Structure and physicochemical properties of Ghanaian grewia gum

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Abstract

Grewia polysaccharides were isolated using sodium metabisulphite and phosphate buffers and the influence of the different extraction techniques on the chemical composition and structural characteristics of the extracts were determined. Structure and chemical composition of the resulting polysaccharide extracts were determined using FT-IR and NMR spectroscopy, neutral sugar analysis, size exclusion chromatography coupled to multi-angle light scattering (SEC-MALS), dilute solution viscometry and steady shear rheology. Chemical composition was similar irrespectively of the extraction solvent used and ranged between 11.1–16.5 % for protein, 53.4–66.9 % for total carbohydrate, 18.5–35.1 % for total uronic acid and 23.5–28.6 % for rhamnose. Predominate sugars in the extracts were rhamnose and uronic acids with spectroscopy showing the presence of esterified groups. Intrinsic viscosity varied between 6.5–9.1 dL g^{-1} and related with molar mass (754–2778 $x10^3$ g mol^{-1}). Grewia polysaccharide dispersions at 1 g dL^{-1} exhibited a shear thinning flow behaviour with crude and sodium metabisulphite extracts having higher viscosities. Overall, differences in extraction techniques produced grewia samples with tailored bulk properties for use in the food and pharmaceutical industries.

Keywords: *Grewia mollis,* polysaccharides, sugars, isolation, viscosity

1. Introduction

Polysaccharides are abundant in nature and form the major constituent of the cell wall material of plants (e.g., cellulose or pectin) [1]. Plant polysaccharide extracts have been widely used in food and pharmaceutical applications due to their valuable functional properties [2, 3]. In addition, they may also display bioactivity including antidiabetic, antitumor, or immunomodulatory properties [1, 4-7]. These functional characteristics have been related to their chemical composition, molar mass, branching characteristics, and functional groups [7].

Grewia mollis is a tropical shrub which belongs to the *Malvaceae* family and is widely distributed in Africa [8]. Polysaccharide extracts from the inner stem bark of the grewia plant have been useful to the food and pharmaceutical industries as a thickening agent, emulsion stabilizer, or as hydrophilic matrix for tablets [8-10]. For example, in Ghana, the crushed grewia stem bark is used as a clarifying agent during the processing of an indigenous beverage referred to as pito [11]. Natural plant-based polysaccharides have been known to demonstrate heterogeneity in structural characteristics depending on the plant genotype and stage of ripening [3, 12]. The physicochemical and rheological properties of polysaccharides also depend on the method, conditions of extraction and purification, which subsequently produce biopolymers with unique functionality [13, 14]. The extraction procedure used influences the yield, quality, structure and bioactive properties of the resulting polysaccharides [1, 14]. Although polysaccharides from other members of the *Malvaceae* family such as okra have been isolated to produce polysaccharides with varied structural and molecular characteristics [3, 15-22], few studies have evaluated the effect of different extraction strategies on the structure and chemical composition of grewia gum. The origin of a plant material is a critical determinant of the chemical, macromolecular and functional characteristics of its polysaccharide extracts. The presence of cellulose, hemicellulose, proteins,

fibre and lipids in a plant polysaccharide extract is influenced by the botanical source of the plant [14, 23]. Grewia is widely distributed in different locations of Africa including Nigeria, Sierra Leone, Somalia, Angola, Zambia and Ghana. The quest for standardization of extraction protocols as a requirement for the application of polysaccharides in food and pharmaceutical products have made it imperative to characterize the physicochemical properties of the gum extracts from the *Grewia mollis* plants. Grewia polysaccharides have been previously isolated from plants obtained from Nigeria [8]. However, the understanding of how different plant sources (e.g., Ghana) and different extraction protocols affect the molecular characteristics of grewia gum would be informative to tailor extracts that meet a specific functionality. The present work aims to investigate and characterize the structure and chemical constituents of polysaccharides from the Ghanaian *Grewia mollis* isolated using different solvent extraction methods. The understanding of the impact of sodium metabisulphite and phosphate buffer extraction solvents on macromolecular characteristics would be relevant in selecting an appropriate extraction medium to isolate polysaccharides with specific functionality.

2. Material and Methods

2.1 Materials

The dried *Grewia mollis* inner stem bark was purchased from the local market in the Northern Region, Ghana. L-Rhamonose (Rha), D-glucose (Glc), D-galactose (Gal), L-arabinose (Ara), D(+)-galacturonic acid (GalA), buffer salts, ethanol and sodium metabisulphite were purchased from Sigma-Aldrich (Poole, UK). Deionized water was used throughout the extraction experiments. All reagents used were of analytical grade.

