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Research Paper

Structural characterization and thermal behavior of a gum extracted from *Ferula assa foetida* L.

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ABSTRACT

The gum asafoetida, an oleo-gum-resin from root of *Ferula assa foetida*, was extracted through alcoholic procedure followed by water extraction and then biochemically characterized using colorimetric assays, Fourier infrared spectroscopy, gas chromatography coupled to mass spectrometry, and 1D and 2D nuclear magnetic resonance. The gum was mainly composed of carbohydrates (67.39% w/w) with a monosaccharide distribution of 11.5: 5.9: 2.3: 1 between Gal, Ara, Rha and GlcA (molar ratio) and proteins (arabinogalactan protein). The polysaccharide consisted of a (1 → 3)-β-D-galactan backbone ramified predominantly from *O*-6 but also from *O*-4 and *O*-4,6. Side chains included terminal-α-L-Ara*f*, terminal-α-L-*Rha*p, (1 → 3)-α-L-Ara*f*, (1 → 5)-α-L-Ara*f*, terminal-β-D-Gal*p*, β-D-GlcA and traces of (1 → 4)-β-D-GlcA. X-ray diffraction pattern showed a semi crystalline microstructure. Thermal behavior of the gum was evaluated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) revealed temperatures below and upper 200 °C as dominant regions of weight loss.

1. Introduction

Depending on their structural diversity, numerous plant polysaccharides have been extensively studied and identified as novel sources of biological and/or techno-functional agents. Asafoetida gum is an exudate oleo-gum-resin obtained from the root and rhizome of *Ferula assa foetida* L., a perennial herb from Umbelliferae family. *Ferula assa foetida* is a plant with 1.5–2 m tall with fleshy thick roots and yellow flowers that appear in large umbels. The plant body is scraped on the bottom of the root and stem ring at the end of spring resulting in milky exudates extracting during the summer. The exudates oleo-gum-resin dries as exposes to the air and the color changes to yellowish-brown (Zargari, 1996).

The plant originated from Iran and Afghanistan. It is also cultivated in Kyrgyzstan, Turkmenistan, Uzbekistan and India. It is named "Anghouzeh", "Khorakoma" and "Anguzakoma" in Iran (Iranshahy & Iranshahi, 2011). Other names are heltit or tybe (Arabic), a-wei (Chinese), duivelsdrek (Dutch), asafetida (English), asa foetida (French), stinkasant or teufelsdreck (German), hing (Hindi), kama (Pakistan and Afghan) and asafétida (Spanish). Asafoetida is traditionally used as folk phytomedicine and food spice in many countries. Indian people use the exudates as a flavoring spice in curries, meatball and pickles (Mahendra & Bisht, 2012). As an herbal medicine, it is used to cure asthma, nervous disorders, flatulence, intestinal parasites, weak digestion and influenza. In recent years, new therapeutic aspects of the gum-resin, especially antioxidant and cancer chemopreventive properties, have been revealed (Iranshahy & Iranshahi, 2011).

Asafoetida gum is chemically consisting of three main fractions: resin (40–64%), gum (25%) and essential oils (10–17%). The resin fraction includes ferulic acid, coumarin, terpenoids and sesquiterpene coumarins (Kajimoto, Yahiro, & Nohara, 1989). The gum fraction contains glycoproteins and polysaccharides composed of d-galactose, l-arabinose, l-rhamnose and d-glucuronic acid as main monosaccharides (Jones & Thomas, 1961). The volatile fraction is mostly composed of disulfides, monoterpenes, free folic acid, valeric acid and trace of vanillin (Lee et al., 2009; Mahendra & Bisht, 2012). The resin and volatile

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components which are soluble in some organic solvent particularly ethanol and methanol are usually used as the herbal remedy. Jones and Thomas (1961) studied the structure of the polysaccharide using paper chromatography and several derivatizations of monosaccharides obtained from acidic degradation. The authors found that the backbone of polysaccharide is consisting of (1 → 3)-*β*-D-galactopyranose in the main chain and d-galactopyranose, l-arabinofuranose, and l-arabinopyranose residues in the side chains. The side chains also contain d-glucuronic acid, its 4-*O*-methyl ether, l-rhamnose and d-galactose (all in the pyranose form). The polysaccharide is also reported to contain smaller percentage of uronic acids than that of frankincense or myrrh (Jones & Thomas, 1961). This paper deals with the extraction, purification and characterization of the chemical structure of asafoetida gum from both its polysaccharidic and proteic parts.

2. Materials and methods

2.1. Plant material

Oleo-gum-resin of *Ferula assa foetida* L. was obtained in September 2015, from Tabas city, South Khorasan province, Iran. Taxonomic group of the herb was determined at herbarium center of the Natural Resources department in Isfahan University of Technology. The roots were scraped in the summer to collect the gum exudates. Then the exudates were stored at −18 °C freezer until the assays. Initial chemical composition of the oleo-gum-resin was determined. Moisture content was obtained by distillation in toluene, proteins by Kjeldahl method. Total ash content was measured by calcinations at 550 °C (A.O.A.C., 1984). The ashes were dissolved in HNO_3, heated on water bath and passed through paper filter to determine metal cations prior to injection to inductive coupled plasma (ICP) instrument (PerkineElmer OptimaTM 7300DV ICP-OES). Fat content was determined based on A.O.A.C method (A.O.A.C., 2005). Carbohydrate content was determined by difference.

