

Received:  
20 August 2015  
Revised:  
9 January 2016  
Accepted:  
3 February 2016

Heliyon (2016) e00076



# Partial structural characterization of pectin cell wall from *Argania spinosa* leaves

Kadda Hachem<sup>a,b,\*</sup>, Yasmina Benabdesslem<sup>b</sup>, Samia Ghomari<sup>c</sup>,  
Okkacha Hasnaoui<sup>b</sup>, Meriem Kaid-Harche<sup>a</sup>

<sup>a</sup> Laboratoire des Productions, Valorisations Végétales et Microbiennes, Département de Biotechnologie, Université des Sciences et de la Technologie d'Oran, BP 1505, El Mnaouar, 31000 Oran, Algeria

<sup>b</sup> Département de Biologie, Faculté des Sciences, Université Docteur Moulay Tahar de Saida, BP 138 cité ENNASR, 20000 Saida, Algeria

<sup>c</sup> Faculté des Sciences, Université de Djillali Liabès Sidi-Bel-Abbès, BP 89, 22000 Sidi-Bel-Abbès, Algérie

\* Corresponding author at: Département de Biologie, Faculté des Sciences, Université Docteur Moulay Tahar de Saida, BP 138 cité ENNASR, 2000 Saida, Algeria.

E-mail address: [kadda46@hotmail.com](mailto:kadda46@hotmail.com) (K. Hachem).

## Abstract

The pectin polysaccharides from leaves of *Argania spinosa* (L.) Skeels, collected from Stidia area in the west coast of northern Algeria, were investigated by using sequential extractions and the resulting fractions were analysed for monosaccharide composition and chemical structure. Water-soluble pectic (ALS-WSP) and chelating-soluble pectic (ALS-CSP) fractions were obtained, de-esterified and fractionated by anion-exchange chromatography and characterized by sugar analysis combined with methylation analysis and <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy. The data reveal the presence of altering homogalacturonan (HG) and rhamnogalacturonan I (RG-I) in both pectin fraction. The rhamnogalacturonan I (RG-I) are consisted of a disaccharide repeating unit [ $\rightarrow \alpha$ -D-GalpA-1,2- $\alpha$ -L-Rhap-1,4  $\rightarrow$ ] backbone, with side chains contained highly branched  $\alpha$ -(1  $\rightarrow$  5)-linked arabinan and short linear  $\beta$ -(1  $\rightarrow$  4)-linked galactan, attached to O-4 of the rhamnosyl residues.

Keywords: Biochemistry, Applied sciences, Plant biology, Natural product chemistry, Biological sciences

## 1. Introduction

Argan tree (*Argania spinosa* (L.) Skeels), also known as “iron tree,” is a woody species belonging to the Sapotaceae family and is endemic to Algeria and Morocco (Peltier, 1983; Baumer and Zeraia, 1999). Its geographic distribution covers a relatively large area of southwestern Algeria in northern Tindouf, where it is the second most common tree after *Acacia radianna* (Kaabèche et al., 2010). Some feet Argan tree is also observed in the west coast of northern Algeria (Stidia area). The argan tree is important for ecological sustainability. The woodlands protect against soil erosion and desertification owing to their deep-growing roots, they shade different types of crops, and help maintain soil fertility in arid zones (Sebaa and Kaid Harche, 2014). The argan tree also supports indigenous populations economically since almonds are used to produce argan oil (Morton and Voss, 1987), which is largely used for cooking and believed to have various medicinal properties (e.g., decreasing cholesterol level, stimulation of vascular circulation). Argan oil is also widely incorporated in many cosmetic products (Charrouf and Guillaume, 1998; Charrouf et al., 2002).

Plant cell walls are known to be potential sources of pharmacologically active polysaccharides (Gloaguen and Krausz, 2004; Paulsen, 2002). Recently, pectins, a group of anionic polysaccharides that are used in traditional pharmaceutical applications have attracted a lot of attention and have been subjected to extensive structural study (Paulsen and Barsett, 2005). Pectins are polydisperse macromolecules having high heterogeneity in terms of molecular mass and chemical structure. Their composition is affected by their origin, localization within the plant, and the extraction method used to obtain them. Pectins have a complex structure (Ridley et al., 2001), consist of three major classes namely, homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). The fine structures of the pectin occupy a biological role(s) in the cell wall. First, pectin lend strength and support to a plant and yet are very dynamic structures, especially rhamnogalacturonan type I (RG-I) which is found in the primary cell wall and provides structural support (Harholt et al., 2010). Second, pectin influences various cell wall properties such as porosity, surface charge, pH, and ion balance and therefore is of importance to the ion transport in the cell wall (Mcneil et al., 1984). Furthermore, pectin oligosaccharides are known to activate plant defense responses: they elicit the accumulation of phytoalexin which has a wide spectrum of anti-microbial activity (Hahn et al., 1981; Nothnagel et al., 1983). Finally, pectin oligosaccharides induce lignification (Robertsen, 1986) and accumulation of protease inhibitors (Bishop et al., 1984) in plant tissues.

