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Compositional Analysis and Rheological Properties of Gum Kondagogu (*Cochlospermum gossypium*): A Tree Gum from India

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Gum kondagogu (*Cochlospermum gossypium*) is a tree exudate gum that belongs to the family Bixaceae. Compositional analysis of the gum by HPLC and LC-MS revealed uronic acids to be the major component of the polymer (~26 mol %). Furthermore, analysis of the gum by GC-MS indicated the presence of sugars such as arabinose (2.52 mol %), mannose (8.30 mol %), α-D-glucose (2.48 mol %), β-D-glucose (2.52 mol %), rhamnose (12.85 mol %), galactose (18.95 mol %), D-glucuronic acid (19.26 mol %), β-D-galactouronic acid (13.22 mol %), and α-D-galacturonic acid (11.22 mol %). Gum kondagogu, being rich in rhamnose, galactose, and uronic acids, can be categorized on the basis of its sugar composition as a rhamnogalacturonan type of gum. The rheological measurements performed on the gum suggest that above 0.6% (w/v) it shows a Newtonian behavior and shear rate thinning behavior as a function of gum concentration. The viscoelastic behavior of gum kondagogu solutions (1 and 2%) in aqueous as well as in 100 mM NaCl solution exhibits a typical gel-like system. The *G′* (viscous modulus)/*G″* (elastic modulus) ratios of native gum kondagogu (1 and 2%) in aqueous solution were found to be 1.89 and 1.85 and those in 100 mM NaCl to be 1.54 and 2.2, respectively, suggesting a weak gel-like property of the polymer. Crossover values of *G′* and *G″* were observed to be at frequencies of 0.432 Hz for 1% and 1.2 Hz for 2% for native gum in aqueous condition, indicating a predominantly liquid- to solid-like behavior, whereas crossover values of 2.1 Hz for 1% and 1.68 Hz for 2% gum in 100 mM NaCl solution suggest a larger elastic contribution.

KEYWORDS: Gum kondagogu; *Cochlospermum gossypium*; compositional analysis; HPLC; LC-MS GC-MS; rheology

INTRODUCTION

Gum kondagogu (*Cochlospermum gossypium*) is a tree exudate derived from the Bixaceae family, originating from India. Natural gums are obtained as exudates from different tree species, which exhibit unique and diverse physicochemical properties and have a wide variety of applications (*1*). Commercially important tree gums include gum arabic, gum karaya, and gum tragacanth (*2*). Karaya polysaccharide (*Sterculia urens*) and gum kondagogu (*C. gossypium*) are used as food additives (*3, 4*). The physicochemical properties and toxicological evaluation of gum kondagogu has been established earlier (*5, 6*). Morphological and structural characterization and physicochemical aspects of gum kondagogu have been elucidated recently, suggesting that this gum belongs to the group of substituted rhamnogalacturonans (*7*).

Understanding of the rheological properties of gum is essential for their application and use as food thickeners, stabilizers, and emulsifiers. Sugar composition has a direct bearing on the rheological character of the gum. Composition and rheological characterizations of many plant exudates have been reported in the contemporary literature. These include acacia gums (*8*), *S. urens* (*9*), *Albizia lebbeck* (*10*), mucilage gum (*Opunita ficus Indica*) (*11*), *Enterolobium contortisilliqum* (*12*), *Sterculia straiata* (*13*), *Aeromonas* gum (*14*), and cashew gum (*15*). Rheological characteristics of arabic gum in combination with guar and xanthan gum (*16*) and cashew gum with gum arabic (*17*) were studied. Dynamic rheology is one of the methods most extensively used to study polysaccharide gel viscoelasticity (*18*). Changes in rheological behavior depend on appropriate conditions of these gum exudates. Rheological properties are sensitive to variations in molecular structure, and they are useful

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in developing structure–function relationships for systems of polysaccharide solutions and intermolecular interactions, as the gelling property of the gum polysaccharide depends upon the rheology of its solution (*19*). The rheological properties of hydrocolloids are particularly important when they are used in the formulation of any food for their effects on the textural attributes (*20*).

A basic understanding of the composition and rheological behavior of gum kondagogu is essential for its potential application as a thickening agent and stabilizer in the food and cosmetic industries. However, the rheological behavior of gum kondagogu is yet to be explored. The aim of the present study was to determine the sugar composition and to characterize the rheological properties of gum kondagogu, as this information is essential to gain an insight into the functional properties of this biopolymer. In this paper, we report the sugar composition of gum kondagogu by three physical methods (HPLC, LC-MS, GC-MS) and its dynamic rheological properties.

MATERIALS AND METHODS

Plant Materials and Standards. Gum kondagogu samples were collected from Girijan Co-operative Corp., Hyderabad, a Government of Andhra Pradesh undertaking, Hyderabad, India, and gratis samples were provided by M/s D.K. Enterprises, Hyderabad, India. Gum kondagogu (grade 1; hand picked, fresh, clean with no extraneous material) was used in the experimental analysis. Gum samples collected were stored in airtight polypropylene jars in desiccated condition. Deionized (Milli-Q) water was used for all experimental work. D-Glucose, D-galactose, L-rhamnose, L-arabinose, L-mannose, D-xylose, D-glucuronic acid, D-galacturonic acid monohydrate, trifluoroacetic acid (TFA), sodium cyanoborohydride, *p*-aminobenzoic acid ethyl ester (ABEE), *N*-*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), Dowex 50W-8X (cross-linking grade of 8% and granulometry comprised between 100 and 200 mesh), and trimethylsilylated sugar reference standards were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). HPLC grade acetonitile and methanol were procured from E. Merck (Mumbai, India). All other chemicals used were of analytical reagent grade.