2.2. Extraction and purification

The crude asafoetida gum was air dried and then extracted with ethanol (96% v/v) at 75 °C for 5 h under reflux. The ratio of ethanol to crude gum was 35:1 (w/w) and the solvent was used in two cycles. Ethanol insoluble materials were separated and oven dried at 80 °C. The resulting white powder was mixed with five fold (w/w) of distilled water and was stirred for 30 min at 45 °C, followed by 2 h at room temperature. Water insoluble materials were separated using filtration (cloth filter) and then centrifugation at 1500*g*, 15 min. In order to purify gum, the supernatant was mixed with three volumes of ethanol letting to precipitate overnight at 4 °C. Then, the resulting precipitate was air dried and mixed with five volumes of acetone, pressed three times and dried at 50 °C overnight. The purification step was performed for one more time after resolubilizing gum powder in ultra pure water. The final purified gum powder was milled and kept for structural analysis.

2.3. Composition analysis

The chemical composition of asafoetida gum was analyzed. The moisture content was measured drying the gum at 110 °C, 24 h. Total sugar was determined according to phenol sulfuric method (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956) with glucose as standard. Total uronic acid was colorimetrically quantified by m-hydroxydiphenyl assay using glucuronic acid as standard (Blumenkrantz & Asboe-Hansen, 1973). Amount of sulphate groups was analyzed according to the method published by Dodgson and Price (1962) using K_2SO_4 as a standard and results were expressed in eq. SO_4. Protein content was obtained using a total nitrogen analyzer (TNM-1, Shimadzu). Water soluble proteins were quantified according to microBradford method (Bradford, 1976) using Bovine Serum Albumin as standard. Total phenolic compounds were measured as described by Singleton, Orthofer, and Lamuela-Raventós (1999) using gallic acid as standard.

2.4. Amino acid analysis

Amino acids were analyzed using high pressure ion exchange chromatography equipped with post-column ninhydrin detection (Hitachi L, 8900). Samples were induced to acidic or basic conditions before injection to the system using NaOH 6 M for tryptophan quantification or HCl 5.5 M for the other amino acids (Ursu et al., 2014).

2.5. Determination of molecular weight

High pressure size exclusion chromatography (HPSEC) with on line three detectors; a multi-angle laser light scattering (MALLS) filled with a K5 cell (50 µL) and a He–Ne laser at 690 nm (HELEOS II, Wyatt Technology Corp., USA), a differential refractive index (DRI) (RID 10A, Shimadzu, Japan) and a viscosimeter (viscostar II, Wyatt Technology Corp., USA) was performed to estimate the weight-average molecular weight (M_w), the number-average molecular weight (M_n), the intrinsic viscosity ([η]), the radius of gyration (R_g), the hydrodynamic radius (R_h) and the polydispersity index (PDI = M_w/M_n) of asafoetida gum using a dn/dc of 0.15 mL/g. Columns (OHPAK SB-G guard column, OHPAK SB806 and 804 HQ columns (Shodex)) were eluted with $LiNO_3$ 0.1 M at 0.5 mL/min. Asafoetida gum was solubilized at 2 g/L in LiNO3 (0.1 M) during 24 h under stirring at room temperature and the solution was filtered through a 0.45 µm filter before injection through a 100 µL full loop. All the data were analyzed using the Astra 6.1 software package.

2.6. Monosaccharide composition analysis by GC/MS-EI

Ten mg of polysaccharide were dissolved in 1 mL of TFA in a glass tube followed by heating at 120 °C for 90 min. The sample was mixed each 30 min. The preparation was then evaporated under nitrogen stream. BSTFA: TMCS (99: 1) derivatization was adapted from Pierre et al. (2014). The samples were kept under stirring during 2 h following the evaporation under nitrogen flow. The derivatized products were dissolved in dichloromethane prior to injection to GC/MS. Standards (l-Rha, d-Rib, l-Fuc, l-Ara, d-Xyl, d-Man, d-Gal, d-Glc, d-GlcA, d-GalA, d-GlcN, d-GalN) were prepared following the same procedure. Analyses were carried out by GC/MS-EI using an Agilent 6890 Series GC System coupled to an Agilent 5973 Network Mass Selective detector. Derivatives are analyzed using an OPTIMA-1MS (30 m, 0.32 mm, 0.25 mm) column, under helium flow of 2.3 mL/min. The helium pressure was set to 8.8 psi and the split ratio to 50:1. The temperature was programmed to 100 ° C for 3 min then raised to 200 °C at 8 °C/min keeping for 1 min continuing with a final temperature increment to 215 °C at 5 °C/min. The ionization was carried out by Electronic Impact (EI, 70 eV). The trap temperature was fixed to 150 °C and the target ion was from 40 to 800 *m/z*.