In this work we describe the chemical composition and structural features of pectic polysaccharides isolated from argan leaves growing in the west coast of northern Algeria (*Stidia aera*).

## 2. Materials and methods

### 2.1. Plant material

The leaves of *Argania spinosa* (L.) Skeels used in this study were collected from *Stidia aera* (Latitude 35° 49' N; Longitude 0° 02' E), west coast of northern Algeria. After collection, leaves were dried in a ventilated oven (40 °C), ground (particle size <200 µm) and stored in desiccators at room temperature.

### 2.2. Cell wall preparation

Fifty grams of milled powder were subjected to two successive extractions with 50:50 ethanol-toluene solutions at room temperature for 14 h. The supernatant was discarded to remove debris, cytoplasmic organelles, and starch granules. The residue was filtered through a blotting cloth and mixed with ethanol 80% by continuous stirring for 2 h to remove any traces of toluene. Then, the residue was washed three times with distilled water and acetone solution, dried in a ventilated oven at 60 °C and weighed (Harche et al., 1991).

### 2.3. Cell wall fractionation

Each dried residue was subjected to extraction with ethanol 80% at 90 °C for 20 min. The residue was dissolved in distilled water, and the supernatant was recovered by centrifugation. The insoluble material was washed twice with distilled water at 100 °C for 2 h to obtain water-soluble pectin (ALS-WSP). The remaining residue was treated with 1% ethylenediaminetetraacetic acid (EDTA) solution at 80 °C for 6 h to obtain chelating soluble pectin (ALS-CSP). All extracts was filtered through a porous glass frit (Porosity 3) and transferred to pre-soaked dialysis tubing (Spectra/Por; molecular weight cutoff 6,000–8,000 Da). Then it was precipitated with ethanol solution (3 volumes), centrifuged and finally lyophilized.

### 2.4. Analytical methods

#### 2.4.1. Gas chromatography

The composition of neutral monosaccharides was determined from their alditol acetates on a Hewlett-Packard 5890A gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA), equipped with a polar-fused silica capillary column (30 m × 0.53 mm) and a flame ionization detector (FID) coupled to a

Hewlett-Packard 3395 integrator. Column temperature was held at 195 °C for 4 min and ramped at 2.5 °C·min<sup>-1</sup> to 225 °C and held for 3 min with a constant flow of 4 ml·min<sup>-1</sup> nitrogen carrier gas. The injector and detector temperature were 260 °C and 280 °C, respectively. Quantification of monosaccharides was conducted using myo-inositol as an internal standard against a mixed standard solution of monosaccharides (rhamnose, fucose, xylose, arabinose, mannose, galactose, and glucose) (Selvendran et al., 1979).

#### 2.4.2. Colorimetric method

The content of uronic acid was estimated using the assay of Blumenkrantz and Asboe-Hansen, (1973). The absorbance was read at 520 nm on a Beckman DU 640 spectrophotometer (Beckman Coulter, Corona, CA, USA).

#### 2.4.3. Chromatography method

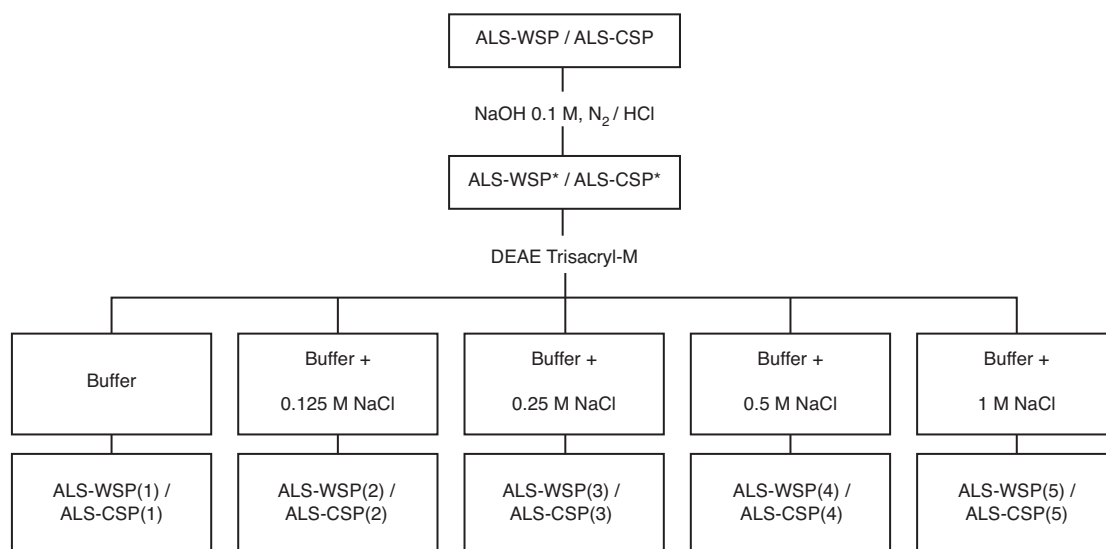
Fractionation of pectin was performed by ion exchange chromatography. A 400 mg pectin sample was de-esterified using 0.1 M sodium hydroxide solution overnight at 4 °C under nitrogen. The solution was neutralized with 1 M (pH 5.0) hydrochloric acid to obtain the acid form of pectin. Extract was dissolved in approximately 100 ml of 0.05 M phosphate buffer (pH 6.3) and applied to a DEAE-Trisacryl M column (2 × 20 cm). Fractions were eluted at a constant flow of 30 ml·h<sup>-1</sup> with 300 ml of 0.05 M phosphate buffer and then eluted three more times with 300 ml of 0.05 M phosphate buffer containing 0.25 M, 0.5 M, and 1 M sodium chloride, respectively. Five fractions were collected for each extract, dialyzed against distilled water, and lyophilized. DEAE-Trisacryl M column was regenerated with 0.5 M sodium hydroxide solution (Fig. 1).

