water for 3 times, evaporated and remethylated to achieve complete methylation of all free OH groups. The methylated samples were then hydrolyzed with 2M trifluoroacetic acid (1 ml) for 1 hr at 121^{0}C, cooled, and evaporated at 40^{0}C. Partially Methylated sugars were then dissolved in dichloromethane (3-5 ml). The dichloromethane phase was washed with water and evaporated to dryness and silylated with silylation reagent (15 µl) and pyridine (5 µl) and analyzed by GC-MS (Agile technologies gas chromatograph and Bruker, mass spectrometer, Germany).

ATR-IR (Attenuated total reflection Fourier Transform Infrared spectroscopy)

IR spectra of the samples were measured using a Bruker Platinum Attenuated total reflection Fourier Transform Infrared (ATR-IR) instrument, with single reflection diamond crystal, all reflective, gold coated optics, in the range of 4000-600 cm^{-1}. Each spectrum was baseline corrected (22).

XRD (X-ray Diffraction)

The X-ray diffraction patterns of the galactomannan was obtained using Philips/PW1830 powder X-Ray diffractometer (Amsterdam, Netherlands) with Ni- filtered Cu radiation, 40 kv and 25 mA. The spectra measurements were carried out at a goniometer speed of 1^o (2θ)/min (5).

TG-DTA (Thermogravimetric Differential Thermal Analysis)

The TGA and DSC were carried out using METTLER DSC1, TGA/DSC1 thermogravimetric analyzer (Switzerland). Briefly, around 4-6 mg of the sample was weight in 70 ml ceramic crucible. Experiments were conducted under Nitrogen atmosphere, at a heating rate of 10^{o}C min^{-1} over a temperature range of 50-600^{o}C. Data were treated using Origine 8 software. Enthalpy was calculated between the onset temperature and the end set temperature (23,24).

Results

The total yield of polysaccharides obtained by total dry weight method was 21.05%. The granule morphology of the galactomannan was analyzed by SEM (**Fig. 3**). The SEM images of the sample exhibited smooth, elongated and irregular granular structure patterns like in commercial guar gum (25), which is clearly soluble in water. The surface structure and topology of a polysaccharide may effect by different extraction and purification methods (26).

The structure of the polysaccharide was confirmed using NMR spectroscopy. The ^{1}H NMR spectrum of polymer showed signals corresponding to β-D-galactopyranose, D-mannopyranosides, a peak at 3.95-4.10 ppm due to anomeric protons and at 4.8-4.9 ppm due to other sugar proton. The dominant peaks at 4.8, 4.9 ppm are ascribed to ^{1}H group of Galactose, 3.95 - 4.10 ppm are ^{1}H group of Mannose (**Fig. 4**).

Methylation and GC-MS analyses

Partially methylated compound analyzed by GC-MS revealed a polysaccharide total chemical composition. The mannose and galactose are the major monosaccharides present in the polysaccharide. The total content of mannose and galactose are 65.2% and 27.6%. The polysaccharide containing nonreducing terminal units of Manp with 0.14% and Galp with 31.03 as