2.7. Glycosyl-linkage composition analysis

Pellets of solid NaOH/DMSO were prepared following the method of Ciucanu and Kerek (1984) and used for the methylation step. The methylation step was adapted from Peña, Tuomivaara, Urbanowicz, O'Neill, and York (2012). The conversion of methylated residues to partially-*O*-methylated alditols acetate (PMAA) derivatives was adapted from the protocols of Blakeney, Harris, Henry, and Stone (1983), Carpita and Shea (1989) and Peña et al. (2012). PMAA derivatives were finally re-suspended into 200 µL of dichloromethane. Analyses were carried out by GC/MS-EI as previously described in section 2.6.

2.8. FT-IR spectroscopy

FT-IR spectra were observed using a VERTEX 70 FT-IR instrument. Samples were dispersed on ATR A225 diamante. The IR spectra were recorded at 15 °C (referenced against air) in the wave range of 400–5000 cm^{-1} with a resolution of 4 cm^{-1} (50 scans). Data were analyzed by OPUS 7.2 software.

2.9. NMR spectroscopy

The dried polysaccharide of asafoetida was dissolved in 99.9% D_2O and freeze-dried in order to replace exchangeable proton with deuterium (this step was repeated 3 times). Polysaccharide was then dissolved in D_2O (50 g/L) prior to NMR analysis. One and two dimensional NMR spectra were recorded on a Brucker Advance 400 (Germany), operating at 400.13 MHz (number of scans = 64) for 1H and 100.61 MHz (number of scans = 8192) for ^{13}C. The spectra of ^{1}H, ^{13}C, homonuclear $^{1}H/^{1}H$ correlation experiments (COSY), heteronuclear multiple-quantum coherence (HMQC) assays were performed at 30 °C. All the chemical shifts were in relative with Me_4Si.

2.10. X-ray diffraction (XRD)

X-ray diffraction was performed using a XRD-6000 diffractometer operating at 40 kV and 40 mA, Cu Kα radiation (λ = 1.54 Å) in the range of 2θ = 10 − 100° with a step size of 0.066845. Crystallinity index was calculated based on equation given by Patterson (1939), using Origin software to calculate as Eq. (1):

$$Crystallinity\ Index = \frac{Crystalline\ area}{(crystalline\ area + amorphous\ area)} \times 100 \quad (1)$$

2.11. Themogravimetric analysis (TGA)

Thermogravimetric analysis was performed using a TA Instrument (TGA 2050, USA). One hundred mg of product (powder) was weighted into platinum pan and the temperature was recorded in the range of 20–500 °C (10 °C/min) under nitrogen atmosphere. Differential of the weight loss (% °C) was reported in order to have a well conception of heat stability.

2.12. Differential scanning calorimetry (DSC)

A TA Instrument (DSC Q2000, USA) was employed to analyze the thermal behavior of the gum during heat flow from −50° to 400 °C at a rate of 10 °C/min under nitrogen atmosphere. The sample (14.5 mg) was sealed into aluminum hermetic pans prior to test. Data was treated using Universal Analysis 2000 software.

3. Results and discussion

3.1. Chemical composition

Chemical composition of crude asafoetida gum exudate was determined. Moisture and ash contents were 29.44% and 2.75%, respectively. These values were considerably high in comparison with oleo-gum-resin from *Ferula gummosa* (Bioss.) that were reported 6.7% for moisture and 1.1% for ashes using similar methods of analysis (Jalali, Ebrahimian, Evtuguin, & Pascoal Neto, 2011). Soluble materials in ethanol which can be considered as resins (non-volatile) and volatile compounds were 40.65% for gum asafoetida. This value is lower than that of oleo-gum-resin from *Ferula gummosa* (Jalali et al., 2011). The insoluble material in ethanol (27.17% w/w) was composed of carbohydrate (≈23.15%) and protein (4.02%, determined by Kjeldhal and considering a conversion factor of 6.25). This oleo-gum-resin contained no extractable lipids using petroleum ether (AOAC method, 2005). As described in methods, ethanol insoluble materials were solubilized in water and then precipitated by ethanol to obtain the purified gum. The yield of purified gum was 27.1% based on dry matter of initial raw material. Table 1 shows the metal cation content of this asafoetida purified gum. Calcium (82.01%) is the predominant mineral followed by magnesium (11.65%), potassium (10.68%) and sodium (5.20%). The variety of metal cations was approximately the same for oleo-gum-resin from two species of *Ferula* (*assa foetida* and *gummosa*) (Jalali et al., 2011).

Table 1
Metal cation composition in asafoetida gum.