2.2 Extraction of grewia gum

The dried Ghanaian *Grewia mollis* inner stem bark was milled to a particle size of 450 µm and then subjected to extraction procedure using sodium metabisulphite solution (1 mg mL^{-1}), pH 4.5) [8] or 100 mM phosphate buffer at pH 6 [16]. The extraction protocols used are shown in **Fig. 1**. The first extraction step yielded crude polysaccharides (SMB crude, PB crude) and upon exhaustive dialysis (molecular mass cut-off 12.000) against deionized water for 3 days produced purified polysaccharides, which are referred throughout the manuscript as SMB pure or PB pure.

2.3 Chemical composition of grewia gum

Protein quantification was determined by Bradford assay [24] using bovine serum albumin as standard, whereas the total sugar content of the polysaccharide extracts were determined by phenol-sulphuric acid method [25] using D-galactose as standard. All determinations were done at least in triplicate. The total uronic acid content of the polysaccharides was determined using *m*-hydroxydiphenyl method [26]. The neutral sugar composition of the grewia gum extracts was determined using methanolysis conducted with 1 M methanolic HCl at 85 °C for 24 h, as described previously [27]. Sugar derivatives were analysed using an Agilent 7890A GC system (Santa Clara, CA, USA) coupled to an Agilent 5675C quadrupole MS. The samples were eluted from an HP-5 column (30 m x 0.25 mm, 0.25µm film) using helium as carrier at a flow rate of 1 mL min^{-1} by applying the following temperature setting: start temperature 140 °C, hold time 1 min, and final column temperature 220 °C with 25 °C min^{-1} gradient.

2.4 Spectroscopic analysis

FTIR spectra were obtained between 400 and 4000 cm^{-1} for all the grewia gum samples in attenuated total reflection (ATR) mode at a resolution of 4 cm^{-1} using 128 scans (Nicolet 380, Thermo Scientific, UK). Spectral smoothing was applied using instrument software (OMNIC 3.1. Thermo Scientific, UK). ^1H NMR was conducted using a Bruker AV 500 spectrometer (Bruker Co., Switzerland) by dispersing grewia gum extracts (3 g dL^{-1}) overnight in D_2O (99.9% D, Goss Scientific Instruments Ltd., Essex), and run as described in our previous investigation [3].

2.5 Molar mass determination

The weight average molar masses (M_w) of the extracts were estimated using size exclusion chromatography coupled to multi–angle light scattering (SEC-MALS) at 25 °C. Extracts were solubilised in 0.1 M $NaNO_3$ solution (3 mg mL^{-1}) at room temperature with stirring overnight. Samples were subsequently injected onto a SEC system (15 μm particle size, 25 cm × 4 mm, Agilent, Oxford, UK) which consisted of a PL Aquagel guard column linked in series with PL Aquagel-OH 60, PL Aquagel-OH 50 and PL Aquagel-OH 40. The samples were eluted with 0.1 M $NaNO_3$ solution at a flow rate of 0.7 mL min^{-1}. The eluent was then detected online firstly by a DAWN EOS light scattering detector (Wyatt Technology, Santa Barbara, U.S.A.) and finally by a rEX differential refractometer (Wyatt Technology, Santa Barbara, U.S.A.). The refractive index increment, dn/dc was taken to be 0.146 mL g^{-1} [28, 29].

2.6 Intrinsic viscosity and steady shear measurements

Samples were dispersed at 0.01–1.0 g dL^{-1} in deionized water. The polysaccharide solutions were stirred overnight and intrinsic viscosity measurements were performed at 20 °C using an Ubbelohde capillary viscometer (PLS Rheotek OB. C 80705). At least three efflux times at each

concentration were monitored. Determination of the intrinsic viscosities were obtained by extrapolation to infinite dilution using [30]:

$$\frac{\eta_{sp}}{c} = [\eta] + k_H[\eta]^2 c \qquad (1)$$

where η_{sp} and η are the specific and intrinsic viscosities, c the biopolymer concentration in g dL^{-1} and k_H the Huggins constant. Steady shear measurements were carried out at 20 °C using a Bohlin Gemini 200HR Nano rotational rheometer equipped with a cone-and-plate geometry (55 mm diameter, cone angle 2°). Flow curves were determined in the range of 0.01-1000 s^{-1} at 20 °C.

2.7 Data Analysis

Data obtained were analysed using Statgraphics (Graphics Software System, STCC, Inc. USA). Comparisons between the different extracts were done using analysis of variance (ANOVA) with a probability $p < 0.05$.