Native and Deacetylated Gum Kondagogu Sample Preparation. Gum kondagogu was powdered in a high-speed mechanical blender (Philips, Mumbai, India) and later sieved using a bin (mesh size = 250 μm) so as to obtain a fine and uniform sample. Gum kondagogu powder (1 g) was accurately weighed and dispensed into a clean glass beaker containing 1 L of deionized water. The entire gum solution was kept on a magnetic stirrer at room temperature and gently stirred overnight. Later, the gum solution was allowed to stand at room temperature (30 °C) for 12 h, so as to separate any undissolved matter, The gum solution was filtered through a sintered glass funnel G-2 followed by a G-4 sintered funnel (*21*). The clear solution so obtained was freeze-dried (Dry Winner, Heto-Holten, A/S, Allerd, Denmark) and stored, until further use. This processed sample was designated "native" polysaccharide. Gum powder (1 g) was dispersed in 1 L of deionized water. After stirring for 5 h, the pH of the gum solution was adjusted to 10 by using ammonia solution and once again stirred for 2 h. The gum solution was allowed to stand for 24 h to separate any suspended particle. The gum solution was filtered through sintered glass filters (G-2 and then G-4), and the soluble polysaccharide was freeze-dried (*7, 21*). Deacetylation was monitored by infrared spectroscopy.

Sample Preparation for RP-HPLC, LC-MS, and GC-MS Analysis. Native gum kondagogu sample (50 mg) was hydrolyzed by TFA (2.0 M) at 100 °C for 8 h. After cooling, it was filtered through 0.45 μm membrane filter, and the contents were rotary evaporated at 45 °C to remove excess TFA, by the addition of methanol. The dried material was dissolved in deionized water and passed through Dowex 50 W-8X cation-exchange resin, packed in a chromatographic column. The sugars were eluted with 3 mL of 0.02 M HCl (*22*). The acidic solution containing monosaccharides and uronic acids were freeze-dried and dissolve in Milli-Q water to get a final sample concentration of 10 mg/mL. All samples were filtered with a 0.45 μm membrane filter before HPLC and LC-MS analysis.

For GC-MS analysis, a 5 mg sample of the lyophilized gum powder was mixed with 200 μL of acetonitrile and 200 μL of BSTFA for silylation. The mixture was heated at 80 °C for 1 h, in a vacuum-sealed glass ampule (*23*). These solutions were stored at 4 °C and used after appropriate dilutions. Trimethylsilylated sugar reference standards were obtained from Sigma-Aldrich. All reference standards and samples were analyzed three times each.

Preparation of Standards for RP-HPLC and LC-MS Analysis. The procedures for preparation of ABEE−sugar derivatives were based on the earlier reported method (*24, 25*). Briefly, ABEE reagent (165 mg) and sodium cyanoborohydride (35 mg) were mixed in 41 μL of glacial acetic acid and 350 μL of methanol. Before use, the reagent was warmed to dissolve any crystals formed during storage. To 10 μL of gum hydrolysate or reference sugar standards in a Reacti-vial was added 40 μL of ABEE reagent, and the mixture was incubated at 80 °C for 50 min; subsequently, 200 μL of Milli-Q water and 1 mL of chloroform were added to the reaction mixture. The sugar derivatives formed were partitioned into the upper aqueous phase after the vial had been shaken and the two phases allowed to separate, after the sample had been centrifuged at 5000 rpm for 5 min. An aliquot of 20 μL sample of the separated aqueous phase was injected into HPLC and LC-MS for the analysis of sugars. Standard sugars (glucose, galactose, arabinose, rhamnose, mannose, lactose, xylose, glucuronic acid, and galacturonic acid) in the range of 50−200 nmol were dervatized with ABEE reagent. Lactose was used as an internal standard to monitor the degree of completion of ABEE−sugar derivatization reaction (*24*). The calibration graphs were based on linear regression analysis of peak area versus sugar concentration.

HPLC. HPLC analysis was carried out using a Jasco HPLC system (Jasco 1580i gradient pump and a Jasco MD-1515 photodiode array detector, Jasco, Tokoyo, Japan). The separation was carried out using a Hibar, LiChrospher 100 RP-18 column (250 × 4.6 mm i.d.; particle size = 5 μm). Neutral and acidic sugars were detected by reverse-phase HPLC of ABEE−sugar derivative of these sugars (*24, 25*). A sample of 20 μL of ABEE−sugar derivative was injected into the C-18 column at room temperature. Chromatography was performed in the isocratic mode with 20 mM potassium phosphate buffer (pH 6.0) and methanol (70:30) at the flow rate of 1.0 mL/min. Identifications of the separated sugars were based on the retention time, co-injections, and spectral matching with the reference standards. ABEE−sugar derivatives were detected at 305 nm.

LC-MS. An Aglient 1100 series LC/MSD Trap SL (ion trap) instrument (Agilent Technologies, Palo Alto, CA) with electrospray ionization (ESI) interface was used in the positive ionization mode. Separation was performed on an Eclipse, XDB, RP-18, 150 mm × 4.6 mm i.d., particle size = 5 μm column with photodiode array (PDA) detector, at room temperature. Gradient elution was employed using 20% acetonitrile and 80% water in 0.1% acetic acid for 5 min, 40% acetonitrile, and 60% water in 0.1% acetic acid for 5–8 min and 20% acetonitrile and 80% water in 0.1% acetic acid for 10 min at a flow rate of 1 mL/min. Data were collected using Chemstation software version LC 3D Rev.A. 09.03 [1417]. The MS signal was collected in both the scan and selected ion monitoring (SIM) modes. ABEE−sugar derivatives (20 μL) were injected into the LC-MS system. The sample and standard sugar derivatized with ABEE were analyzed by LC-MS, and mole percent was calculated on the basis of a peak area ratio of the SIM signal of the analyte and the known quantity of standard sugars. $[M + H]^+$ ions for the ABEE−sugar standards were monitored in SIM mode and the peak areas calibrated versus the concentration of individual standards. Standard curves were generated for individual standards by serial dilutions. The scan signal was used to verify the identities of the chromatographic peaks. The mass spectrum analysis was carried out by SIM for ABEE−sugar derivative $[M + H]^+$ *m*/*z*. Characteristic *m*/*z* signals and retention times were determined by analyzing individual standards.

