Surface Morphology and Physicochemical Characterization of Thermostable Moringa Gum: A Potential Pharmaceutical Excipient

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ABSTRACT: An efficient protocol for physico-chemical characterization of gum exudates collected from the drumstick tree (Moringa oleifera Lam.) has been reported in the present study. Extraction of gum metabolites was done using a series of water, alcohol, acid, and alkali solvent systems. The gum was sparingly soluble in water at room temperature and formed a colloidal solution. Solubility of the gum gradually increased in the solvent gradient (80% ethanol, deionized water, 0.05 M HCl, and 0.05 M NaOH) at 90 °C. Further, electron microscopy revealed that the acetyl group is essential in maintaining the structural integrity, and deacetylation of gum resulted in formation of a mesh of scattered and fibrous particles. Treatment of gum with deionized water resulted in development of a hydrocolloidal matrix with a pore size of 0.5 μm, which upon deacetylation was reduced up to 0.2 μm. The polymer was amorphous in nature and showed maximum thermal stability in ethanol. Gas chromatography−mass spectrometry of the gum polymer revealed that carbohydrate derivatives constituted its major part (>75%). Maximum carbohydrate concentration was obtained in the ethanol soluble fraction, along with fatty acids (10%) and secondary metabolites (9%). The results provided very first confirmation of the hydrocolloidal properties and thermostability of the gum exudates obtained from the drumstick tree, which can further be used to develop an eco-friendly and nontoxic bioligand.

1. INTRODUCTION

Plant gums, resins, and latexes are highly used and traded non-woody products after essential oils.1 Plant gums are hydrophilic polymers composed of complex mixtures of branched polysaccharides, primarily formed by breakdown of cellulose in a process called “gummosis” and are secreted from the bark or endosperm of the seed.2 These carbohydrates are often present with minor concentrations of proteins and minerals depending upon the type of gum.3 Plant gums are either seed gums biosynthesized in embryos as a food reserve or exudate gums secreted from the bark as a defense mechanism in response to injury.3 Exudate gums are either water-soluble or water-dispersible hydrocolloids and exhibit suspending, dispersing, and stabilizing properties. Therefore, use of plant gum exudates as emulsifying, gelling, thickening, suspending, binding, swelling, bulking, encapsulating, and flocculating agents in food, pharmaceutical, textile, petroleum, paper, and cosmetic industries has increased exponentially.145

The past few years have witnessed extensive use of plant-based gums at an industrial scale, which posed a threat toward sustainability of these economically important gum yielding trees. Among various gum-producing trees, members of family Leguminosae (Acacia spp., Prosopis spp.), Sterculiaceae (Sterculia urens), and Combretaceae (Anogeissus latifolia) have been highly recognized for commercial applications; therefore, studies can be planned to explore the potential of various tree species for gum secretion and utilization of gum in various food and medicinal formulations.67

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Moringa oleifera Lam. commonly known as “drumstick tree” is a gum-yielding tree species belonging to family Moringaceae. The tree exudes gum from the traumatic ducts developed lysigenously in the secondary phloem and phelloderm of the bark of the tree.8,9 The gum exudes in the form of tears or stalactite pieces from the bark and gradually hardens due to environmental exposure.10,11 It is sparingly soluble in almost every solvent and tends to swell in water due to its mucilaginous nature, much similar to the tragacanth or hog gum series.11,12

Moringa gum exudates have been traditionally used in treatment of various chronic diseases, and their utility as an alternative to synthetic suspending and binding agents has also been well established in the past few decades.5,13,14 Various preformulation and toxicity studies demonstrated that the gum can serve as a potent non-toxic, biodegradable binding, and suspending agent in drug delivery. Being a mucoadhesive polymer, it can also be used as a stabilizing, thickening, and gelling agent.15−17 Recent studies reported that physical and chemical modifications of the Moringa gum polysaccharide aided in development of hydrogels with biomedical applications such as wound dressings,18 controlled drug delivery,19,20 and nanometric carriers.21 Apart from these, gum derived from M. oleifera has also been used to develop biosorbents for efficient and rapid removal of heavy metals22 and toxic dyes.23

Even though Moringa gum has a wide range of applications in food and medicine, but due to tedious harvesting methods and less knowledge of physicochemical properties, its uses have been restricted to the research laboratories only. Thus, in order to optimize uses of these gum exudates at an industrial scale, a detailed knowledge about structural and physicochemical constituents is the first and foremost requirement, which can lead to preparation of stable and non-reactive formulations.