3. Results and discussion

3.1 Chemical composition of grewia gum

Sodium metabisulfite is a reducing agent that may aid the extraction of polysaccharides by disrupting the protein matrix of inner stem bark whereas phosphate buffer does not have reducing capacity. In addition, the solvents have been chosen so as to evaluate whether different polysaccharide structures could be obtained at different mildly acidic pH values (4.5 *vs.* 6.0). The isolation method used had a rather muted impact on the protein and carbohydrate contents of grewia gum extracts. The phosphate buffer extraction protocol resulted in polysaccharides with

relatively high total carbohydrate content and moderate amounts of protein (**Table 1**). It has been reported that polysaccharides with different chemical compositions can be extracted depending on the pH and temperature of the extraction medium [16, 19]. Higher solvent temperatures increases the ability of the solvent to penetrate the raw material and solubilize the polysaccharides [19]. The mildly acidic nature (pH 6) and high temperature (80 °C) of the phosphate buffer separated successfully grewia polysaccharides from the other cell wall materials resulting in relatively high total carbohydrate content. However, extraction at room temperature (25 °C) with metabisulfite also yields comparable amounts of total carbohydrates, which is a particular advantage when considering scaling up the isolation process. The extraction of polysaccharides from plants usually results in protein-carbohydrate mixtures and the presence of these proteins either as contaminants or structurally linked moieties to the polysaccharide is not well elucidated [31]. Nonetheless, further purification is mostly required to reduce the protein content and isolate functional polysaccharides [32]. In this study, further purification was achieved by dialysis of the crude sample against deionized water with subsequent polysaccharide precipitation with ethanol. Dialysis reduced significantly protein content and increased total carbohydrate in both sodium metabisulphite and phosphate buffer extracts (**Table 1**). The ecological source of the grewia plant seems to influence the protein-polysaccharide biopolymer composition of the extracts, as sodium metabisulphite-extracted grewia polysaccharides obtained from Ghana had comparatively higher protein content (14.5 to 16.5 %) than those previously obtained from samples obtained in Nigeria (2.3 to 5.2 %) [8].

The constituent sugar composition of the samples is shown in **Table 1**. The total uronic acid content varied from 18.5 % to 35.1 % (**Table 1**). The extraction protocol used significantly affected the total uronic acid content of the different grewia polysaccharide extracts. Grewia gum extracted

using sodium metabisulphite solution (34.5 to 35.1 %) generally had a higher total uronic acid content than the phosphate buffer extracts (18.5 to 27.4 %), which is attributed to its lower pH (~ 4.5). Total uronic acid content (34.5 to 35.1 %) of grewia gum extracted with sodium metabisulphite was comparable to values previously reported (~30 %) [8] but remarkably lower in phosphate buffer extracts. The difference in total uronic acid is attributed to variations in the source of the raw material, extraction conditions and method of determination [16]. It should be noted, however, that the mol% of total uronic acids is not particularly different for the samples after dialysis. This could be due to free uronic acids or small oligomers that are lost during the dialysis process. The total uronic acid content of *Grewia mollis* gum, although lower than polysaccharides from *Abelmoschus esculentus* (42.8 to 63.4%) [3], *Hoheria populnea* (40.5%) [33], *Abelmoschus manihot* (38.8 to 43.4%) [34] and *Althaea officinalis* (37.5%) [35] were higher than polysaccharides extracted from the mallow *Malva aegyptiaca* (5.7 to 6.1 %) [36]. The main neutral sugar present was rhamnose (~44 mol%), followed by arabinose (~10 mol%), glucose (~3 mol%), and galactose (~ 0.3 mol%) that also contributed into the neutral sugar make-up of the samples. The low glucose content indicates lower amounts of α-glucans (e.g., starch) than those observed in our previous investigation [8]. Although the chemical composition of grewia gums extracted in this study is very similar to those characterised previously [8], it is not unexpected that there are some differences, as polysaccharide composition is influenced by extraction conditions (metabisulphite *vs.* phosphate buffer), growing conditions (Ghana *vs.* Nigeria) as well as seasonal, climatic or genetic variations. It should be also noted that the overall composition of dialysed samples is essentially invariable between the two solvents revealing that similar polysaccharides are obtained with either protocol. Having explored the compositional

characteristics of the extracts we proceeded to explore other physicochemical parameters that are described in the next sections.

3.2 FT-IR and NMR spectroscopy

FT-IR spectra (4000 to 800 cm^{-1}) were used to compare the different extracts and the overlapping of their infrared spectra confirmed that they had similar functional groups (**Fig. 2**). Samples displayed the characteristic broad and intense band within the range of 3600 to 3200 cm^{-1} for the stretching absorption of the hydroxyl group. A similar O-H stretching absorption peak has been reported within the range of 3600 to 3000 cm^{-1} for lacebark polysaccharides [33] and in the region of 3200 cm^{-1} for polysaccharides extracted from *Malva aegyptiaca* [36]. This absorption band has been attributed to the inter- and intra- molecular hydrogen bonding of the D-GalA backbone [3, 16]. The peak in the range of 3000–2800 cm^{-1} is characteristic of the C–H stretch of methyl groups and corresponds to CH, CH_2 and CH_3 stretching vibrations [2, 5]. For polysaccharide extracts from *Abelmoschus manihot* an intense peak around 2888 cm^{-1} was assigned to the C-H stretch vibration [37]. The spectra of all grewia extracts revealed two critical peaks associated with the carboxyl group esterification. A band that occurred around 1600 cm^{-1} and thus corresponds to the symmetrical stretching vibration of the carboxylic group (COO^-). The second band which corresponds to esterified groups occurred at around 1731 cm^{-1} [8, 38]. These two major peaks of esterification are typical of polysaccharides from other members of the *Malvaceae* family such as okra [3, 16], lacebark mucilage [33] and marshmallow [35]. The bands at 1416, 1380 and 1230 cm^{-1} correspond to bending of CH_2, OH and $-CH_3CO$ stretching respectively [39, 40]. Polysaccharides have generally shown specific bands between 1200 and 800 cm^{-1}, hence signals in this region correspond to the fingerprint of carbohydrates as described in the literature [5].