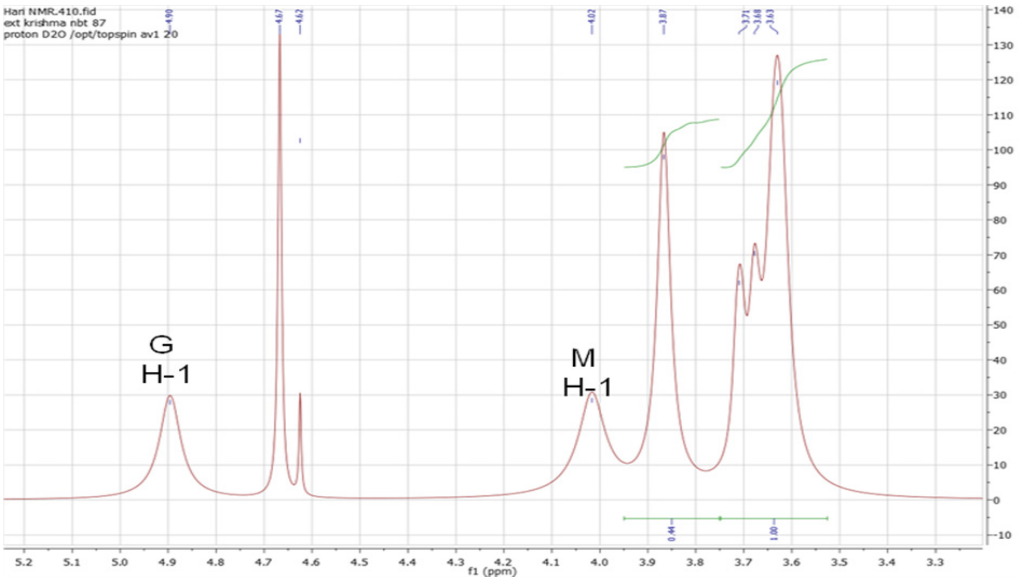

Figure 4. ^{1}H NMR spectrum of *Mimosa pudica* polymer concentration 10mg/ml in D_2O at 30 ^{0}C.

well as 4-Manp with 28.09%, 4,6-Manp 37.76% and 4-Galp 2.98. The methylation analysis results confirm the structure and linkage profile of the galactomannans as a 1-4 mannose linkages with galactose side chains attached at C6 position presented. The galactomannan contain minor amounts of other monosaccharides such as arabinose (Ara), glucose (Glc), rhamnose (Rha) and xylose (Xyl). These minor components present in the galactomannan could be attributed to a more complex polysaccharide composition. These results showed the total chemical composition in which mannose and galactose are the major constituents.

From the observation of ATR-IR spectra shown in **Fig. 5**, a weak absorption peaks at 550-750 cm^{-1} was attributed to protein absorption determining the presences of minute amount of protein or polypeptide which may be useful for the interaction with other compounds. The major peaks appeared in three regions, 3700-3000 cm^{-1} which are attributed to the hydroxyl groups. The peak at 3000-2800 cm^{-1} could be attributed to - C-H stretching of alkane. The absorption of an amino group was confirmed by the presences of peaks at 1650-1350 cm^{-1} (27). The peak present at 1150-950 cm^{-1} was associated with -CO and C-O-H stretching. The peaks at 870-810 cm^{-1} confirmed the characteristic absorption of mannose which is similar compared to reference compound LBG (28) (**Fig. 6**).

X-ray diffraction patterns determine the percentage of crystallinity, size and orientation of the galactomannans powder. The galactomannans have two scattering peaks at about 20^{o} and 44^{o} (**Fig. 7**). Four major peaks similar to that of standard LBG was observed at 2θ - 20.02, 44.05, 52.05 and at 73.01. The broad peak at 2θ - 20.02 indicates that the powder sample is amorphous in nature at room temperature as reported

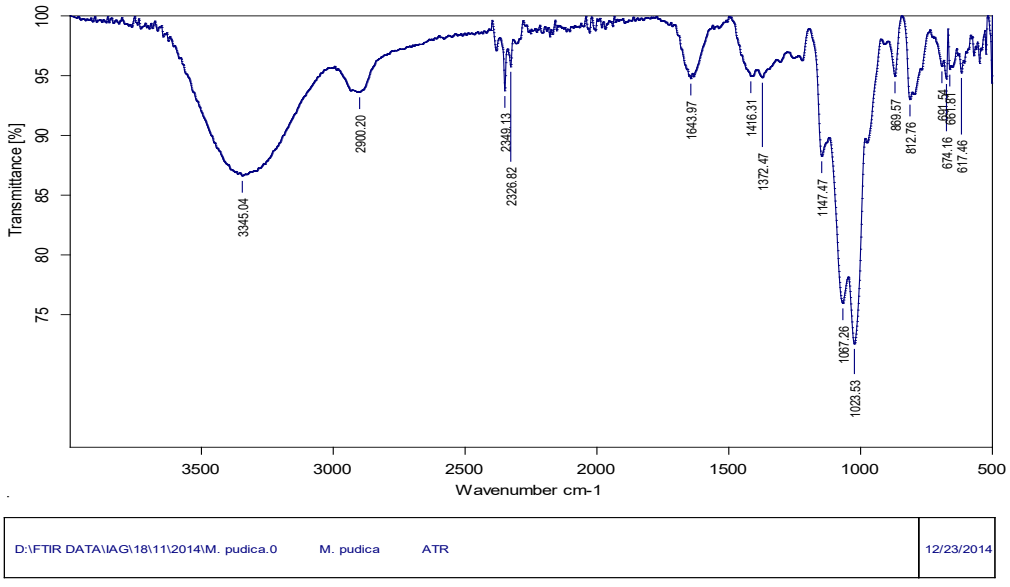

Figure 5. ATR-IR spectra of *Mimosa pudica* galactomannans with different absorption peaks.