There are several reports available on morphological, structural, and physicochemical characterization of various plant gum exudates including “cashew gum”24, “malva nut...
gum, but a comprehensive report on Moringa gum characterization, which could further establish its gelling and binding potential, is still lacking.

The present study hypothesizes that the gum polymer of the drumstick is a complex mixture of carbohydrates, which, being non-toxic, could serve as an efficient encapsulating and gelling agent in food and pharmaceuticals. Analysis of its structure and chemical configuration can be an important step to establish its gelling properties. Sometimes, crude gum exudate is resistant to structural changes, and its morphological characterization could be difficult; therefore, sequential extraction of gum carbohydrates using deionized water, absolute ethanol, dilute acid (HCl), and alkali (NaOH) would be tried for detailed structural and biochemical characterization. Further, deacetylation of the gum could be useful to identify the essential groups for maintaining the structural integrity of the gum, and its characterization using SEM and TEM would reveal the presence of nano-sized gaps or voids, which would be used for drug loading and delivery.

Therefore, in this study, physicochemical characterization of Moringa gum has been reported to establish its hydrocolloidal properties. In addition, imaging techniques were used to study the surface morphology and matrix properties of the gum. This study presents the first preliminary report on the characterization of Moringa gum, which could further be used to evaluate its structural, functional, and rheological characteristics.

2. RESULTS AND DISCUSSION

The plant secretes gum throughout the year; however, for the present investigation, typical brownish-red and hardened gum was collected from the bark of the plant. Gum exudates were subjected to two different extraction methods: extraction with water at room temperature (cold extraction) and sequential extraction with alcohol (EtOH), water, acid (HCl), and alkali (NaOH) at high temperature (hot extraction). Cold extraction resulted in partial solubilization and swelling of gum components in water. Solubility of the gum in water increased with the hot extraction process.

On the contrary, DI gum particles were more uniformly and compactly arranged, forming matrix-like structure with smaller pore size (≤200 nm) (Figure 2e,f). Further magnification of the clustered region showed cross-linking of these matrix-like structures into clusters with reduced size (≤50 nm) (Figure 2g). DI molecules were also found to be amorphous in nature (Figure 2h).

SEM and TEM micrographs of Moringa gum thus indicated that gum particles formed a mesh-like structure in water due to swelling, while their deacetylation resulted in loss of particulate native gum morphology and formed a more compact matrix. Change in the morphology of deacetylated gum has been accounted to loss of acetyl groups, which indicated that acetyl groups are an essential factor in maintaining the structural integrity of the gum. Various experimental studies demonstrated that the size and surface area of the gum particles are an important factor in controlling the hydration kinetics of gum particles, which could be attributed to the changes that occurred in the intrinsic viscosity and molecular mass of the gum. Also, swelling is the primary mechanism in diffusion-controlled drug release, and this property makes Moringa
gum as a suitable matrix substrate along with sustained release of dosage of drugs. The differences in matrix properties of deacetylated gum and water swollen gum can be useful in preformulation studies for optimization of drug release.

2.2. Functional Group Analysis. To identify the effect of the extraction method on chemical composition of the gum, FTIR spectra was recorded for crude gum and its different extracts (WS-1, WS-2, AS, AcS, and AkS) to identify the presence of different functional groups. The IR spectra were similar to those previously reported spectra of the gum but showed minor differences in peak numbers and intensities.