Metal cation	Quantity (g/100 g ash)
Ca	66.76
Mg	9.48
K	8.69
Na	4.24
Fe	1.44
Al	1.02
Sr	0.36
Zn	0.15
Mn	0.12
Ba	0.10
Cu	0.05
Ni	0.01
Bi	0.01
Total	92.43

The pure asafoetida gum contained 67.39 (± 1.24, % w/w) of total sugar, a slight amount of uronic acids (5.2 ± 0.20% w/w) and traces of sulphate content (0.17 ± 0.02% w/w). The total protein content obtained from total nitrogen amount was 6.80% (± 0.04, % w/w); while it was estimated around 4.90 (± 0.29, % w/w) for water soluble proteins. This protein content is high compared to some other sources of gum (Anderson, Gill, Jeffrey, & McDougall, 1985; Islam, Phillips, Sljivo, Snowden, & Williams, 1997; Petera et al., 2015; Sims & Furneaux, 2003). For example, gums from acacia species like *A. senegal*, *A. laeta*, *A. seyal* and *A. jennerae* contains, respectively, 0.29, 0.65, 0.14 and 0.11% of total nitrogen in comparison with 1.09% of the asafoetida gum. The presence of few amounts of phenolic compounds (2.16 ± 0.68% w/w) may be due to ethanol soluble materials remained from extraction step. As it was mentioned for crude gum, asafoetida had relatively high ashes content that had been recovered with the water-soluble gum during the purification (4.16 ± 0.07% w/w).

Monosaccharide composition of asafoetida is shown in Table 2. Gal, Ara, Rha and GlcA were detected as the constitutive monosaccharides by GC/MS-EI with a ratio 11.5: 5.9: 2.3: 1, respectively. Based on this monosaccharide composition, the polysaccharide was identified as an arabinogalactan as already reported for similar gum such as those from acacia and albizia specious (Jurasek, Kosik, & Phillips, 1993). Gum asafoetida contained more pentose than gum from *Ferula gummosa* which contained 17% Ara (Jalali et al., 2011). However, the quantity of uronic acids (GlcA) was lower than that of some other arabinogalactans such as Arabic and Ghatti gums (Islam et al., 1997; Kang et al., 2011).

3.2. Molecular weight analysis

Results of SEC MALLS analysis are summarized in Table 2. The average molecular weight (M_w) was higher than that observed by Jalali, Ebrahimian, Evtuguin, & Neto (2011). Average M_w of some samples of gum acacia (*senegal*) were reported to range from 6.22×10^5 to 2.54×10^6 g/mol (Cui, Phillips, Blackwell, & Nikiforuk, 2007). The value of polydispersity index (M_w/M_n) suggested a slight polydisperse nature for the asafoetida gum. It was a higher value in comparison with gum from *A. mearnsii* (PDI = 1.7) (Grein et al., 2013) but lower than gum from *A. senegal* (PDI = 2.2-2.7) (Assaf, Phillips, & Williams, 2005). Theoretical critical concentration (C*) is defined by 1–4/[η]. Here, the

Table 2
Monosaccharide composition and molecular weight of pure asafoetida.

M_w[(a)] (g/mol)	M_n[(b)] (g/mol)	PDI[(c)]	R_h[(d)] (nm)	[η][(e)](mL/g)	Monosaccharides[(f)] (mol%)			
					Gal	Ara	Rha	GlcA
1.5×10^5	8.0×10^4	1.88	6	10.7	58.3	30.0	11.7	5.05

[(a)] M_w: Molecular weight was measured by SEC-MALLS-DRI.
[(b)] M_n: Number average molecular weight was measured by SEC-MALLS-DRI.
[(c)] PDI: Polydispersity index M_w/M_n.
[(d)] R_h: Hydrodynamic radius was measured by SEC-MALLS-DRI.
[(e)] [η]: Intrinsic viscosity was measured by SEC-Visco-DRI.
[(f)] Monosaccharides composition was measured by GC/MS-EI. Gal: Galactose; Ara: Arabinose; Rha: Rhamnose; GlcA: Glucuronic acid. All analyses were run in triplicate and the relative standard deviations are less than 5%.

Table 3
Amino acid composition of pure asafoetida.

Amino acid	mg/100 g
Asp	835.66
Thr	445.42
Ser	687.09
Glu	615.56
Gly	331.82
Ala	392.09
Val	318.22
Ile	334.16
Leu	494.42
Tyr	790.83
Phe	397.88
His	139.76
Lys	313.78
Arg	311.36
Pro	463.25
Total	6871.3

theoretical C* value calculated from this equation could range from 93 to 373 g/L, traducing the Newtonian behavior of the gum in water solution.

3.3. Amino acid analysis

Amino acid composition of gum asafoetida is depicted in Table 3. Fifteen types of amino acids were detected among which Asp (12.2%), Tyr (11.5%), Ser (10.0%) and Glu (9.0%) were the most abundant. This is not in agreement with other arabinogalactans such as gum from *A. jennerae* that have higher proportions of Hyp (13.44%) and Pro (12.0%) comparing to Tyr (1.9%) (Anderson et al., 1985). However, the amount of Asp, Glu and Ser are high similar to Arabic gum (Akiyama, Eda, & Katō, 1984; Anderson & Yin, 1988) due to high protein content. It has been established for Arabic gum from *A. aestivalis* and *A. microbotryd* that major amino acids are Asp and Ser (Anderson et al., 1985). The proteinaceous structure of complex gums could then consist of a mixture of proteins that vary in amino acid composition.