GC-MS. The GC-MS analyses were carried out on an Agilent 6890 GC system equipped with a 5973 inert mass selective detector and a 7863 autosampler (Agilent Technologies, Palo Alto, CA). A CP-SIL 8

Table 1. Sugar Composition of Gum Kondagogu As Determined by HPLC and LC-MS[a]

ABEE derivative of	sugar composition (mol %) by HPLC	sugar composition (mol %) by LC-MS
glucose	8.54 ± 0.22	7.84 ± 0.15
galactose	18.52 ± 0.54	19.52 ± 0.42
uronic acid	25.53 ± 0.95	ND
glucuronic acid	ND	16.25 ± 0.32
galacturonic acid	ND	10.5 4 ± 0.18
rhamnose	17.56 ± 0.74	15.85 ± 0.36
arabinose	2.19 ± 0.04	1.82 ± 0.02

[a] Values are mean ± SD, $n = 3$; ND, not detectable.

CB (5% phenyl, 95% dimethylpolysiloxane) (Varian, Middleburg, The Netherlands) column of 30 m length, 0.25 mm i.d., and 0.25 μm film thickness was used. The oven was programmed from an initial temperature 50 °C (hold time = 2 min) and to the final temperature 280 °C at the rate of 10 °C/min. The final temperature hold was 5 min. Helium at the rate of 1 mL/min was used as the carrier gas at a constant flow mode. The inlet and interface temperature were kept at 280 °C. The EI source was operated at 230 °C, and the quadrupole temperature was 150 °C. The MS was scanned from *m/z* 30 to 600 for recording full-scan spectra. One microliter of the sample was injected in split mode at a split ratio of 5:1 by the autosampler. The compounds were identified by the NIST and Wiley library database supplied by the Agilent Technologies along with instrument software. The analytical data were also compared with the trimethylsilylated sugar reference standards (Sigma-Aldrich).

Quantitative GC-MS Analysis. GC-MS data were acquired and processed with the Agilent Chemstation software, supplied by the instrument manufacturer. Compound identification was performed by comparison with the chromatographic retention characteristics and mass spectra of authentic standards, reported mass spectra, and mass spectral library of the NIST and Wiley library databases supplied by Agilent Technologies along with the GC-MS system. Sugars were quantified using total ion current (TIC) peak area and converted to sugar mass to concentration corresponding to the peak area of standard sugars. In addition to the retention time of the sugar standards, their Kovats indices (where the retention times are related to the *n*-alkane distribution) were also determined.

Preparation of Solutions for Rheological Analysis. Aqueous and salt solutions (100 mM NaCl) at different concentrations ranging from 0.5 to 4% (w/v) were prepared by dissolving lyophilized native gum and deacetylated sample, respectively.

Rheological Measurements. All of the rheological measurements were performed in a Physica MCR 51 rheometer (Anton Paar GmbH, Ostfildem, Germany). The solutions were characterized for their steady-shear viscosity function, $\eta(r)$, using a unidirectional steady-shear flow, with shear rate ranging from 0.3 to 1000 s^{-1}. The dynamic viscoelastic properties, (i) viscous or storage modulus (G') and (ii) elastic or loss modulus (G'') were determined through small-amplitude oscillatory shear flows at frequencies ranging from 1 to 50 rad/s.

Statistical Analysis. Analytical values are based on the mean and standard deviation of three replicates. For all rheological measurements, reported values are based on the mean of at least three replicates.

RESULTS AND DISCUSSION

Sugar Composition by HPLC and LC-MS. The sugar composition of gum kondagogu as determined by RP-HPLC method is depicted in **Table 1**. **Figure 1a** shows the chromatogram of the corresponding separated ABEE−sugar derivatives. In comparison to other tree gums such as gum karaya (*S. urens*), *A. lebbeck*, and *S. striata*, the neutral and acidic sugar compositions of gum kondagogu (*13*) were found to be different. Earlier, the sugar composition of karaya gum was reported to contain galactose (26.3%), rhamnose (29.2%), and uronic acid (37.6%) (*26*). The neutral sugar components such as galactose (52%) and arabinose (36%) were reported to be higher in *A. lebbeck* gum exudates, whereas the uronic acid (10.5%) content was found to be lower (*10*). The presence of galactose (23.4%), rhamnose (28.8%), xylose (5.6%), and uronic acid (42.2%) has been reported in *S. striata* (*13*). In comparison to the compositional analysis of these tree gums, a detailed investigation was undertaken to establish the sugar composition of gum kondagogu by LC-MS and GC-MS analyses.

One of the limitations of HPLC methods in determining the composition of uronic acids is that acidic sugar residues such galacturonic acid and glucuronic acid coelute during the separation. However, the separation of acidic sugars can be successfully achieved by GC-MS analysis, as reported earlier (*7*), wherein the gum kondagogu was hydrolyzed by H_2SO_4 and subsequently analyzed by GC-MS for the presence of galacturonic and glucuronic acid residues. Thus, HPLC analysis may not reflect the true composition of uronic acids.

The sugar residue analysis of TFA-hydrolyzed gum kondagogu and its subsequent derivatization with ABEE and carried out by LC-MS are depicted in **Table 1**, and **Figure 1b** gives the chromatographic separation profile of ABEE−sugar derivatives. The major sugars identified in the gum were glucose (7.84 mol %), galactose (19.52 mol %), rhamnose (15.85 mol %), arabinose (1.82 mol %), glucuronic acid (16.25 mol %), and galacturonic acid (10.54 mol %). The full-scan mass spectrum of ABEE−sugar derivatives as analyzed by LC-MS is indicated in **Figure 2**. The *m/z* values for the separated sugars were observed to be ABEE−glucose (RT, 2.7 min, *m/z*, 330.1), ABEE−glucuronic acid (RT, 3.5min, *m/z*, 344.1), ABEE−galactose (RT, 5.5 min, *m/z*, 330.1), ABEE−arabinose (RT, 6.3 min, *m/z*, 300.1), ABEE−rhamnose (RT, 8.0 min, *m/z*, 314.1), and ABEE−galacturonic acid (RT, 9.1 min, *m/z*, 362.1). As compared to HPLC analysis, the uronic acids (ABEE−glucuronic acid and ABEE−galacturonic acid) separation was achieved in LC-MS by employing gradient elution system.