Significant similarity was observed among the IR spectra of crude gum and different gum extracts (Figure 3a,b). Sharp, weak to medium peaks in the region beyond 3000 cm⁻¹ indicated the presence of secondary amines (=NH) and unsaturated bonds. Peaks at 3130 and 2924 cm⁻¹ confirmed the presence of O−H stretching in CG and WS-1. The presence of hydroxyl groups can be due to the presence of sugar alcohols and aldehydes, while that of N−H groups could be due to fatty amides. On the contrary, previous studies have reported the presence of peaks at 2928 and 2925 cm⁻¹ due to −C−H stretching of arabinose.19,32 Multiple broad peaks at 2362 cm⁻¹ were attributed to the presence of N−H bonds, prominently due to ammonium ions present in CG, WS-1, WS-2, AS, AcS, and AkS fractions. A strong sharp peak near 1521 cm⁻¹ indicated the presence of nitro compounds. The presence of fatty acids and cyclic sugar compounds was further ascertained by the medium sharp C−H and weakly sharp C= C peaks at 1696 and 1650 cm⁻¹, respectively. Appearance of weak to medium peaks in the region of 540−760 cm⁻¹ is usually due to the presence of haloalkanes (C−X). Singh and Kumar19 reported that peaks in this region often correspond to the aromatic C−H out-of-plane bending vibrations, which is more likely in the present samples under study. Timilsena et al.27 reported that the region between 1800 and1500 cm⁻¹ also corresponds to the presence of carboxylic acid groups, indicating the presence of ketonic sugars in gum polysaccharides. As per the literature, peaks at 1417 cm⁻¹ in AcS and AkS can be attributed to the −CH₂ bending vibrations,32 while those at 1743 cm⁻¹ in AS, AcS, and AkS can possibly be due to −C=O group of esters.19

2.3. GC−MS Characterization. Metabolite profiling of M. oleifera crude gum and its extracts (WS-1, WS-2, AS, AcS, and AkS) was done through GC−MS. Compounds were identified by comparing the retention time and mass fragmentation pattern of separated compounds with those of standard TMS derivatives of sugars and other metabolites using the NIST17 library.

The chromatogram of 50 compounds was generated through GC for each sample, and a detailed description of metabolites identified in each sample can be obtained from supplementary data tables. The chromatogram revealed the presence of various types of sugars and alcohols as major constituents, while other metabolites including fatty acids, flavonoids, and terpenes were identified as minor constituents in all samples. Sugars were relatively the most abundant (46.6%) followed by sugar acids (7.4%), secondary metabolites (7.4%), and sugar alcohols (4.1%). Among the 15 sugars detected, d-arabinose (30.2%), d-talose (20.6%), and ribofuranose (14.6%) constituted approximately 65%, whereas monosaccharides including glucose, fructose, xylose, ribose, and allole constituted 22.4% of the total sugars identified. Myo-inositol (15.5%), glycerol (8.6%), arabinol (6.5%), and sorbitol (4.4%) were the major sugar alcohols, while glucuronic acid (7.8%), palmitic acid (10.8%), and protocatechuic acid (7.3%) were the major sugar acid, fatty acid, and secondary metabolite compounds present in the gum, respectively.

The mass spectrum of CG revealed the presence of sugars, alcohols, fatty acid derivatives, alkane, phenolic acid, and flavonoids in crude gum (Table S1). The peak area percent and ratio of the peak area to peak height represented sugars as one of the major component of CG. d-Arabinose was relatively the most abundant sugar (3.11%) followed by myo-inositol (2.34%), d-talose (1.19%), d-fructose (0.9%), l-arabinol (0.58%), d-ribofuranose (0.48%), D-trehalose (0.45%), and α-arabinopyranose (0.45%). Fatty acids and their derivatives including myristic acid, palmitic acid, 9-octadecenitrile, and

Figure 2. Transmission electron micrograph of (a–d) water insoluble fraction (WI) of gum at (a) 0.5 μm, (b) 200 nm, (c) 100 nm, and (d) 101 nm. (e–h) Deacetylation insoluble fraction (DI) of gum at (e) 0.5 μm, (f) 200 nm, (g) 50 nm, and (h) 101 nm.
13-docosenamide constituted around 4.23% of the gum sample (Supporting Information, Table S1).