^{1}H-NMR spectra of both extracts revealed comparable resonance patterns suggesting similarities in compositional characteristics (**Fig. 3**). The ^{1}H-NMR spectra for the pure polysaccharide extracts from both solvents showed proton signals in the low field region around 5.0 ppm. These signals have been assigned to protons originating from anomeric sugars [6, 40, 41]. The acetyl groups were detected in the region of 2.45 – 2.60 ppm for all extracts [3, 42]. Similar peaks indicative of the presence of *O*-acetyl groups have been reported in lacebark mucilage at 2.14 – 2.22 ppm [33]. The methyl group of the rhamnosyl residues were detected as a dominant signal at 1.64 ppm confirming the high rhamnose content in polysaccharide as determined by the neutral sugar analysis. In the case of phosphate buffer, only one clear signal is present, however, in the case of the metabisulfite extracts there is a doublet (1.57, 1.65 ppm) indicating different rhamnosal branching patterns. Comparable peaks have been previously reported from a Nigerian crude grewia extract [43]. Overall, it appears that grewia extracts tend to have similarities with polysaccharides extracted from other members of *Malvaceae* family (e.g. okra [3, 16], lacebark [33], or cola [44]). Spectroscopy has revealed the presence of acetyl groups, however, ^{1}H-NMR also reveals the presence of a peak at 3.64-3.75 ppm, which could be indicative of uronic acid methyl esterification.

3.3 Molar mass of grewia gum

The weight-average molar mass values of the samples ranged widely from 0.75 to 2.8 x10^6 g mol^{-1} (**Table 2**). The crude polysaccharide samples recorded relatively high molar masses and this may be due to the presence of other aggregates such as proteins or hemicelluloses [14, 23], considering the crude nature of the samples. The pure grewia polysaccharides were also obtained by precipitation at two successive stages with two volumes of ethanol. It has been reported that the continuous exposure of polymer chains to organic solvents, for instance, ethanol [16] or

isopropanol [32], facilitates the cleavage of polysaccharides. Hence the main reason for the molar mass reduction of the polysaccharides in the purified samples is attributed to the breakdown of the biopolymer chains in the presence of successive ethanol precipitation and protein removal. Extraction solvent has also influenced the molar mass of the polysaccharides. Samples extracted using sodium metabisulphite at a relatively lower temperature (25 °C) recorded higher molar masses (1.7 to 2.8 x 10^6 g mol^{-1}) than the phosphate buffer extracts (0.75 to 0.92 x 10^6 g mol^{-1}). This variation in molar mass of the SMB and PB polysaccharide extracts is attributed to temperature differences, duration of extraction, and pH [45,46]. The phosphate buffer extraction at 80 °C and pH 6.0 may result in limited acid hydrolysis or β-elimination reactions resulting in low molar mass polymers. On the contrary, even though metabisulfite is more acidic (pH 4.5) the milder extraction temperatures (~25 °C) affords protection to the size of the extracted macromolecules. Molar masses of the polysaccharides studied were higher than polysaccharides from *Abelmoschus esculentus* (5.0 – 6.0 x 10^4 g mol^{-1}) [3, 20, 47], *Abelmoschus manihot* (8.8 x 10^3 g mol^{-1}) [37], *Hibiscus sabdariffa* (8.7 x 10^3 – 1.4 x 10^5 g mol^{-1}) [48], but lower than *Althaea officinalis* polysaccharides (33.3 x 10^6 g mol^{-1}) [35] revealing that a range of macromolecular sizes can be obtained from members of *Malvaceae* family.