3.4. FT-IR spectroscopic analysis

The FTIR analysis of asafoetida was performed (data not shown). The band in region 3287 cm^{-1} is corresponding to O—H vibration of polysaccharide or associated water. The band in 2924 cm^{-1} region indicates the C—H stretching vibration (Zhao, Kan, Li, & Chen, 2005). The band at 1598.1 cm^{-1} is due to asymmetrical stretching of carboxylate groups of uronic acid residue (Fadavi, Mohammadifar, Zargarran, Mortazavian, & Komeili, 2014)). The band in area 1200 cm^{-1} to 820 cm^{-1} is attributed to specific "fingerprint" of the gum (Cui et al., 2007). Moreover, the band observed in area 820 cm^{-1} to 400 cm^{-1} was due to symmetrical and asymmetrical ring breathing vibration (C—C-O and C—O—C) as suggested by Cui et al. (2007). The wave number at 1030.51 cm^{-1} arose from the C—C function of carbohydrate. The bands lying from 1490 cm^{-1} to 1200 cm^{-1} can be ascribed to amide III functional groups suggesting the presence of protein in the structure of gum as was confirmed by the result of Kjeldal analysis. These data were in agreement with the literature published by Zhou, Sun, Bucheli, Huang, and Wang (2009).

3.5. Glycosyl-linkage analysis

Methylation profile, linkage type and peak area of each partially methylated alditol acetate (PMAA) are presented in Table 4. According to the observed quantities (%), Gal with 53% of total peak area was the most abundant monosaccharide available in the polysaccharide chain that was in good agreement with GC–MS results (see Section 3.1.). Thus, the main chain of polysaccharide consists of →3)-Gal*p*-(1→ linkages with side chains ramified from *O*-6, *O*-4 and/or *O*-4,6 of Gal. The side chains were constituted of Ara with various types of linkages including →3)-Ara*f*-(1→, →5)-Ara*f*-(1→ and traces of →4)-Glc*p*-(1→ which can be attributed to Glc*p*A. Similarly, Glc*p* was ascribed to Glc*p*A as uronic acid. The observed terminal units were T-Ara*f*, T-Gal*p*, T-Rha*p* and T-Glc*p* that presented in the side chain as terminal units. This result is in accordance with Jones and Thomas (1961) that found Gal both in the main chain or branched residue. The galactose linkages found for asafoetida gum is similar to those of other arabinogalactans described in literature such as Arabic (Cornelsen et al., 2015; Grein et al., 2013; Nie et al., 2013) and Ghatti gums (Kang et al., 2011).

^{1}H NMR and ^{13}C NMR assignments are depicted in Fig. 1A and B. It is clear from this figure that signals related to anomeric carbon are located in the range δ 100–110 ppm for ^{13}C NMR and δ 4.2-5.4 ppm for ^{1}H NMR spectra.

2D NMR was used in order to uncover the details of overlapped peaks and correlations (Fig. 1C and D). The peak area δ 4.3-4.5 ppm in ^{1}H NMR included two anomeric signals of β-Gal that can be tracked in

Table 4
Methylation analysis of the polysaccharide extracted from *Ferula assa-foetida*.

Partially *O*-methylated alditol acetate[a]	%[b]	Linkage type[c]
2,3,5-Me_3-Ara	22.7	Ara*f*-(1→
2,3,4,6-Me_4-Gal	6.9	Gal*p*-(1→
2,3,4,6-Me_4-Glc[d]	5.3	Glc*p*-(1→
2,3,4-Me_3-Rha	10.8	Rha*p*-(1→
2,5-Me_2-Ara	4.6	→3)-Ara*f*-(1→
2,3-Me_2-Ara	3.6	→5)-Ara*f*-(1 →
2,4,6-Me_3-Gal	3.1	→3)-Gal*p*-(1→
2,3,6-Me_3-Glc[d]	trace	→4)-Glc*p*-(1→
2,6-Me_2-Gal	10.5	→3,4)-Gal*p*-(1→
2,4-Me_2-Gal	23.0	→3,6)-Gal*p*-(1→
2-Me-Gal	9.5	→3,4,6)-Gal*p*-(1→

[a] 2,3,5-Me_3-Ara = 2,3,5-tri-*O*-methylarabitolacetate.
[b] % of peak area of *O*-methylated alditol acetates relative to total area, determined by GC–MS.
[c] Based on derived *O*-methylated alditol acetates.
[d] from Glc*p*A and 4-Me-Glc*p*A units.