GC-MS Analysis. Trimethylsilylation of gum kondagogu and its subsequent analysis by GC-MS revealed that the major components present in the gums were neutral sugars and uronic acids. The chromatograms of major sugars identified in the gums are shown in **Figure 1c**. The retention times of individual sugars and their Kovats indices are depicted in **Table 2**. The sugar residues identified were arabinose (2.52 mol %), mannose (8.3 mol %), α-D-glucose (2.48 mol %), β-D-glucose (2.52 mol %), rhamnose (12.85 mol %), galactose (18.95 mol %), D-glucuronic acid (19.26 mol %), β-D-galactouronic acid (13.22 mol %), and α-D-galacturonic acid (11.22 mol %). Detection of sugars was based on peak retention times of separated compounds, comparison with external reference standards of trimethylsilylated derivatives of sugars, and similarity with the NIST and Wiley MS databank/libraries. Furthermore, the EI mass spectra of the TMSi sugar derivatives were also compared with the *m/z* values of the trimethylsilyl sugar derivatives reported in the literature (*23, 26–30*).

GC-MS is one of the widely used sensitive analytical tools as compared to HPLC or LC-MS for the detection of subnanomolar concentrations of sugars. Additionally, this method provides information on both the identity and amount of component monosaccharides and allows the detection of α- and β-anomers (*31*). The volatile nature of TMSi−sugar derivatives has a superior sensitivity and resolution in GC-MS analysis, as compared to the separation of ABEE-derivatized sugars, achieved in LC-based systems. Furthermore, LC-MS analysis in the ESI mode gives good response to the polar analytes, whereas the other nonpolar components show a poor response. In the present investigation GC-MS analysis was found to be superior in

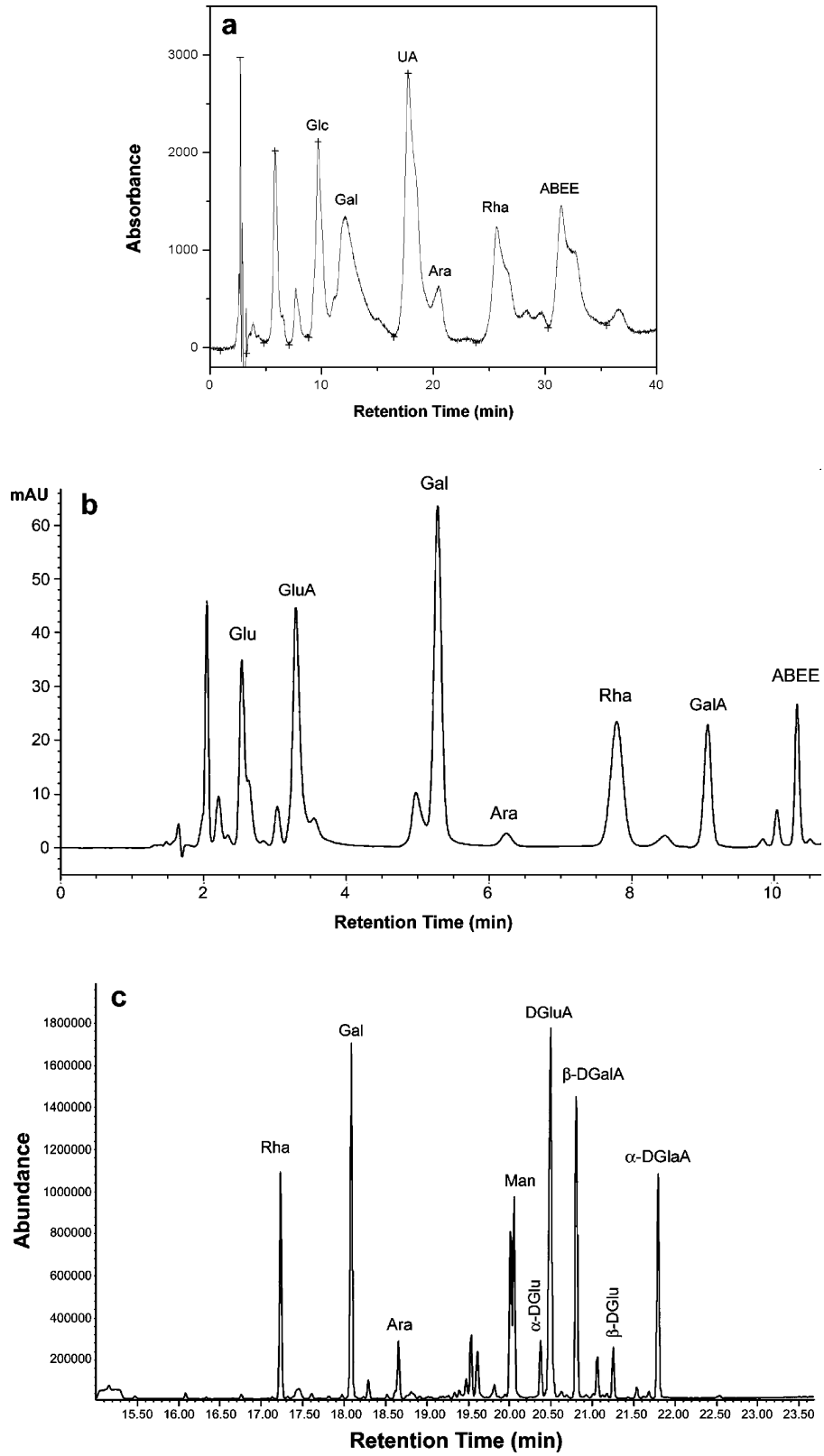

Figure 1. Chromatograms of TFA-hydrolyzed gum kondagogu and its sugar composition as analyzed by (**a**) HPLC, (**b**) LC-MS, and (**c**) GC-MS. Glu, glucose; Gal, galactose; UA, uronic acid; Ara, arabinose; Rha, rhamnose; ABEE, *p*-aminobenzoic ethyl ester; DGluA, D-glucuronic acid; DGlaA, D-galacturonic acid.

comparison to HPLC and LC-MS for the compositional analysis of gum kondagogu. The GC-MS method allows the distinction among sugars with identical molecular weights (e.g., glucose, mannose, galactose), which is not possible by LC-ESI-MS- or HPLC-based methods. In the present study, the GC-MS data suggest that sugar residues such as mannose, α-D-glucose, β-D-glucose, α-D-galacturonic acid, and β-D-galactouronic acid were identified and quantified additionally as compared to both the HPLC and LC-MS methods.