The water extract prepared through cold extraction facilitated better elution of compounds than that through hot extraction (Supporting Information, Tables S2 and S3). The percent abundance of sugars increased in the water extracts of crude gum. Use of water as a solvent also facilitated identification of some important sugars (D-xylose, xylitol, D- and L-rhamnose, D-ribose, and lactose) and sugar alcohols (sorbitol) along with fatty acids. Similar to the crude extract, D-arabinose was the most abundant (6.2%) compound present in the water extracts.

In total, 72 metabolites were identified in the alcoholic, acidic, and alkaline gum extracts (Supporting Information, Tables S4–S6). D-Arabinose was the most abundant carbohydrate in alcohol-soluble and acid-soluble fractions with a 6.76 and 12.83% relative abundance, respectively. Alkaline extracts consisted of L-rhamnopyranose, benzeneethanamine, D-psicose, 1-hexadecanol, oleic acid, 18-methylnonadecanol, and 1-monopalmitin along with other sugar and fatty acid derivatives (Supporting Information, Table S6).

The relative percent abundance of sugars increased up to 3 times (30.4%), which can be attributed to the increased solubility of carbohydrate compounds in water. A higher number of compounds were detected in the hot extraction method, which is possibly due to breakdown of the covalent linkages present between sugar molecules as well as among different biomolecules at high temperature. Separation of similar compounds at different retention times in different extracts can be due to the differential breakdown of the corresponding covalent linkages in each solvent, causing selective solubilization of gum components during each extraction step.

These findings corroborated a previous study reporting arabinose, galactose, xylose, rhamnose, and glucuronic acid in a molar ratio of 64:25:4:3:4, respectively, as major constituents of a 190 kDa polysaccharide isolated from the Moringa gum.33 Further, detailed investigation of the linkage pattern between sugars, sugar derivatives, lipids, and other metabolites will be carried out to deduce the chemical structure of the gum polymer.

2.4. Carbohydrate Estimation. The maximum concentration (16.6 g L−1) of carbohydrates was noted in the alcohol-soluble fraction followed by deacetylated gum (15 g L−1), alkaline-soluble (13.84 g L−1), water-soluble (11.6 g L−1), and acid-soluble (10.34 g L−1) fractions. The number of metabolites (sugar) detected in each extract through GC–MS clearly coincided with their sugar content, further providing affirmation for ethanol being the most suitable solvent system for extraction of gum metabolites. Differential solubility of sugar compounds in different solvents can be accounted for the variations in sugar content of different extracts. The differences in solubility can be attributed to the differential hydrolysis of the corresponding glycosidic linkages in the presence of each solvent. With such a high sugar concentration, it would be adequate to say that Moringa gum is...
dominantly a polysaccharide polymer. These are the first and preliminary findings on chemical composition of *Moringa* gum; however, a detailed study to elucidate the polymer structure of gum using NMR and other spectrometric techniques will also be carried out.

2.5. Thermal Analysis. Thermal curves obtained for change in mass by TGA and enthalpy by DTA with respect to temperature were analyzed for different gum extracts and compared with those of crude gum. Thermograms of all the samples have been provided in the Supporting Information (Figure 4–6).