#### 2.4.4. Methylation analysis

Prior to methylation analysis, the carboxyl groups of the uronic acids were reduced according to the method of Taylor and Conrad, (1972). Then the polysaccharides samples were methylated twice using sodium hydroxide and methyl iodide in dry dimethyl sulfoxide, according to the method described by Hakomori, (1964). Permethylated polysaccharides were hydrolyzed with 2 N trifluoroacetic acid at 100 °C for 3 h. Subsequently, they were converted into their corresponding alditol acetates by successive treatments with sodium borohydride and were acetylated with acetic anhydride in the presence of pyridine that acts as a catalyst.

#### 2.4.5. Mass spectrometry

Permethylated polysaccharides were characterized by a gas chromatograph (HP-Agilent 6850) using a 530 µm capillary column (SP 2380). The injector



**Fig. 1.** Ion-exchange chromatography fractionation of (ALS-WSP) and (ALS-WSP).

temperature is 260 °C with a constant flow of 4 ml·min<sup>-1</sup> nitrogen carrier gas. Column temperature was held at 165 °C for 4 min and ramped at 2.5 °C·min to 225 °C and held for 3 min. After separation in capillary column, methylated derivatives were analyzed by a mass spectrometer (Agilent 5975C, Agilent technologies Inc., Santa Clara, CA, USA).

#### 2.4.6. Nuclear magnetic resonance (NMR) spectroscopy

Samples of 15 mg were dissolved in 0.5 ml deuterated water (NMR <sup>1</sup>H: 5 mg in 0.5 ml solvent, NMR <sup>13</sup>C: 15 mg in 0.5 ml solvent, tube diameter of 5 mm) and NMR spectra were recorded on a Bruker Avance 400 spectrometer equipped with a quadruple nucleus probe at room temperature or at 60 °C, at frequency 400.13 MHz for the <sup>1</sup>H and 100.62 MHz for <sup>13</sup>C. Chemical shifts δ were expressed in ppm relative to tetramethylsilane.

### 3. Results and discussion

#### 3.1. Yield and composition of monosaccharides

Monosaccharide extraction yield and composition of different polysaccharide fractions, determined by GC as alditol acetates, are presented in Table 1. These results revealed that the largest extract (ALS-CSP) was solubilized by EDTA (11%), the fractions solubilized by distilled water (ALS-WSP) corresponded to 5.5% of the dried material. ALS-WSP and ALS-CSP were rich in arabinose (45.3% and 75%, respectively), uronic acid (51.4% and 67.9%, respectively) and the neutral sugars were present in varying amounts.

**Table 1.** Monosaccharide yield and composition of water soluble ALS-WSP and chelating soluble ALS-CSP pectins obtained by gas chromatography.

Fractions	Yield <sup>a</sup>	Monosaccharide composition <sup>c</sup>							
		UA <sup>b</sup>	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
ALS-WSP	5.5	51.4	25.5	–	45.3	1.5	0.5	21	6
ALS-CSP	11	67.9	6	5	75	1.5	–	6	6.5

<sup>a</sup>Percentage weight of the 15 g starting leaf powder (dry weight).

<sup>b</sup>Percentage weight of fraction dry weight.

<sup>c</sup>Percentage of peak area relative to total peak areas, determined by GC.

The presence of arabinose, galactose and rhamnose suggested the lack of homogalacturonans in most of the polysaccharide fractions. Thus the high Arabinose content relative to Galactose suggests the presence of side chains of arabinan and/or arabinogalactan, while the ration of Rha/AU reported for ALS-WSP (0.20) and ALS-CSP (0.06) fractions indicating a predominance of rhamnogalacturonan-I. According to [Voragen and Schols, \(1996\)](#), the Rhamnogalacturonan-I can be distinguished from homogalacturonans by its ratio Rha/GalA that ranges from 0.05 to 1. These results do not exclude the possibility of the presence of rhamnogalacturonan type II as well.