The major observation made in the present investigation suggests that gum kondagogu contains very high concentration of uronic acids [D-glucuronic acid (19.26 mol %), β-D-galactouronic acid

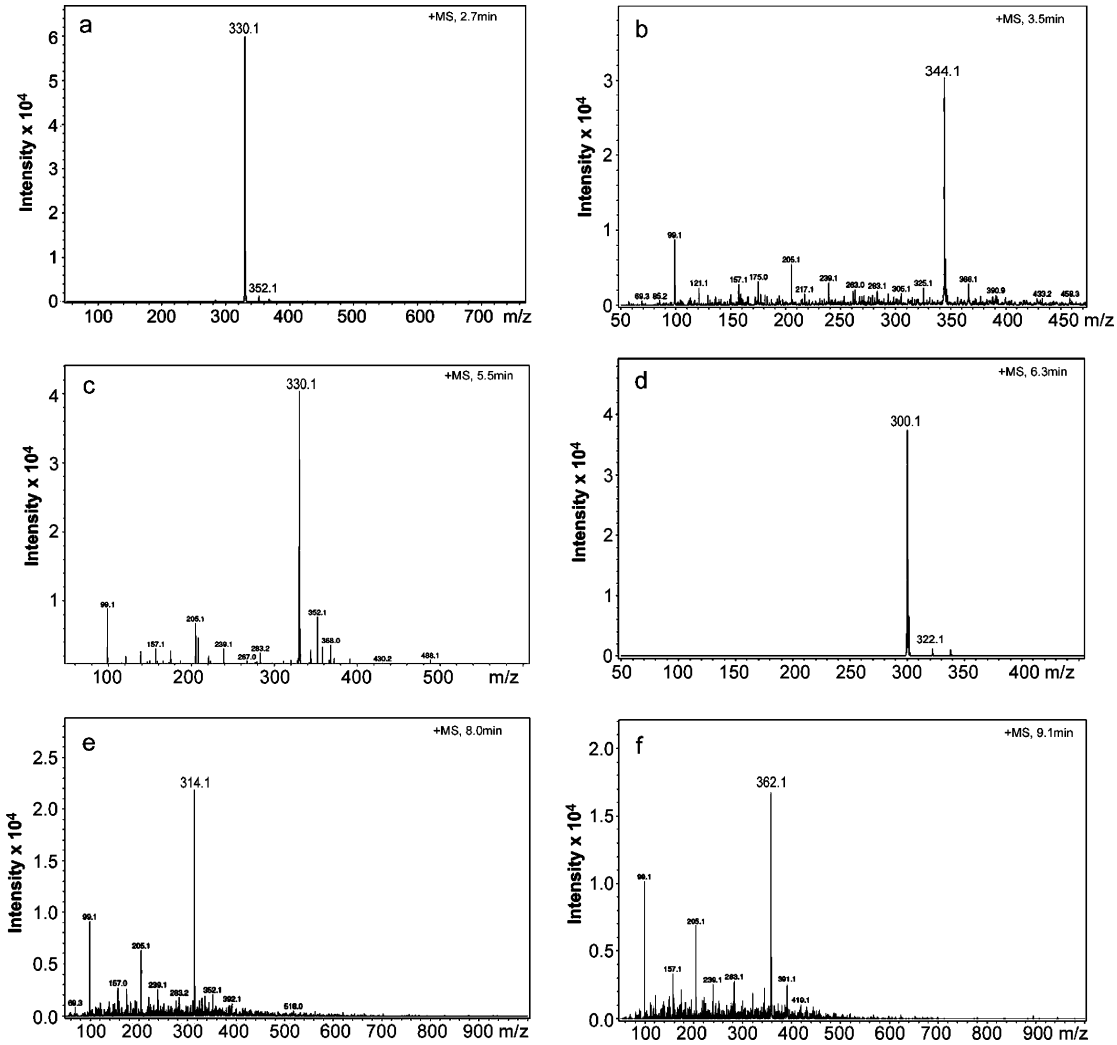

Figure 2. Mass spectra (*m*/*z*) of corresponding ABEE−sugar derivatives as determined by LC-MS: (**a**) ABEE−glucose; (**b**) ABEE−glucuronic aicd; (**c**) ABEE−galactose; (**d**) ABEE−arabinose; (**e**) ABEE−rhamnose; (**f**) ABEE−galacturonic acid.

Table 2. Sugar Profile of TFA-Hydrolyzed Gum Kondagogu As Determined by GC-MS Analysis

identified sugar residue	retention time (min)	Kovats index	composition[a] (mol %)
rhamnose	17.24	1658	12.85 ± 0.15
galactose	18.09	1857	18.95 ± 0.18
arabinose	18.66	1644	2.52 ± 0.01
mannose	20.06	1840	8.3 ± 0.09
α-D-glucose	20.38	1925	2.48 ± 0.03
D-glucuronic acid	20.50	1934	19.26 ± 0.17
β-D-galacturonic acid	20.81	1965	13.22 ± 0.12
β-D-glucose	21.26	2009	2.52 ± 0.01
α-D-galacturonic acid	21.80	2025	11.02 ± 0.12

[a] Values are mean ± SD, $n = 3$.

(13.22 mol %), and α-D-galacturonic acid (11.22 mol %)], as compared to other tree gums such as (i) arabinogalactan types of gums [gum arabic (*Acacia senegal*) (*32*), gum tragacanth (*Astralagus gummifer*) (*33*), *Spondias dulsis* gum (*34*), *Merta sinclairii* (*35*)], (ii) glucuromannan types of gums [gum ghatti (*Anogeissus latifola*) (*36*)], and (iii) gum karaya, a rhamnogalacturonan gum (*37*), when analyzed by GC-MS. The high acidity contributed by the presence of uronic acids in gum kondagogu is indicative of its higher zeta (ζ) potential value (*7*).