TGA of crude gum exhibited significant loss of mass (42%) at initial temperature (∼29 °C) and followed multistage degradation (Figure 4). The thermogram pattern of the compound indicated a possible degradation without formation of intermediates. Major weight loss of 5.46 and 5.6% occurred between 67–131 and 165–252 °C, respectively; however, only 38% of the initial weight of the crude gum persisted until the highest temperature (300 °C) was reached. First, the derivative of weight percentage (DTG) curve showed occurrence of simultaneous endothermic and exothermic reactions precisely at 102.5 and 115.66 °C, stage-I; 93.26 °C (stage-II); 203.95, 234.09, and 258.47 °C (stage-III); and 281.88 °C (stage-IV). Further, a single endothermic peak with an onset at 26.75 °C and offset at 92.73 °C and two exothermic peaks between 221.87–259.17 and 274.51–301.57 °C were observed in the differential thermogram of WS-1 (Figure 5a). As evident from the DTA and first derivative graph, loss of weight in WS-1 at multiple temperatures in the third stage can majorly be attributed to the exothermic processes (Figure 5a). On the contrary, apparent change in wt % of WS-2 was
observed at 49.89 °C (stage-I); 71.33, 83.53, 95.98, 105.44, and 121.33 °C (stage-II); 163.72 °C (stage-III); and 267.70 °C (stage-IV) (Figure 5b). The DTA curve of WS-2 was characterized by the presence of single endothermic and exothermic peaks with $\Delta \mu V$ of $-205.36$ and $49.55$, respectively (Figure 5b). The onset and offset of these endothermic and exothermic peaks revealed that the weight loss at stage-I began with endothermic processes and extended until stage-II, whereas change in weight percentage due to exothermic processes started with a rise in temperature to 234.97 °C later in stage-III. The degradation pattern of both WS-1 and WS-2 exhibited differences in their decomposition process, which is explained by the difference in their chemical composition as reported in previous sections.

Decomposition of the AS extract occurred in three stages, and maximum degradation (12.23%) occurred between 142.04 and 301 °C, with apparent weight change at 271.42 and 277.11 °C (Figure 6a). The thermogram pattern that indicated decomposition could possibly be due to degradation of bonds at high temperature and vaporization and melting at low temperature (Figure 6a). Among all, the AS extract was comparatively the most stable throughout the test range, resulting in $\sim 25\%$ net weight loss. The stability of the compound indicated toward the absence of any major decomposition reactions. The presence of a sharp endothermic peak between 30 and 117 °C and two weak endothermic peaks ranging between 122–209 and 256–297 °C further supported the TGA and DTG thermograms (Figure 6a). The presence of a sharp endothermic peak can be ascribed to the abundance of volatile –OH groups due to the presence of solvent remnants.

TGA of the acid extract (AcS) showed a similar decomposition pattern as that of WS-1 and WS-2, but the former (33.23% weight loss) showed greater thermal stability than the latter (41.5 and 53.06% weight loss) even at higher temperatures. Unlike water-soluble extracts, the acid-soluble extract showed maximum loss of weight (13.73%) at stage-IV (212.95–301 °C) of decomposition (Figure 6b). As per DTG of AcS, weight changes in all the three stages occurred at 26.69, 49.21, 66.77, and 196.25 °C, while at stage-IV no specific temperature could be identified (Figure 6b). The absence of a specific peak in the stage-IV of degradation could be explained as a consequence of release of volatile less-tightly bound molecules, leading to a very less energy change. DTA was characterized by one endothermic and two exothermic regions, which extended between 26.57–117.6 and 120.55–261.22 °C, respectively (Figure 6b).

AkS showed five-stage degradation similar to that of crude gum, with 33.1% net weight loss (Figure 6c). Unlike crude gum, the AkS was thermally more stable and showed maximum weight loss (10.65%) between 59.03 and 113.46 °C. The differential curve was also similar to that obtained for crude gum, and two temperatures (102.50 and 115.84 °C) were identified for the prominent weight loss at stage-I and stage-II, respectively (Figure 6c). Unlike crude gum, AkS did not

![Figure 6](https://dx.doi.org/10.1021/acsomega.0c03966)
exhibit any weight loss at a lower temperature range (∼28 °C). The DTA curve was also similar to that of crude gum, with the only difference being in the number of detectable peaks (five for crude gum, four for the alkaline-soluble extract). Two endothermic peaks and two exothermic peaks in the temperature ranges of 29.84–142.66 and 165.74–287.40 °C, respectively, were recorded in its differential thermogram (Figure 6c). Since the thermograms of AkS were quite similar to those of crude gum, therefore decomposition of both the samples occurred through similar mechanisms, but higher thermal stability of AkS can be due to the presence of more compounds, thereby forming a more complex and heat-stable structure.