### 3.2. Fractionation

After removal of the salts, 74.5% of ALS-WSP<sup>+</sup> and 55% of ALS-CSP<sup>+</sup> were recovered. The percentages of ALS-WSP and ALS-CSP fractions obtained with different eluting solutions are given in [Table 2](#). These results showed that ALS-WSP and ALS-CSP fractions appear as mixtures and sub-fractions contain acidic polysaccharides. However, each of them was characterized by the

**Table 2.** Mass yield of water soluble ALS-WSP and chelating soluble ALS-CSP pectins obtained by ion-exchange chromatography.

Elution solutions	Fractions <sup>a</sup>	
	ALS-WSP	ALS-CSP
(1) Buffer	7	4
(2) Buffer + 0.125 M NaCl	17	7
(3) Buffer + 0.25 M NaCl	35.5	31.5
(4) Buffer + 0.5 M NaCl	7	9
(5) Buffer + 1 M NaCl	8	3.5
Total:	74.5	55

Buffer: Phosphate buffer (0.05 M, pH 6.3).

<sup>a</sup>Expressed as % of 400 mg ALS-WSP and ALS-CSP.

dominance of a single sub-fraction. Most of the sub fractions were eluted with 0.25 M NaCl with total percentage of ALS-WSP(3) and ALS-CSP(3) to be 35.5% and 31.5%, respectively.

### 3.3. Study of ALS-WSP and ALS-CSP

#### 3.3.1. Content of monosaccharides

Monosaccharide compositions of ALS-WSP(3) and ALS-CSP(3) are presented in Table 3. ALS-WSP(3) and ALS-CSP(3) were acidic and contained approximately 27% and 25.4% uronic acid, respectively. They also had significant amounts of arabinose (64% and 74% in ALS-WSP(3) and ALS-CSP(3), respectively). The presence of rhamnose and galactose (on an average, 17.2% and 11.2% in ALS-WSP(3) and ALS-CSP(3), respectively) were suggesting that the chains of arabinan and galactan on the rhamnogalacturonan skeleton are connected.

#### 3.3.2. Methylation analysis

Methylation analysis of ALS-WSP(3) and ALS-CSP(3) was performed in the carboxy-reduced sample due to the presence of galacturonic acid. The results given in Table 4 showed similar composition in both fractions, the main observed derivatives were those of arabinose, (52.7% and 60.4% respectively) which was in agreement with monosaccharide analysis. The presence of 6,6-d2-2,3,6-Me<sub>3</sub>-Gal-ol acetates (25.6% and 19% respectively) in approximately equal amount to the sum of the 3-Me-Rha and 3,4-Me<sub>2</sub>-Rha-ol acetates (15.5% and 10.6% respectively), indicated that (39.5 and 44.2% respectively) of galacturonic acid residues were involved in galacturonan blocks and (60.5 and 55.8% respectively) in rhamnogalacturonan blocks. Among the rhamnose residues, 45% and 40% respectively were substituted by arabinan or galactan side-chains (McNeil et al., 1980). The presence of high amounts of 2,3-Me<sub>2</sub>-Ara-ol acetate, indicating the presence of (1 → 5)-linked Araf units.

**Table 3.** Monosaccharide composition of water soluble ALS-WSP(3) and chelating soluble ALS-CSP(3) pectins.

Fractions	Yield <sup>a</sup>	Monosaccharide composition <sup>c</sup>							
		UA <sup>b</sup>	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
ALS-WSP(3)	35.5	27	21.7	–	64	2	–	12.5	–
ALS-CSP(3)	29.5	25.4	12.7	–	74	1.5	–	10	1

<sup>a</sup> Percentage weight of the 400-mg AL-WSP(3) and AL-CSP(3) eluted with 0.25 M NaCl.

<sup>b</sup> Percentage weight of fraction dry weight.

<sup>c</sup> Percentage of peak area relative to total peak areas, determined by GC.

**Table 4.** Partially methylated alditol acetates of water soluble ALS-WSP(3) and chelating soluble ALS-CSP(3) pectins.

Partially O-methylalditol acetate	ALS-WSP(3) <sup>a</sup>	ALS-CSP(3) <sup>a</sup>	Mode of linkage
2,3,5-Me <sub>3</sub> -Ara <sup>b</sup>	17.3	18.4	Araf-(1 →
2,5-Me <sub>2</sub> -Ara	–	–	→ 3)-Araf-(1 →
2,3-Me <sub>2</sub> -Ara	21.3	23.8	→ 5)-Araf-(1 →
2-Me-Ara	10.7	14.5	→ 3,5)-Araf-(1 →
3-Me-Ara	3.4	3.7	→ 2,5)-Araf-(1 →
3,4-Me <sub>2</sub> -Rha	8.5	6.4	→ 2)-Rhap-(1 →
3-Me-Rha	7.0	4.2	→ 2,4)-Rhap-(1 →
2,3,4,6-Me <sub>4</sub> -Gal	3.0	4.9	Galp-(1 →
2,3,6-tri-Me <sub>3</sub> -Gal	3.2	5.1	→ 4)-Galp-(1 →
6,6-d2-2,3,6-Me <sub>3</sub> -Gal <sup>c</sup>	25.6	19.0	→ 4)-Galp-A-(1 →

<sup>a</sup> Percentage of peak area of O-methylalditol acetates relative to total area, determined by GC-MS.