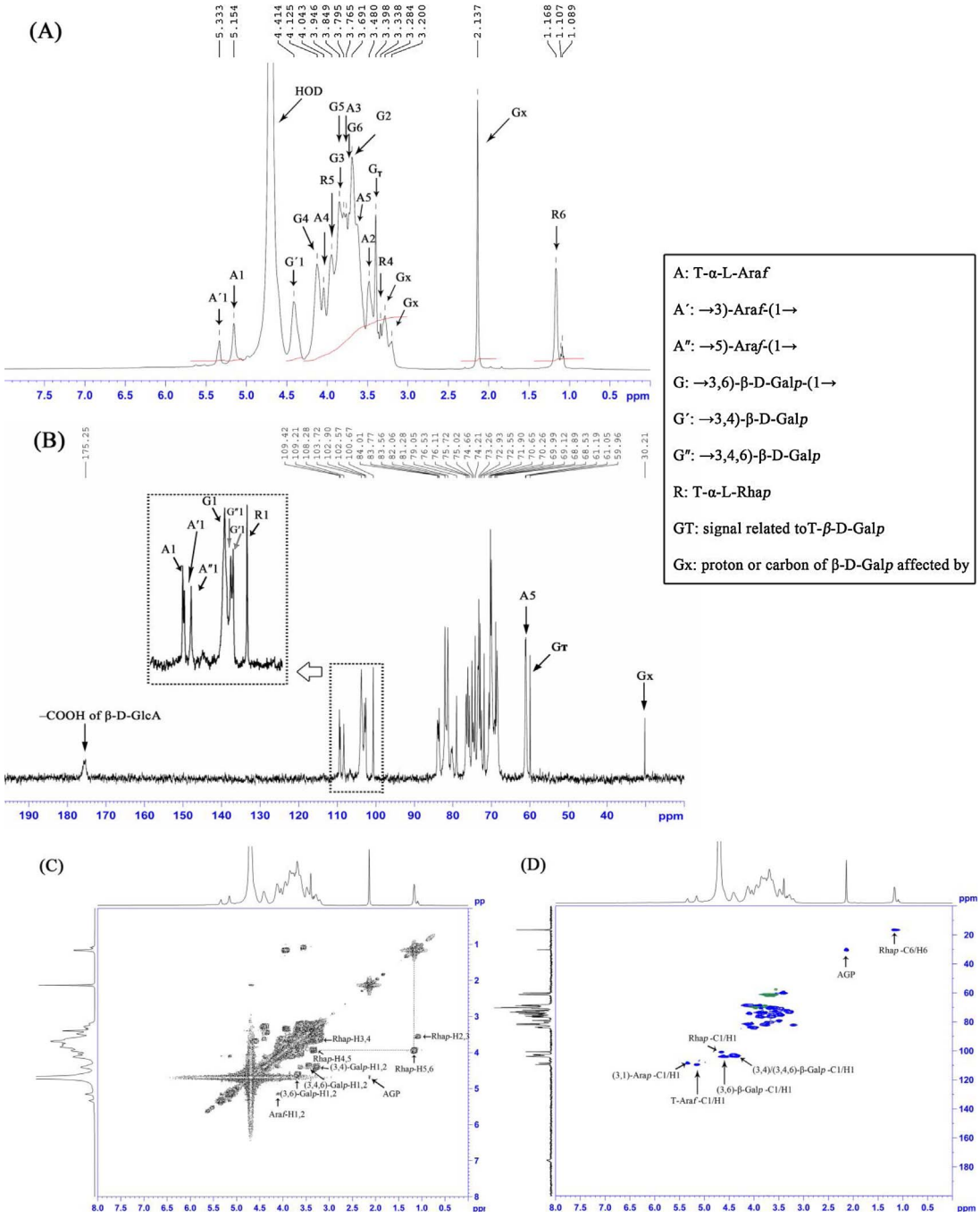

Fig. 1. (A) ^{1}H NMR, (B) ^{13}C NMR, (C) $^{1}H/^{1}H$ COSY and (D) $^{13}C/^{1}H$ HSQC spectra of asafoetida polysaccharide. Analyses were recorded at 50 g/L in D_2O.