The thermal properties varied greatly for each extract with respect to that of crude gum except alkaline extract. These variations can be explained as a result of the presence of different combinations of compounds in each extract. Thermograms revealed gradual decomposition of all the samples subjected to TGA. Higher thermal stability of AS, AkS, and AcS extracts could be due to the presence of a comparatively large number of metabolites, resulting in formation of more complex structures through strong inter- and intramolecular interactions. Maximum weight loss in a single stage occurred at higher temperatures (120–300 °C) in AS, AcS, WS-1, and WS-2 and at moderate temperatures (50–100 °C) in AkS and crude gum. All the extracts exhibited a characteristic endothermic peak between 26 and 120 °C, which could primarily be associated with the loss of moisture. A broader peak in the alcohol-soluble extract also corresponds to the loss of hydroxyl groups present as solvent remnants and to desorption of the moisture content.

Loss in mass of the compound is usually due to gas desorption and phase transitions (vaporization and sublimation) or decomposition, breakdown reactions, gas reactions, and chemisorption. Further, loss of weight around 25–195 °C usually corresponds to the loss of water formed by inter- and intramolecular condensation of polymeric hydroxyl groups31 and that at early stage corresponds to desorption of moisture.34

3. CONCLUSIONS

This study presents the first report on thermal, chemical, and matrix properties of gum exudates obtained from the drumstick tree (M. oleifera). Comparative analysis of different extraction methods was done to identify the most efficient strategy for optimum recovery of gum metabolites. Moringa gum was sparingly soluble in cold water, and the solubility was further improved by deacetylation of the water gum extract. Maximum recovery of gum metabolites was attained by sequential extraction with alcohol, water, acid, and alkaline, respectively, at high temperature. Microscopic analysis of soluble and insoluble fractions of deacetylated and water-soluble gum provided insights into the matrix-forming potential of Moringa gum, thereby making it a suitable substrate for controlled drug release. Identification of functional groups and gum constituents along with TGA and DTA revealed important facts about the gum. Physicochemical properties of Moringa gum indicated towards its hydrocolloidal nature and its suitability as a potent binding, suspending, and thickening agent. Being non-toxic and biodegradable, Moringa gum could serve as an excellent substrate for formulation of various gum-based products, thereby confirming the hypothesis proposed in the present study.

This study will further be extended to structural and rheological characterization of the gum for establishment of its applications in food and pharmaceuticals. Further, in vitro and in vivo toxicity assays of Moringa gum will be performed to identify its maximum permissible dosage and study its toxic effect on the host.

4. MATERIALS AND METHODS

4.1. Collection of Gum. Moringa gum was collected from drumstick trees located in Jaipur by making cut marks on the bark. Collected gum samples were then air dried, pulverized into granular powder, packed into airtight zip-lock bags, and stored at room temperature in a dry place until further use.

4.2. Deacetylation of Moringa Gum. Deacetylation of Moringa gum prior to extraction was done as per the method reported by Vinod et al.28 Gum powder (2 g) was dispersed in 100 mL of deionized water and continuously stirred at room temperature. After 5 h, pH of the gum solution was adjusted to 10 with ammonia solution (HiMedia, Mumbai, India) and stirred again for 2 h at room temperature. The mixture was then allowed to settle and filtered using a muslin cloth to obtain clear red tinted deacetylated gum extract (DG). The remaining pellet after filtration was stored as deacetylation-insoluble gum (DI) in a separate vial.