<sup>b</sup> 2,3,5-Me<sub>3</sub>-Ara = 2,3,5-tri-O-methylarabinitolacetate, etc.

<sup>c</sup> The carboxy-reduced GalpA.

The presence of 3-Me- and 2-Me-Ara-ol acetates indicated that this arabinan was branched at O-2 and O-3, respectively. The presence of 3-Me-Rha-ol acetates indicated that some rhamnose residues were at O-4 position of the side chains (Voragen et al., 1995; Ridley et al., 2001). These results also indicated that 36.5% and 33.8% respectively of rhamnose units were substituted. The side chains contained long arabinan chains (52.7 and 60.4% respectively) and short galactan chains (6.2% and 10% respectively) (Ros et al., 1998; Habibi et al., 2004); however, longer galactose chains as compared to arabinose side chains were also obtained (Yapo et al., 2007; O'Donoghue and Somerfield, 2008; Vriesmann and Petkowicz, 2009). Analysis revealed the presence of two galactose monomers, 2,3,6-Me<sub>3</sub>-Gal and 2,3,4,6-Me<sub>4</sub>-Gal-ol acetates, both in the same proportion. We assume that these two galactose units are (1 → 4) linked on the same side chain and given the no detection of other Me-Gal-ol acetates residues which indicates the absence of arabinogalactan side chain, already detected in other rhamnogalacturonan (do Nascimento et al., 2015; Leivas et al., 2015). Several arabinose monomers were also identified in variable percentages: 2,3,5-Me<sub>3</sub>-Ara (32.8% and 30.5% respectively), 2,3-Me<sub>2</sub>-Ara (40.4% and 39.4% respectively), Me<sub>2</sub>-Ara (20.3% and 24% respectively), and Me<sub>3</sub>-Ara-ol acetates (6.5% and 6.1% respectively). These results showed that 68.2% and 69.5% respectively of arabinose monomers were (1 → 5) linked on the same side chain. This suggests that the arabinan side chain may be mainly composed of (1 → 5) linked arabinose units, which can be substituted at the O-3 and/or O-2 position by other arabinofuranose units and, as a result, arabinan units are (1 → 3) linked to oligoarabinoses (Cardoso et al., 2002; Habibi et al., 2005).



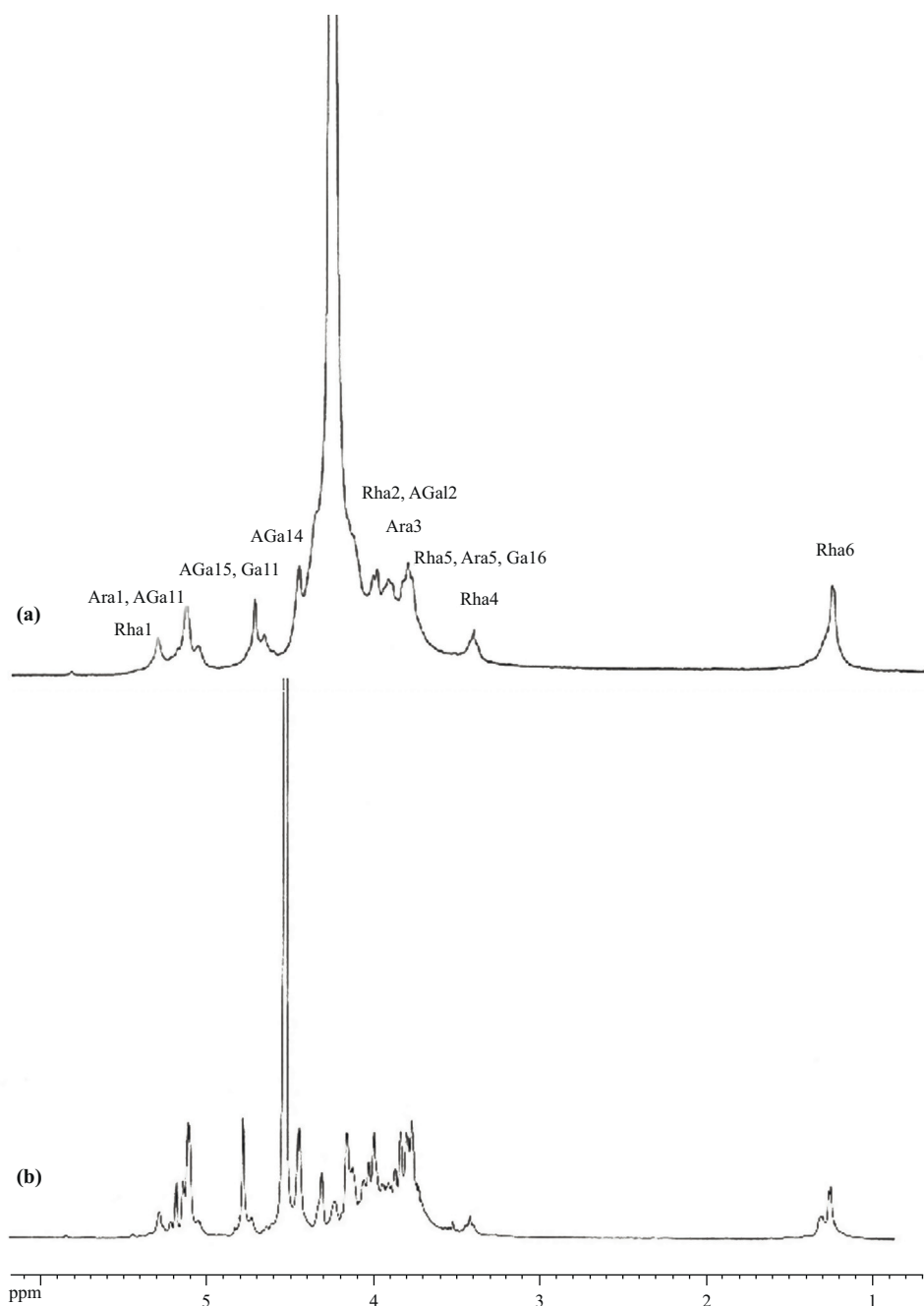
These data confirms the presence of type I rhamnogalacturonan with side chains contained highly branched  $\alpha$ -(1  $\rightarrow$  5)-linked arabinan and short linear  $\beta$ -(1  $\rightarrow$  4)-linked galactan, attached to O-4 of the rhamnosyl residues.