3.4 Intrinsic viscosity and flow behaviour

Dilute polymer solutions are characterized by negligible interactions between polymer chains, hence intrinsic viscosity gives a measure of the hydrodynamic volume of the polymer in dilute solutions [49]. Intrinsic viscosity ranged from 6.5 to 9.1 dL g^{-1} (**Table 2**) and sodium metabisulphite extracts recorded higher values. Intrinsic viscosity of samples obtained in this study were higher than reported values for grewia samples in the presence (3.78 dL g^{-1}) or absence (4.40 dL g^{-1}) of starch [8] with differences in plant sources contributing to this variation, although the

molar mass and solution conformation of the polysaccharides are also important. The intrinsic viscosity values of the polymers were in agreement with molar mass of the samples. The solvent extraction method used likewise influenced the intrinsic viscosity of grewia polysaccharides, where polymers extracted with phosphate buffer recorded decreased intrinsic viscosity values (6.5 – 8.3 dL g^{-1}). The K_H value is indicative of polymer interactions with the solvent and reflects the state of aggregation of the polymer [50]. In a good solvent and for flexible polymers, K_H values range between 0.3 and 0.5, 0.5 – 0.8 in theta solvents whereas higher than 1 in the case of aggregated polymers [51, 52]. K_H values for SMB samples were above 1 in crude samples indicative of possible polymer aggregation and were alleviated after dialysis (SMB pure). This trend was not consistent, as in the PB samples removal of low molecular mass species after dialysis seems to have changed the specific interaction forces between macromolecules resulting in partial aggregation.

The final step of the present investigation was to explore the steady shear viscosity of the samples that gives first insights of the bulk properties of the isolated polysaccharides. Samples were dispersed in deionized water (1 g dl^{-1} at 20 °C) and the effect of polymer type on flow behaviour was examined (**Fig. 4**). All the polymers exhibited shear thinning flow behaviour with sodium metabisulphite extracts demonstrating flow curves at higher viscosities relative to the phosphate buffer extracts. At neutral pH, previous investigations have reported that polymers with repeating units of uronic acids are deprotonated resulting in anionic polyelectrolytes exhibiting intra- and inter- chain repulsions [53]. Irrespectively of the extraction solvent used, the crude samples demonstrated higher viscosities than the purified extracts. The samples showed decreasing viscosities in the order of SMB crude extracts > SMB pure extracts > PB crude extracts > PB pure extracts. The key molecular characteristics of the grewia gum that are relevant in relating structure

and viscosity appears to be molar mass and the uronic acid content of the samples. In the present study, a corresponding decreasing trend was generally observed in uronic acid and molar mass, as reported for viscosity. Polymers extracted with phosphate buffer recorded lower uronic acid and molar masses with correspondingly decreased viscosities (Fig. 4). Overall, it becomes evident that initial bulk properties, such as viscosity, is easily tailored (to one order of magnitude) for grewia polysaccharides by choosing the appropriate extraction solvent. This is a significant development, as viscosity is in most cases critical factor in applications of natural biopolymers.

4. Conclusions

In the present study grewia polysaccharides were extracted using different solvents to produce biopolymers as functional ingredients for the pharmaceutical and food industries. The isolated biopolymers had similar chemical composition but different physicochemical properties due to the differences in size and the specific interactions of the polymer chains. The dominant neutral sugar in the extracts was rhamnose, and irrespective of extraction solvent employed the samples had high rhamnose and total uronic acid contents and spectroscopy revealed the presence of esterified groups. Intrinsic viscosity of the polymers related with molar mass and extraction solvent used, with phosphate buffer extracts recording the least intrinsic viscosity and molar mass values. The sodium metabisulphite extracts showed higher viscosities attributable to their higher molar masses. The present findings show that different physicochemical properties and functionality of grewia extracts are obtained depending on the source and extraction techniques employed.

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FIGURE CAPTIONS

Figure 1: Isolation of grewia polysaccharide gum with two different extraction solvents.

Figure 2: FTIR spectra of grewia gums extracted with different solvents.

Figure 3: Typical ^{1}H-NMR spectra of (a) phosphate buffer (PB) grewia gum extract and (b) sodium metabisulphite (SMB) grewia gum extract.

Figure 4: Apparent viscosity dependence on shear rate of grewia gum dispersions at 1 g dl^{-1}.