4.3. Extraction of Gum Metabolites. Gum metabolites were extracted using previously reported protocols: (i) cold extraction28 and (ii) the sequential hot extraction method.25

Two grams of gum powder was extracted with 100 mL of deionized water by continuous stirring of the mixture for 24 h at room temperature. The resulting gum solution was allowed to stand for 12 h and filtered through a muslin cloth, and the resulting filtrate was collected as the water-soluble (WS-1) fraction. The resulting insoluble pellet was stored as the water-insoluble fraction (WI) in a separate vial.

Extraction of metabolites from Moringa gum was also done using the sequential hot extraction method given by Somboonpanyakul et al.25 with some modifications. Concisely, 5 g of fine-powdered gum was boiled (90 °C) in 200 mL of 80% (v/v) ethanol (HiMedia, Mumbai, India) for 90 min to obtain the alcohol-soluble (AS) fraction. The insoluble gum fraction was filtered into separate beaker using Whatman’s filter paper no. 1 and extracted with 100 mL of deionized water (Merck-Millipore, India) at 90 °C for 2 h. The water-soluble (WS-2) fraction was then obtained through centrifugation at 10,000 rpm (25 °C) for 20 min. The insoluble pellet was then extracted with 100 mL of 0.05 M HCl (Rankem, India) for 30 min at 85 °C, and the acid-soluble (AcS) fraction was obtained by centrifugation of the gum mixture at 8000 rpm for 15 min (25 °C). The remnant pellet was then finally extracted with 100 mL of 0.05 M NaOH (HiMedia, India) for 30 min at 85 °C. The gum solution was then centrifuged at 8000 rpm (25 °C) for 15 min, and the resulting supernatant was collected to obtain the alkali-soluble (AkS) fraction.

All the five fractions were concentrated in a hot air oven at an optimum temperature (∼80 °C) and stored at 4 °C until further use.

4.4. Scanning Electron Microscopy. Crude gum powder (CG), water-soluble (WS-1), deacetylated (DG), water-insoluble (WI), and deacetylation-insoluble (DI) gum extracts were imaged using a Nova Nano FE-SEM 450 (FEI, US). Samples were made conductive by gold coating using a Q105T ES sputter coater. For the gold sputter coating, a double-sided adhesive carbon tape was pasted on the aluminum stub from
one side, and the samples were adhered on the other side. The carbon tape facilitated the enhancement in conductivity required for imaging. The aluminum stubs containing gold-coated samples were then placed on the stage of the SEM and imaged under high vacuum mode at magnification ranging from 1 to 100 μm.

4.5. Transmission Electron Microscopy. Both WI and DI were subjected to electron microscopy using a Tecnai G2 20 S-TWIN transmission electron microscope (FEI, USA). Prior to imaging, samples (10–20 mg) were dispersed in water and ethanol, respectively, by sonication for 1 h using an ultrasonic water bath (Merck, India). The homogeneously dispersed sample was loaded on the copper grid (Sigma, India) and was allowed to air dry for 12 h. TEM images of samples was captured at various magnification levels (20–200 nm), and the diffraction pattern was captured at 101 nm to identify the nature of the specimen.

4.6. FTIR Spectroscopy. All the gum fractions were subjected to FTIR (Bruker, USA) spectroscopy for the functional group identification, and the spectra of the samples were recorded within the range of 700–4000 cm\(^{-1}\).

4.7. GC–MS Analysis. 4.7.1. Chemicals and Reagents. All the reagents and chromatography-grade solvents required for GC–MS analysis were procured from Sigma-Aldrich, India. The methoxyamination reagent required for derivatization was prepared by dissolving 20 mg of methoxyamine hydrochloride in 1 mL of pyridine.