### 3.3.3. Study by NMR spectroscopy

Fig. 2 and Fig. 3 represent NMR spectra  $^1\text{H}$  and  $^{13}\text{C}$  pectic ALS-WSP(3) and ALS-CSP(3) fractions having the common characteristics of alternating homogalacturonan and rhamnogalacturonan I blocks which can be substituted by short galactan and arabinan side-chains. The chemical shift assignment was performed by comparison with spectral data already published on the structural characterization of pectins (Vriesmann and Petkowicz, 2009; Habibi et al., 2005; Rondeau-Mouro et al., 2008; Yu et al., 2010). The signals detected in the spectra correspond to the pectins and more specifically to 1,5 arabinan, 1,4 galactan, 1,4 galacturonan and 1,2 rhamnose.

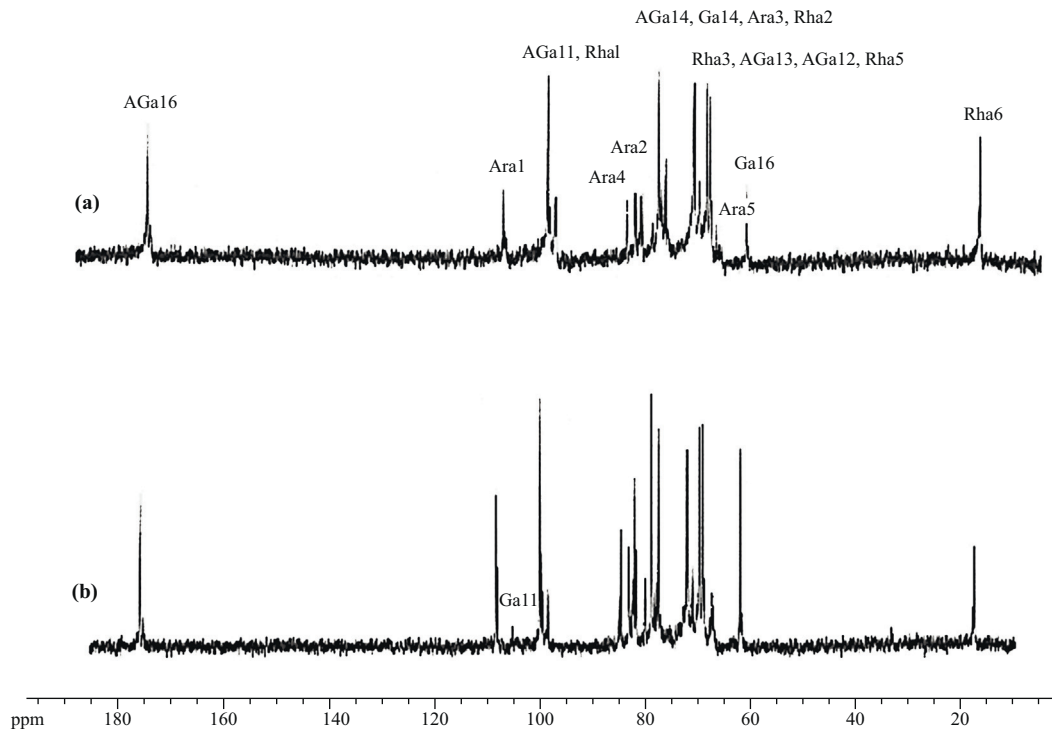
The  $^1\text{H}$  spectrum showed great similarity as already observed in the case of CASF3 (Habibi et al., 2005). The region for anomeric signals in Fig. 2 contained at least four signals at 5.27, 5.17, 5.12, 5.05 and 4.66 ppm for ALS-WSP (3) and at 5.29, 5.19, 5.12, 5.05 and 4.62 ppm for ALS-CSP (3) were assigned, to  $\alpha$ -(1  $\rightarrow$  2) rhamnopyranosyl,  $\alpha$ -(1  $\rightarrow$  5) arabinofuranosyl,  $\beta$ -(1  $\rightarrow$  4) galactopyranosyl and  $\alpha$ -(1  $\rightarrow$  4)-linked galactopyranosyl acid, respectively. We can identify two doublets centered at 1.28 and 1.37 ppm, 1.28 to 1.33 ppm for the ALS-WSP(3) and the ALS-CSP(3) fraction, respectively and which are attributed, respectively, to the H-6 rhamnose residues. The signal at 1.28 ppm corresponds to the units only connected at (1  $\rightarrow$  2) to a galacturonic acid. The signal at 1.33 and 1.37 ppm correspond to the rhamnose units linked by (2  $\rightarrow$  1) bearing a galacturonic acid O-4 branching.