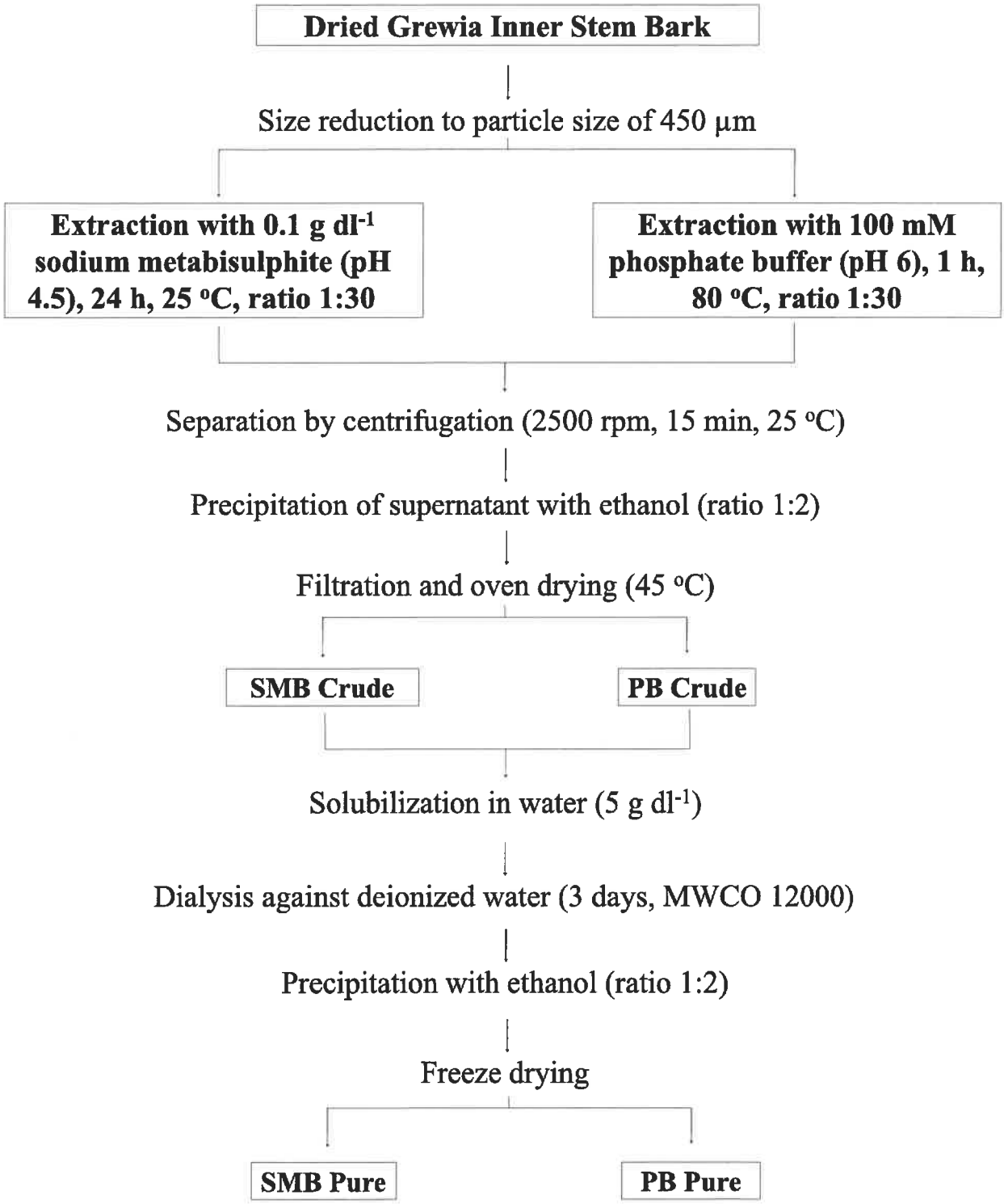
Dried Grewia Inner Stem Bark
Size reduction to particle size of 450 µm
Extraction with 0.1 g dl-1 sodium metabisulphite (pH 4.5), 24 h, 25 °C, ratio 1:30
Extraction with 100 mM phosphate buffer (pH 6), 1 h, 80 °C, ratio 1:30
Separation by centrifugation (2500 rpm, 15 min, 25 °C)
Precipitation of supernatant with ethanol (ratio 1:2)
Filtration and oven drying (45 °C)
SMB Crude
PB Crude
Solubilization in water (5 g dl-1)
Dialysis against deionized water (3 days, MWCO 12000)
Precipitation with ethanol (ratio 1:2)
Freeze drying
SMB Pure
PB Pure