Rheological Analysis. The flow curves of native and deacetylated gum kondagogu at different concentrations were determined as apparent viscosity versus shear rate. The flow curves of native gum kondagogu at three concentrations [0.6, 2, and 3% (w/v)] were studied. **Figure 3a** shows the shear viscosity versus shear rate in the range of $10^{-3}-10^{1}$ s^{-1} on a logarithmic scale. The shear thinning behavior of the gum kondagogu solutions at concentration of 0.6, 2, and 3% were observed to be maximal in the range of 300–500 s^{-1} (shear rate). Gum kondagogu (2%) in water shows a sudden decrease in shear rate at 300–500 s^{-1} and then an increase (**Figure 3a**). The flow curves of gum kondagogu (**Figure 3a**) clearly indicate that a Newtonian behavior was observed above 0.6% of gum concentration in water. The shear-rate thinning behavior was observed in gum kondagogu solution as the concentration increased from 0.6 to 3% in deionized water. For 2% gum solution, a sudden change of shear rate (s^{-1}) at 300–500 s^{-1} was observed, wherein initially there was a decrease in shear rate that was followed by an immediate increase. Thus, the shear-rate thinning behavior is directly related to the gum concentration. This behavior may be attributed to the polyelectrolytic property of the gum (*7*). A similar type of shear thinning behavior was observed for 1% solution of gum tragacanth in water at 25 °C. A low shear Newtonian region was observed in the case of 1% tragacanthin in 0.1 M NaCl (*38*). Flow curves of *Enterolobium contortisilliquum* in aqueous solutions were performed in a concentration range of 0.1–3.5 g/dL. In the case of *E. contortisilliquum* Newtonian behavior was observed for

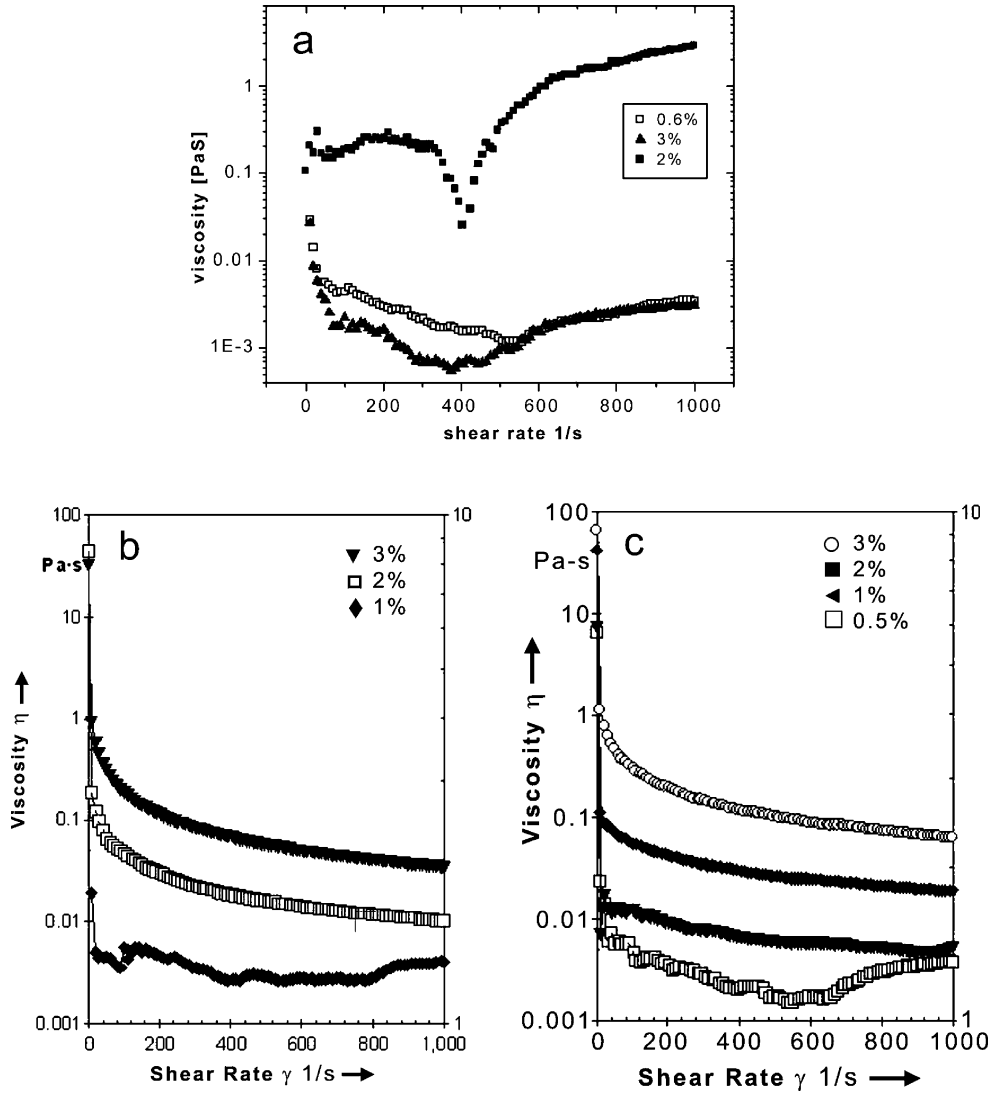

Figure 3. Flow curves at increasing or decreasing shear rates obtained on 0.5−3% (w/v) native gum kondagogu dispersions in (**a**) deionized water, (**b**) 100 mM NaCl solutions, and (**c**) deacetylated gum kondagogu in 100 mM NaCl solutions at 30 °C.

solutions with concentrations as high as 1% over the range of shear rate used, and above this critical concentration, the solution presents a shear thinning behavior (*12*). The steady shear viscous flow properties of commercially important biopolymers such as xanthan gum (*39*), mucilage gum (*O. ficus Indica*) (*11*), fenugreek gum (*40*), and *Aeromonas* gum (*14*) show similar flow curves. The flow curves of acacia gum dispersions at increasing or decreasing shear rates were similar above 0.5–1.0 s^{-1}. Below 0.5–1.0 s^{-1}, flow curves at decreasing shear rates were slightly above flow curves at increasing shear rates, indicating a higher apparent viscosity of dispersions (*8*). The flow curves of native and deacetylated gum kondagogu solutions in 100 mM NaCl solution are shown in **Figure 3**, panels **b** and **c**, respectively. Similar types of curves were observed for native and deacetylated *S. striata* and karaya gums in the range of concentration from 0.1 to 2% in 100 mM NaCl (*13*).