$^{1}H/^{1}H$ COSY and $^{13}C/^{1}H$ HSQC spectra. The correlations of carbon signals at δ 102.57 ppm and δ 102.9 ppm with proton signals approximately at δ 4.3-4.5 ppm in HSQC were contributed to anomeric carbons of →3,4), and →3,4,6) of *β*-D-Gal*p*, respectively. The signals ranging δ 4.5–5 ppm of ^{1}H NMR were masked by HDO signal but correlation of δ 3.69/4.6 ppm (H-1/H-2) in the COSY and 103.72/4.6 in the HSQC were evident of anomeric carbon of →3,6)-*β*-D-Gal*p*-(1→. It can be perceived that $^{13}C/^{1}H$ correlations of δ 103.72/4.60, 70.26/3.69, 73.26/3.85, 82.06/4.12, 72.93/3.80 and 69.12/3.72 ppm were assigned to C-1/H-1, C-2/H-2, C-3/H-3, C-4/H-4, C-5/H-5 and C-6/H-6 of →3,6)-*β*-D-Gal*p*-(1→ (Nie et al., 2013). Furthermore, less intensive signals in the ^{13}C NMR spectrum were identified at δ 69.99, 76.53, 75.02, 74.66, 61.19 as well as 102.57 ppm corresponding, respectively, to C-2, C-3, C-4, C-5, C-6 and C1 of →3,4)-Gal*p*-(1→ (Petera et al., 2015). Our observation about T-α-L-Ara*f* was the correlations at δ 109.42/5.15, 79.05/3.48, 76.11/3.76, 84.01/4.04 and 59.96/ ≈ 3.62 ppm in the HSQC allocated respectively to C-1/H-1, C-2/H-2, C-3/H-3, C-4/H-4 and C-5/H-5 (Nie et al., 2013). Two less intensive signals appeared at δ 109.21 and δ 108.28 ppm of ^{13}C NMR spectrum. They were assigned to C-1 of →3)-Ara*f*-(1→ and →5)-Ara*f*-(1→, respectively, and correlated with H-1 signal at δ 5.33 ppm (Cornelsen et al., 2015). Another identified monosaccharide was T-α-L-Rha*p* that correlations at δ 100.67/ ≈ 4.62, 70.65/ ≈ 3.56, 71.90/ ≈ 3.58, 74.21/3.34, and 68.89/3.95 ppm were attributed to C-1/H-1, C-2/H-2, C-3/H-3, C-4/H-4 and C-5/H-5 respectively. A signal was detected at δ 1.17 ppm of ^{1}H NMR and approximately δ 17 ppm of ^{13}C NMR which can be ascribed to H-6/C-6 methyl group of Rha*p* (Cui et al., 2007). The signal at δ 175.25 ppm was attributed to −COOH group of uronic acid

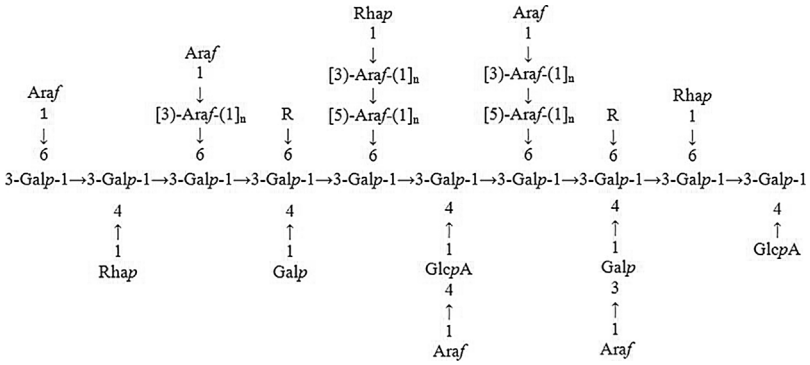

Fig. 2. Structure proposed for the asafoetida polysaccharide.

(Grein et al., 2013).

In the COSY spectrum a correlation of protons was observed at δ 2.14/4.7 ppm could be assigned to β-Gal residue influenced by proximity of protein in a arabinogalactan (Matulová, Capek, Kaneko, Navarini, & Liverani, 2011) as observed in $^{13}C/^{1}H$ HSQC spectrum at δ 30.21/2.14 ppm. Moreover, the proton signals at δ 3.2 and δ 3.8 ppm that correlated with carbon signals at δ 83.56 and δ 72.55 ppm, respectively in HSQC spectrum might be indicative of β-Gal*p* units influenced by peptides. The correlation at δ 3.28/4.41 ppm in COSY spectrum led to the same conclusions.

Taking into account the results of linkage type and NMR analysis, a possible structure of asafoetida polysaccharide is suggested in Fig. 2:

Where R may be one of the following groups: T-Ara*f*, T-Rha*p*, →2)-Ara*p*-(1→, →3)-Ara*p*-(1→, →5)-Ara*p*-(1→, T-Gal*p*, →3)-Gal*p*-(1→, T-Glc*p*A, →4)-Glc*p*A-(1→. Gal are β-D-Gal and Ara/Rha are α-L-Ara/Rha.

3.6. XRD analysis

The XRD pattern of asafoetida gum showed a small sharp peak at 2θ = 17.39 and a broad amorphous plateau (Fig. 3). The crytallinity index was calculated as 15.74%, hence the gum sample has a semi crystalline microstructure with more amorphous nature. Rezaei, Nasirpour, and Tavanai (2016), indicated that the crystallinity of almond gum is related to water solubility of each gum fraction. Authors reported the crystallinity index of water insoluble, water soluble and whole almond gum fractions to be 36.33%, 24.27% and 26.46%, respectively.