4.7.2. Derivatization. Prior to analysis, gum fractions were derivatized through silylation using MSTFA and methoxyamine HCl as per the standard protocol.\(^{35}\) To each concentrated fraction, 40 μL of the methoxyamination reagent was added, and the mix was incubated in the water bath for 2 h at 37 °C. For silylation, 70 μL of MSTFA (N-methyl-N-(trimethylsilyl)trifluoroacetamide) was added to all the samples after incubation. The derivatized samples were then filtered using a 0.22 μm membrane filter (Axiva, India) into screw-capped amber glass vials (Borosil, India) and subjected to GC–MS analysis.

4.7.3. GC–MS Analysis. Analysis of the derivatized samples was carried out on a single quadrupole GCMS QP-2020 Plus (Shimadzu, Japan). The samples were passed through a 30 m-long Rxi-5Sil MS (Crossbond, 5% diphenyl/95% dimethyl polysiloxane) column having 0.25 mm internal diameter and 0.25 μm film thickness. Injection of samples was done at 230 °C in split mode with a split ratio adjusted to 1:10, and the flow rate of the carrier gas (He) was retained at 1.24 mL min\(^{-1}\) throughout the run. The oven temperature was maintained at 40 °C with a 5 min hold followed by a final hold at 280 °C for 5 min.

For MS, the temperature of the ion source and the interface was maintained at 280 °C, and the solvent cut time was set at 2.5 min to avoid detector saturation with the solvent ions. The mass scan ranged from m/z 40 to m/z 400, and the spectrum for each separated compound was recorded and identified through similarity search in the NIST17 library database.

4.8. Quantitative Estimation of Carbohydrates. Carbohydrate concentration in each extract was estimated using the phenol–sulfuric acid method.\(^{36}\) Briefly, 0.05 mL of phenol (5%, v/v) was added to 2 mL of the sample solution. Subsequently, 5 mL of H\(_2\)SO\(_4\) was added rapidly (10–20 sec) to the mixture and was allowed to stand for 10 min following by incubation at 25–30 °C in a water bath for 10–20 min. After incubation, absorbance was taken at 490 nm using a UV–vis spectrophotometer (UV-2600, Shimadzu, Japan). Glucose solutions of varying concentrations (25, 50, 75, 100, and 125 g L\(^{-1}\)) were used as a standard, and sample solutions were prepared by serial dilution using distilled water.

4.9. Thermal Gravimetric and Differential Thermal Analyses. All the soluble and insoluble gum fractions along with the crude gum were subjected to thermogravimetric (TGA) and differential thermal analyses (DTA) using a DTG-60H thermogravimeter (Shimadzu, Japan). The sample (20 mg of the sample) was placed in one of the two alumina crucibles, and the other was kept empty to nullify the weight of the crucible. Both crucibles were then carefully placed on the holder inside the equipment and covered with the lid. TGA and DTA data for all samples were obtained for temperatures ranging from 30 to 300 °C. A rate of 10 °C min\(^{-1}\) under a nitrogen atmosphere was maintained throughout the entire process.

### ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c03966.

(Table S1) Details of compounds identified in crude gum through GC–MS analysis, (Table S2) details of compounds identified in the water-soluble gum extract derived from cold extraction (WS-1) through GC–MS analysis, (Table S3) details of compounds identified in the water-soluble gum extract derived from hot extraction (WS-2) through GC–MS analysis, (Table S4) details of compounds identified in the alcohol-soluble gum extract (AS) through GC–MS analysis, (Table S5) details of compounds identified in the acid-soluble gum extract (AkS) through GC–MS analysis, and (Table S6) details of compounds identified in the alkaline-soluble gum extract (AkS) through GC–MS analysis (PDF)

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**Author Contributions**
Conceptualization was done by R.J.; experimental design and methodology were contributed by R.J.; experimental studies were performed by M.K.B. and S.G.; result analysis was done by M.K.B.; funding acquisition was done by R.J.; writing (manuscript draft preparation) was done by S.G.; writing...
(review and editing) was done by S.K.; and supervision and validation of results were performed by S.L.K. All authors have read and agreed to the published version of the manuscript.

Notes

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