Viscoelastic behavior of gum kondagogu was determined over the frequency range from 0 to 10 Hz at 30 °C. **Figure 4** shows the frequency dependence of the dynamic viscous modulus (G') and elastic modulus (G'') at 30 °C for concentrations of native gum (1 and 2%) in aqueous and in 100 mM NaCl [1 and 2% (w/v)] solutions. The two different concentrations of gum kondagogu (1 and 2%) in aqueous and 100 mM NaCl solutions indicated that the viscous modulus (G') has a weak dependence

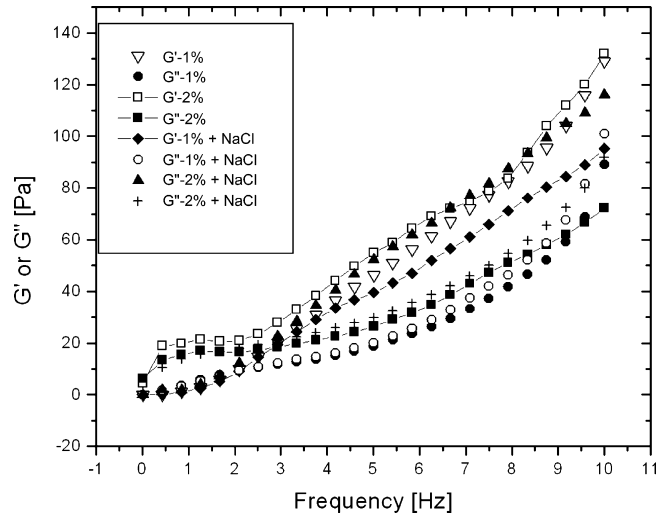

Figure 4. Oscillatory shear rate for 1 and 2% (w/v) gum kondagogu dispersions in deionized water and in 100 mM NaCl at 30 °C.

on frequency and was always higher than elastic modulus (G''). This behavior is typical of a gel-like matrix, as reported in the case of *S. striata* polysaccharide and gum karaya (*18*). Gels

can be classified either as strong or weak on the basis of their G'/G'' ratio (*40*). The G'/G'' ratios of native gum kondagogu (1 and 2%) in aqueous solution were found to be 1.89 and 1.85 and those in 100 mM NaCl, 1.54 and 2.2, respectively. In the case of true gel, the ratio of G'/G'' should be greater than 3 (*18*). The G'/G'' ratio of gum kondagogu (1 and 2%) in aqueous and salt solution was less than 3; thus, its acts as a weak gel. However, higher concentrations of gum kondagogu (>2%) may act as true gels, as earlier studies have indicated that >2% solution of gum karaya and 4% *S. striata* polysaccharide (*18*) have true gel properties.

The frequency sweep for 1 and 2% gum kondagogu dispersions in water exhibits a mechanical spectrum very similar to the one obtained for a weak gel system such as gum tragacanth (*38*). Aged and fresh *S. urens* (gum karaya) gums exhibit gel-like properties as evidenced by mechanical spectrum with $G' > G''$. The fresh gum behaves as a stronger gel than the aged one (*41*). In the presence of 100 mM NaCl, gum kondagogu showed a more liquid-like structure, and the oscillatory data are as expected for a semidilute to concentrated solution of entangled, random coil polymers. A similar behavior was earlier observed in gum tragacanth (*38*).

The frequency versus G' and G'' curves for deacetylated gum kondagogu solutions of various concentrations (1, 2, and 3%) are illustrated in panels **a** and **b** of **Figure 5**. In these plots, a high-frequency value was observed for 2% gum as compared to 1 and 3% gums, respectively. Crossover points of G' and G'' against frequencies of native gum kondagogu in aqueous and 100 mM NaCl solutions are indicated in panels **a** and **b** of **Figure 6**. A crossover value of G' and G'' was observed to be at frequency 0.432 Hz for 1% and 1.2 Hz for 2% native gum in aqueous solution and at 2.1 Hz for 1% and 1.68 Hz for 2% gum in 100 mM NaCl concentrations, respectively. Crossover values provide a good index for the viscoelastic behavior of the material, as lower crossover values reflect a larger elastic contribution (*42*). Previously, it has been reported that 4 and 5% *S. striata* polysaccharide had crossover values (G' and G'') of 6.8 and 3.1 Hz (*18*). The crossover point denotes a change of solution response from predominantly liquid-like to solid-like behavior, which occurs at low frequencies for solutions of high polymer concentrations. A crossover value of G' and G'' was observed to be at frequency 0.432 Hz for 1% and at 1.2 Hz for 2% for native gum in aqueous solution, indicating a predominantly liquid- to solid-like behavior, whereas a crossover value of 2.1 Hz for 1% and 1.68 Hz for 2% gum in 100 mM NaCl solution suggests a larger elastic contribution.

Furthermore, the native gum kondagogu (2%) concentration showed a higher crossover value (1.2 Hz) as compared to 1% gum (0.432 Hz) in aqueous solution. This behavior was dissimilar to the one observed in *S. striata* polysaccharide, wherein 1% gum had a higher frequency than the 2% gum solution (*18*). Possibly, this behavior may be ascribed to the higher content of uronic acids in *S. striata* gum (*13*). However, the native gum kondagogu concentrations at 1 and 2% in 100 mM NaCl show crossover values (2.1 Hz for 1% and 1.68 Hz for 2% solutions) that are similar to the values observed in *S. striata* polysaccharide, suggesting a larger elastic contribution. In the presence of NaCl, the crossover value decreases with an increase in gum concentration. Interestingly, the gel strength (*18*) and the polyelectroyletic effect (*7, 38*) of gum were found to decrease in the presence of sodium chloride.