In the  $^{13}\text{C}$  spectrum (Fig. 3), we can see for the ALS-WSP(3) and the ALS-CSP(3) fraction, a several characteristic signals of arabinose residues linked by (1  $\rightarrow$  5) at 108.17, 82.99, 78.04, 84.67, 67.52 ppm and at 108.51, 83.35, 78.27, 84.92, 67.57 ppm assigned to C-1 – C-5, respectively and confirming the presence of an arabinan-like structure as side-chain. We noticed the presence in the anomeric regions of the characteristic signals of rhamnose and galacturonic acid residues involved in rhamnogalacturonan blocks at 99.69, 98.14 ppm and 100.05, 98.56 ppm assigned to (1  $\rightarrow$  2)-linked rhamnose and (1  $\rightarrow$  4)-linked galacturonic acid residues, respectively. The signal at 175.87, 175.28 ppm and 175.81, 175.17 was characteristic of carboxyl functional groups of (C6) (1  $\rightarrow$  4)-linked galacturonic acid and galacturonic acid (1  $\rightarrow$  2)-linked rhamnose residues, respectively. While the signal at 17.22 and 17.56 ppm was characteristic of (C6) methyl groups of rhamnose residues, respectively.



**Fig. 2.**  $^1\text{H}$  NMR spectra (300.0 K, 400.13 MHz) of (a) ALS-WSP(3) and (b) ALS-CSP(3).

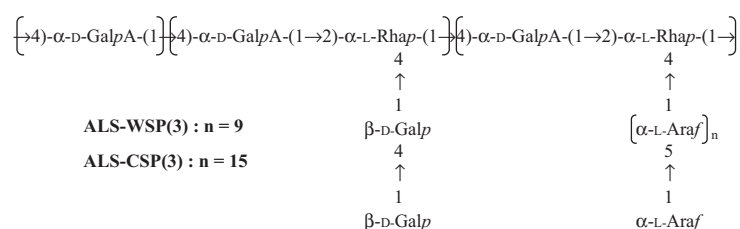
The galactan side-chains were characterized by the minor signals at 105.33, 77.79 and 62.17 ppm assigned to C-1–C-4–C-6, respectively for the ALS-CSP(3) fraction. At 77.37 and 61.75 ppm assigned to C-4–C-6, respectively For the ALS-WSP(3) fraction. The signal at 105 for C-1 of  $\beta - (1 \rightarrow 4)$  galactopyranosyl did not appear.



**Fig. 3.**  $^{13}\text{C}$  NMR spectra (300.0 K, 100.62 MHz) of (a) ALS-WSP(3) and (b) ALS-WSP(3).

The presence of arabinose and galactose in ALS-WSP(3) and ALS-CSP(3) that was detected by NMR and methylation analysis revealed two lateral arabinan and galactan branches that were separate and differed in the length of strings (arabinose strings were longer than those of galactose). In order to estimate the relative importance of the side chains to the rhamnogalacturonan backbone. The arabinose to galactose ratio is an estimation of the proportions of Ara versus Gal-rich side chains (Leivas et al., 2015). The calculated ratio was 5.0 for ALS-WSP and 8.0 for ALS-CSP demonstrating that ALS-CSP contained large arabinan side chains than ALS-WSP (16 and 10 units, respectively).

The structure of rhamnogalacturonan type I is the same in ALS-WSP(3) and ALS-CSP(3) with minor variations in arabinan side chains (Fig. 4). These results are in agreement with previous studies reported in some leaves up



**Fig. 4.** Schematic structure of ALS-WSP(3) and ALS-WSP(3).

to now, in *Mesembryanthemum crystallinum* (M'sakni et al., 2006), in two plants endemic to central Africa, *Fleurya aestuans* (Linnaeus) Miquel and *Phragmenthera capitata* (Spreng) (Aboughe Angone et al., 2009) and in the medicinal tree *Cola cordifolia* (Austarheim et al., 2014) in which rhamnogalacturonan type I structure is variable.