Figure 1

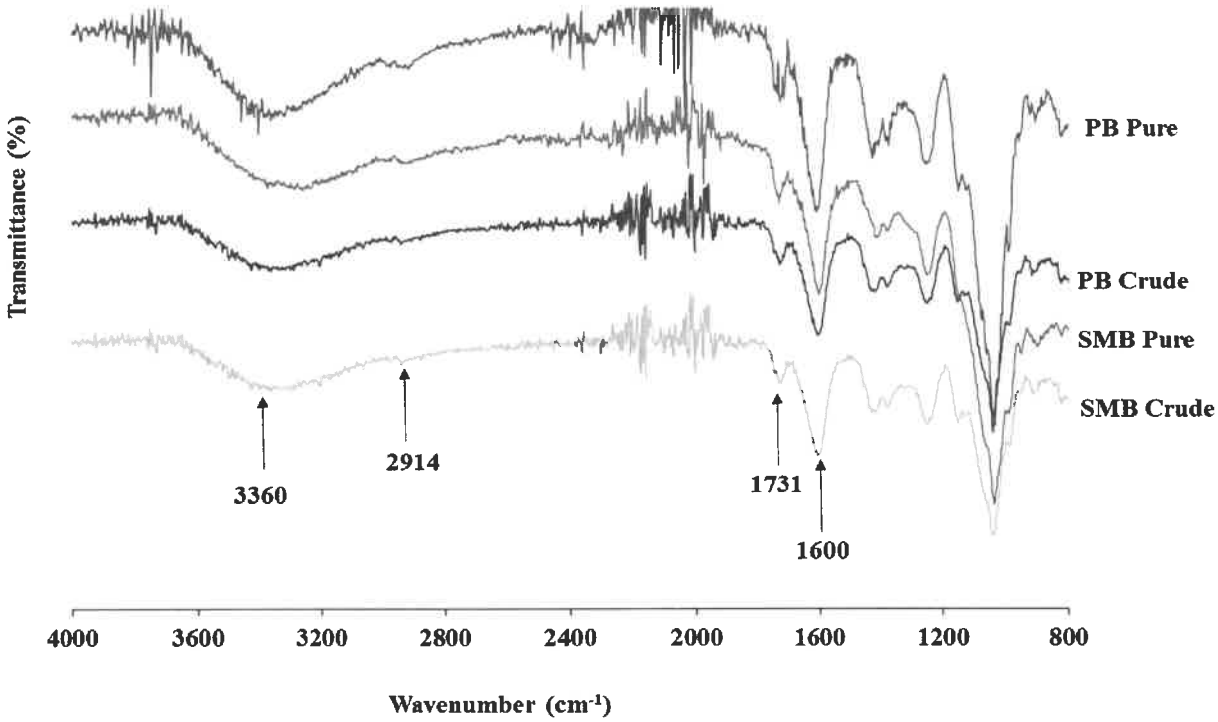
Transmittance (%)
PB Pure
PB Crude
SMB Pure
SMB Crude
3360
2914
1731
1600
4000
3600
3200
2800
2400
2000
1600
1200
800
Wavenumber (cm-1)

Figure 2

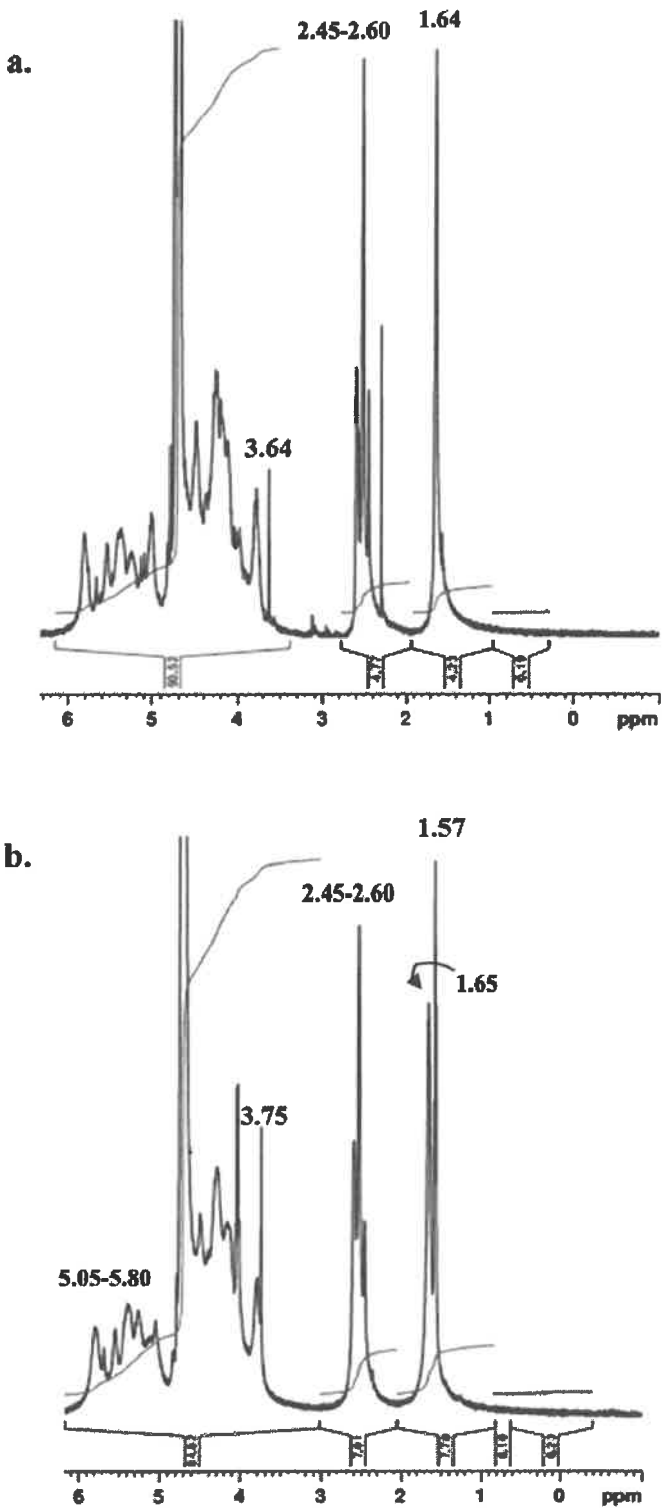
a.
2.45-2.60
1.64
3.64
6
5
4
3
2
1
0
ppm
b.
1.57
2.45-2.60
1.65
3.75
5.05-5.80
6
5
4
3
2
1
0
ppm