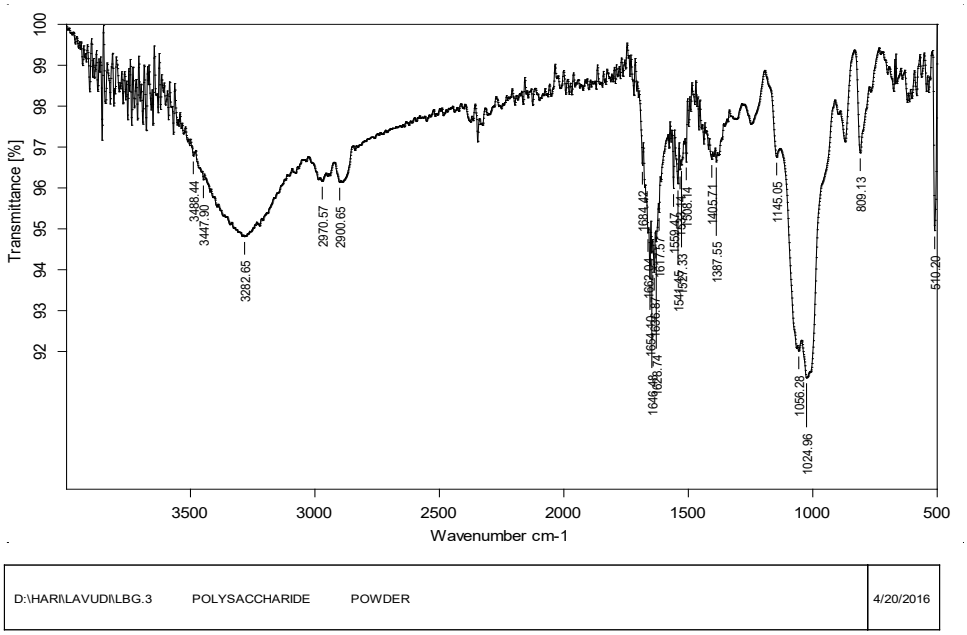

Figure 6. ATR-IR spectra of Locust bean gum showing the various absorption peaks.

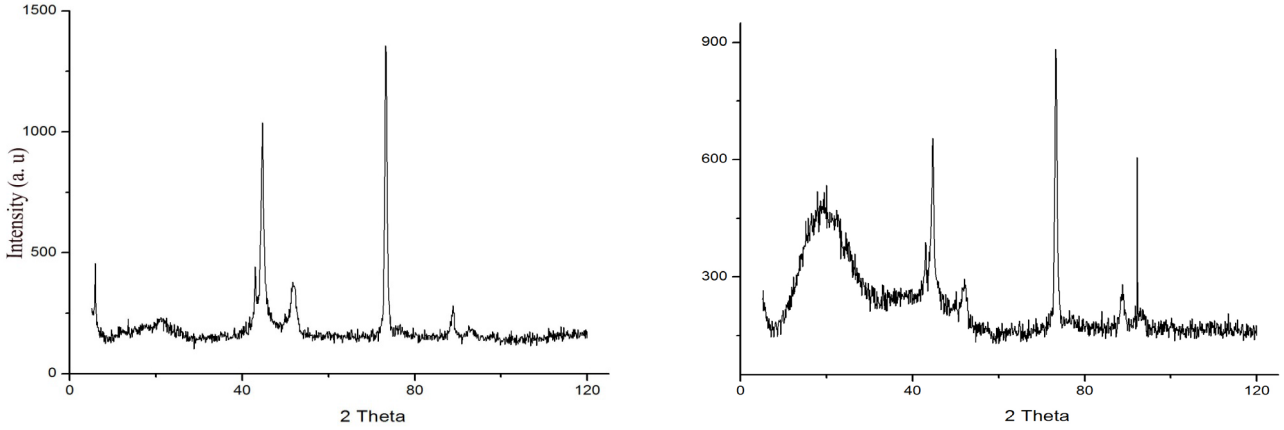

Figure 7. X-ray powder diffraction patterns of galactomannans from *Mimosa pudica* and Locust bean gum.