In summary, the following conclusions can be inferred from the present experimental investigations: (i) Compositional analysis of gum kondagogu by HPLC, LC-MS, and GC-MS, suggests the occurrence of glucose, galactose, arabinose, rhamnose, mannose, glucuronic acid, β-D-galacturonic acid, and α-D-galacturonic acid residues in the gum, indicating that it is an acidic gum. (ii) The rheological measurements performed on the gum kondagogu suggest that above 0.6% (w/v), it shows a Newtonian behavior and shear rate thinning behavior as a function of gum concentration. (iii) The two different concentrations of gum kondagogu (1 and 2%) in aqueous and 100 mM NaCl solutions indicated that the viscous modulus (G') has a weak dependence on frequency and was always higher than the elastic modulus (G''). This behavior is typical of a gel-like matrix, and addition of NaCl decreases the gel strength of gum kondagogu polysaccharide. (iv) In the presence of 100 mM NaCl, gum kondagogu showed a more liquid-like structure, and the oscillatory data are as expected for a semidilute to concentrated solution of entangled, random coil polymers. (v) Crossover values of G' and G'' observed at a frequency 0.432 Hz for 1% and 1.2 Hz for 2% for native gum in aqueous solution indicated a predominantly liquid- to solid-like behavior, whereas crossover values of 2.1 Hz for 1% and 1.68 Hz for 2% gum in 100 mM NaCl solution suggest a larger elastic contribution.

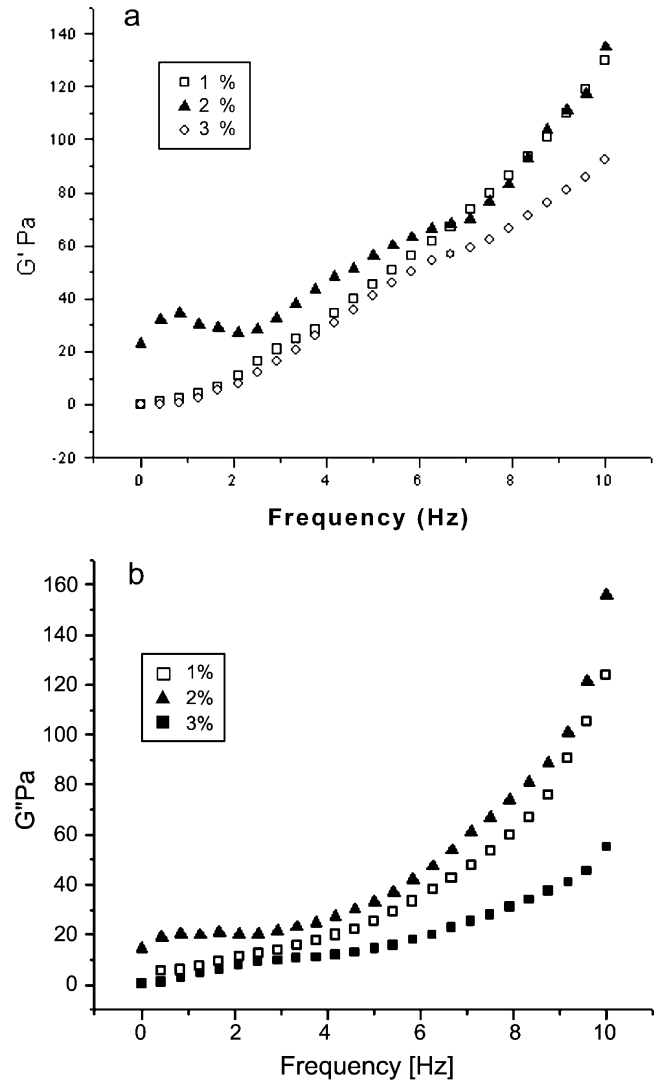

Figure 5. Effect of frequency on (**a**) elastic modulus (G') and (**b**) viscous modulus (G'') of deacetylated gum kondagogu solutions at different concentrations (1, 2, and 3%) at 30 °C, in deionized water.

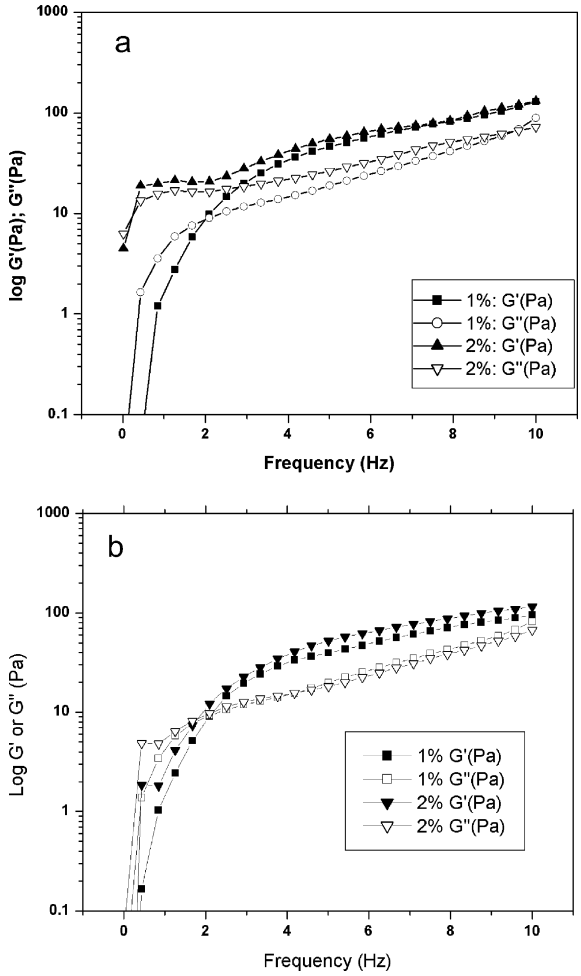

Figure 6. Crossover point frequency on elastic and viscous modulus of native gum kondagogu at 1 and 2% concentrations in (**a**) deionized water and (**b**) 100 mM NaCl.

The results of the experimental investigation permit the exploitation of this biopolymer as a thickener, stabilizer, and emulsifier in the food and cosmetic industries.

ABBREVIATIONS USED

RP-HPLC, reverse-phase high-performance liquid chromatography; LC-MS, liquid chromatography–mass spectrometry; GC-MS, gas chromatography–mass spectrometry; ABEE, *p*-aminobenzoic ethyl ester; TFA, trifluoroacetic acid; BSTFA, *N-O*-bis(trimethylsilyl)trifluoroacetamide; Ara, arabinose; Glc, glucose; Gal, galactose; Rha, rhamnose; Man, mannose; UA, uronic acid; GalA, galacturonic acid; GluA, glucuronic acid; ESI, positive electrospry ionization; SIM, selective ion monitoring; TIC, total ion current.

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