3.7. Thermal behavior

TGA thermograms of gum asafoetida are given in Fig. 4A. Thermal treatment caused the powder weight to decrease in two main steps: the first one occurred at temperatures below 200 °C, resulting from water desorption and the second occurred upper 200 °C due to degradation of gum structure (A line in Fig. 4A). Moreover, the DTG curve related to differential weight loss demonstrates the decomposition rate (B line in

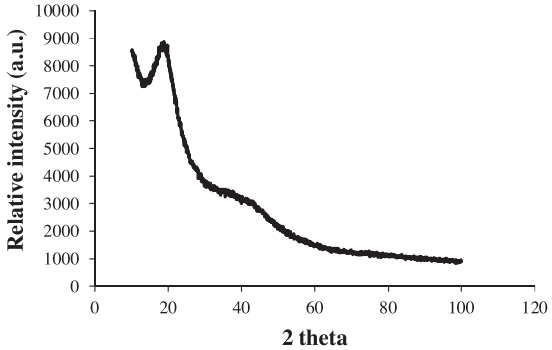

Fig. 3. X-ray diffraction pattern of asafoetida gum.

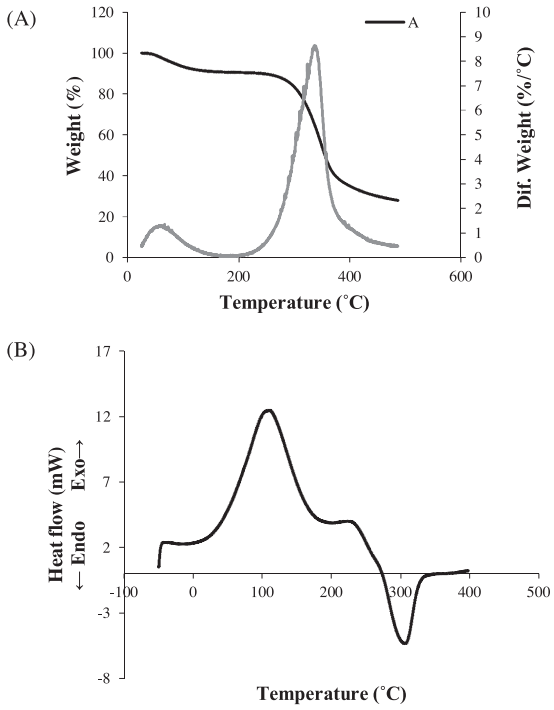

Fig. 4. Thermal behavior of asafoetida gum: (A) TGA (A line) and DTG (B line) thermogram; (B) DSC thermogram.

Fig. 4A). In this respect, rate of weight loss showed a sharper peak (around 90.4% weight reduction) related to the main thermal degradation resulting from calcination of the sample. Our data were in accordance with findings of Cozic, Picton, Garda, Marlhoux, and Le Cerf (2009) about gum acacia that defined the temperatures below and above 200 °C as the main regions of weight decreasing phenomenon. Mothé and Rao (2000) assigned temperatures below 100 °C and above 252 °C as the two distinctive weight reductions of gum acacia and cashew gum. Fadavi et al. (2014) found that 40–140 °C corresponding to dehydration and 250–300 °C to decomposition of the Zedo gum.

The DSC thermogram confirms the result obtained from TGA analysis (Fig. 4). The heating flow started a mild rising by increasing the temperature to 20 °C, and then reached to its highest level at around 110 °C. The endothermic peak at 110 °C is corresponding to water desorption and the exothermic peak which was observed in the range of 225 °C to 331 °C is due to dehydration, depolymerization and pyrolitic decomposition (Zohuriaan & Shokrolahi, 2004). No T_g was detected due to overlapping with endothermic peak. Some parameters including particle size, moisture content and nature of the gums respect to their functional groups are recognized to be effective on DSC pattern of a polymer (Mothé & Rao, 2000; Zohuriaan & Shokrolahi,2004).

4. Conclusion

According to our study, the gum from asafoetida was composed of 67.39% total sugar and 6.8% protein. Based on GC–MS and 1D and 2D NMR, the chemical structure of polysaccharide was proposed. The core residue was composed of (1 → 3,6)/(1 → 3,4)/(1 → 3,4,6) of β-D-galactose with 23%, 10.5% and 9.5%, respectively. The structure of side chain elucidates the presence of T-α-L-Araf, T-α-L-Rhap, (1 → 3)-α-L-Araf, (1 → 5)-α-L-Araf, T-β-D-Galp, β-D-GlcA and low amount of (1 → 4)-β-D-GlcA. Protein including Asp, Tyr, Ser and Glu as the most abundant amino acids was attached to polysaccharide residue from β-Gal side. Consequently, asafoetida was proposed to have an arabinogalactan-protein structure. XRD assessment showed quite a small peak suggesting a semi crystalline microstructure with more amorphous matter. According to TGA and DSC results temperatures below 200 °C, with the most intensity at 110 °C, corresponded to water desorption and temperatures higher than 200 °C, mostly in the range of 225–331 °C, was responsible to structural degradation weight loss.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2017.10.096.

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