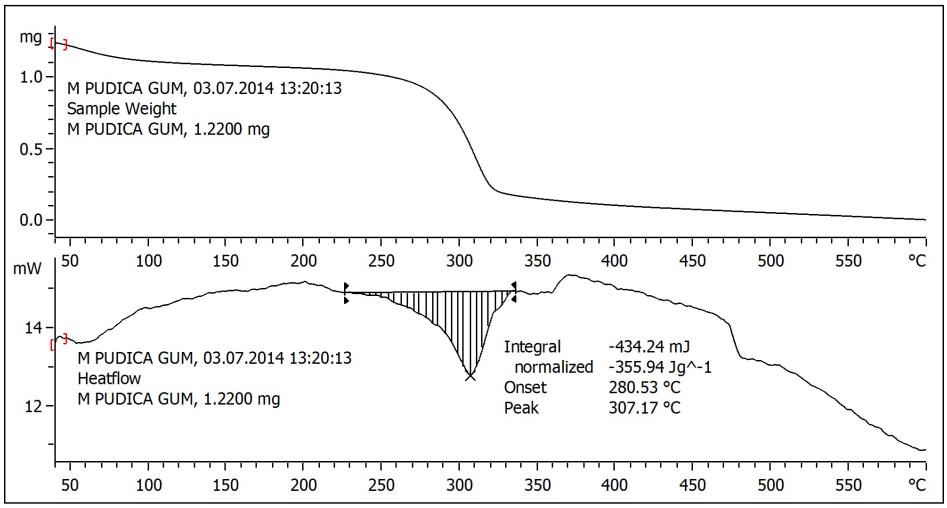

Figure 8. TG-DTA thermogram of polysaccharide powder of *Mimosa pudica* at 10^{0}C per minute upto 600^{0}C.

earlier in native guar gum (29,30), which has a large degree of crystallinity and the reduction of water content.

To determine the thermal stability of the isolated galactomannans, TG-DTA analysis was performed which reveal the decomposition pattern and thermal transition occurring in the course of heating under inert nitrogen atmosphere. The thermogram exhibited three distinct weight loss regions. Early endothermic event was located between 90-120°C which attribute to water evaporation. The second peak was located between 180-270°C which attribute to the degradation of polysaccharide chains indicate the endothermic event and the third stage at 270-330°C is related to the decomposition of the polysaccharide (**Fig. 8**). It clearly explained the thermal stability of the galactomannan allowing its use for several industrial applications.

Discussions

Galactomannans extracted from the seeds of *Mimosa pudica* having excellent properties such as amorphous powdered nature and high stability. The NMR results shown that the more intense signals at 4.8-4.9 ppm arose from H-1 (Gal) are confirmation of the β-D-galactopyranose ring and these results were as similar as of *Prosopis juliflora* galactomannans (31). The signal for H-1 (Man) is observed at 3.95-4.10 ppm corresponds to the monomeric β-D-mannopyranose (32, 33).

The GC-MS and methylation analyses were confirmed that the structure of the galactomannans as 1-4 mannose with galactose side chains linked at the C6 position. The total content of galactosyl and mannosyl residues was resembled the earlier work on monosaccharide composition (14). It also show the presence of arabinose and rhamnose residues in galactomannan extracts as reported earlier (34, 35) in *Gleditsia triacanthos*.

The FT-IR spectra resulted the characteristic absorption bands very similar with the galactomannan as reported by previous workers (36-38). Bands at 3700-3000 cm^{-1} are attributed to O-H stretching of the polysaccharide, and the region 3000-2800 cm^{-1} is representative of - C-H stretching (36-39). X-ray diffraction is widely used to determine the crystallinity, orientation of the crystallites and structural variation of the compound. The results shown the crystalline and powdered nature of polysaccharide, have been reported earlier for native guar gum (40). The typical natural polysaccharide nature presents in TG-DTA thermogram (5) which explain thermal stability of the compound. The results obtained reveal the chemical characteristics and useful to understand the complexity of the polysaccharide, which is an alternative and natural source available in the market in different industrial applications.

Conclusions

Purified galactomannan was isolated from the seeds of *Mimosa pudica* with ethanol and water. SEM pictures clearly explained the granular morphology of the polysaccharide. The ATR-IR spectra revealed the major functional groups present in the compound. TG-DTA analysis indicated that the thermal transition temperature was around 280-300°C. XRD studies of galactomannan powder determined the A-type pattern of crystallinity which is amorphous in nature with a large degree of crystallinity. Thus, the isolated polysaccharide from natural source with similar properties to that of commercial galactomannans e.g. Guar gum, Locust Bean Gum and Tara Gum can be use as alternatives in food and Pharmaceutical industries which is cost effective and readily available.

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Conflict of interest statement

The authors declare there is no conflict of interest.

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