Figure 3

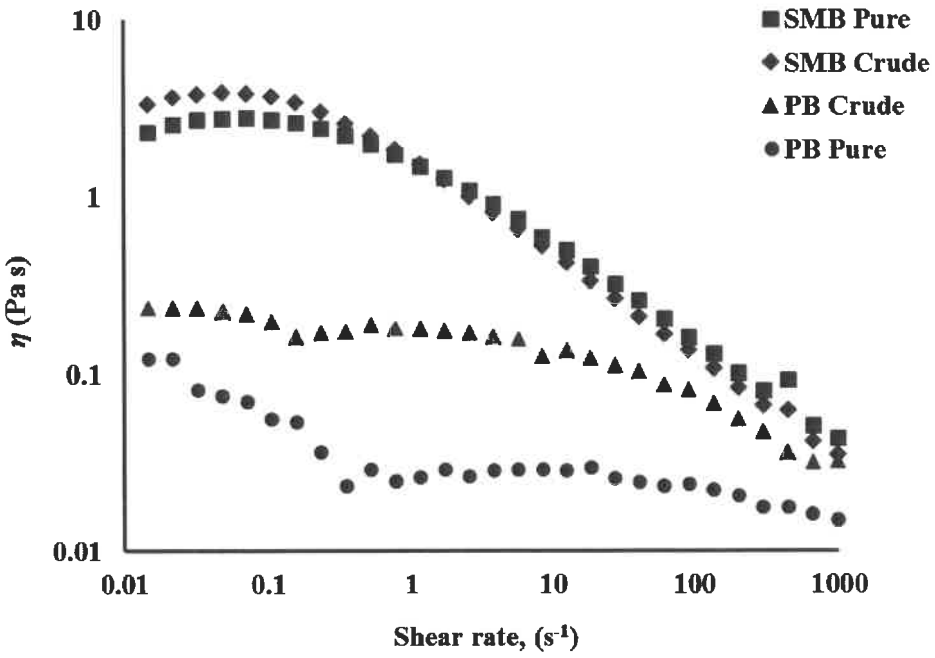
SMB Pure
SMB Crude
PB Crude
PB Pure
10
1
0.1
0.01
η (Pa s)
0.01
0.1
1
10
100
1000
Shear rate, (s-1)

Figure 4

Table 1: Chemical composition of grewia gum samples extracted in different solvents. Means sharing the same letters in a column are non-significant (p>0.05); Values in parenthesis are the standard deviations and in square brackets are mol%. SMB is sodium metabisulphite extract and PB is phosphate buffer extract.

Sample	Protein (g dL^{-1})	Total carbohydrate (g dL^{-1})	Total uronic acids	L-Rha	L-Ara	D-Glc	D-Gal
SMB Crude	16.5 (1.4)^{c}	56.5 (2.3)^{a}	35.1 (0.4)^{c} [45.7]	28.0^{c} [43.9]	2.6^{b} [4.5]	3.8^{d} [5.4]	0.4^{d} [0.6]
SMB Pure	14.5 (1.0)^{b}	65.3 (5.4)^{b}	34.5 (0.1)^{c} [43.5]	28.6^{d} [43.5]	5.5^{d} [9.3]	2.5^{b} [3.4]	0.2^{c} [0.3]
PB Crude	15.5 (1.6)^{bc}	53.4 (3.1)^{a}	18.5 (0.1)^{a} [34.8]	24.7^{b} [24.7]	1.3^{a} [3.3]	2.8^{c} [5.7]	0.1^{a} [0.2]
PB Pure	11.1 (2.4)^{a}	66.9 (4.0)^{b}	27.4 (2.6)^{b} [42.7]	23.5^{a} [44.1]	4.7^{c} [9.8]	1.8^{a} [3.0]	0.2^{b} [0.3]

Table 2: Intrinsic viscosity, Huggins constant, r^2, and *Mw* characteristics of grewia extracts. SMB is sodium metabisulphite extract and PB is phosphate buffer extract.

Sample	$[\eta]$(dL g^{-1})	K_H	r^2	M_w (x10^6 g mol^{-1})
SMB Crude	9.1	1.6	0.99	2.8
SMB Pure	9.6	0.3	0.90	1.7
PB Crude	8.3	0.6	0.79	0.92
PB Pure	6.5	1.0	0.98	0.75