#### 4. Conclusion

From the foregoing results it can be concluded that ALS-WSP(3) and ALS-CSP (3) can be considered as models of the pectin structure synthesized in the cell wall argan tree leaves collected from *Stidia aera* in the west coast of northern Algeria. These fractions have similar type with altering homogalacturonan and rhamnogalacturonan I blocks which can be substituted by short galactan and large arabinan side chains attached to O-4 of the backbone rhamnose units.

It is known that certain cell wall polysaccharide of pectin nature has pharmacological activities such as antibacterial, antiviral, antitumor and immune activities, which have not yet been determined in the cell walls of the leaves of *Argania spinosa* (L.) Skeels. Studies are currently conducted to investigate these possibilities.

#### Declarations

##### Author contribution statement

Kadda Hachem: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yasmina Benabdesslem, Samia Ghomari, Okkacha Hasnaoui: Contributed reagents, materials, analysis tools or data.

Meriem Kaid-Harche: Conceived and designed the experiments.

##### Funding statement

This research was funded by Dr. Moulay Tahar University of Saida, Algeria.

##### Competing interest statement

The authors declare no conflict of interest.

##### Additional information

No additional information is available for this paper.

## Acknowledgments

The authors gratefully acknowledge Pr. Redouane Borsali, director of the Research Center on Plant Macromolecules (C.E.R.M.A.V – UPR5301 – France) for welcoming us and to Gérard Chabmat, who has retired, for his help during our study.

## References

- Aboughe Angone, S., Bardor, M., Nguema-Ona, E., Rihouey, C., Ishii, T., Lerouge, P., Driouich, A., 2009. Structural characterization of cell wall polysaccharides from two plant species endemic to central Africa, *Fleurya aestuans* and *Phragmenthera capitata*. Carbohydr. Polym. 75, 104–109.
- Austarheim, I., Christensen, B.E., Aas, H.T.N., Thöle, C., Diallo, D., Paulsen, B.S., 2014. Chemical characterization and complement fixation of pectins from *Cola cordifolia* leaves. Carbohydr. Polym. 102, 472–480.
- Baumer, M., Zeraia, L., 1999. La plus continentale des stations de l'Arganier en Afriquedu nord. Rev. For. Fr. 51, 446–452.
- Bishop, P.D., Pearce, G., Bryant, J.E., Ryan, C.A., 1984. Isolation and characterization of the proteinase inhibitor-inducing factor from tomato leaves. Identity and activity of poly- and oligogalacturonide fragments. J. Biol. Chem. 259, 13172–13177.
- Blumenkrantz, N., Asboe-Hansen, G., 1973. New method for quantitative determination of uronic acids. Anal. Biochem. 54, 484–489.
- Cardoso, S.M., Silva, A.M.S., Coimbra, M.A., 2002. Structural characterisation of the olive pomace pectic polysaccharide arabinan side chains. Carbohydr. Res. 337, 917–924.
- Charrouf, Z., Guillaume, D., 1998. Ethnoeconomical, ethnomedical, and phytochemical study of *Argania spinosa* (L.) Skeels. J. Ethnopharmacol. 67, 7–14.
- Charrouf, Z., Guillaume, D., Driouich, A., 2002. The Argan tree, an asset for Morocco. Biofutur 220, 54–57.
- do Nascimento, G.E., Corso, C.R., Werner, M.F.P., Baggio, C.H., Iacomini, M., Cordeiro, L.M.C., 2015. Structure of an arabinogalactan from the edible tropical fruit tamarillo (*Solanum betaceum*) and its antinociceptive activity. Carbohydr. Polym. 116, 300–306.
- Gloaguen, V., Krausz, P., 2004. Plant polysaccharides: a biologically active class of molecules. SOFW Journal 130, 20–26.

- Habibi, Y., Heyraud, A., Mahrouz, M., Vignon, M.R., 2004. Structural features of pectic polysaccharides from the skin of *Opuntia ficus-indica* prickly pear fruits. *Carbohydr. Res.* 339, 1119–1127.
- Habibi, Y., Heyraud, A., Mahrouz, M., Vignon, M.R., 2005. Arabinan-rich polysaccharides isolated and characterized from the endosperm of the seed of *Opuntia ficus-indica* prickly pear fruits. *Carbohydr. Polym.* 60, 319–329.
- Hahn, M.G., Darvill, A.G., Albersheim, P., 1981. Host-pathogen interactions. XIX. The endogenous elicitor, a fragment of a plant cell wall Polysaccharide that elicits phytoalexin accumulation in soybeans. *Plant Physiol.* 68, 1161–1169.
- Hakomori, S., 1964. Rapid permethylation of glycolipids and polysaccharides catalysed by methylsulfinyl carbanion in dimethylsulfoxide. *J. Biochem.* 55, 205–208.
- Harche, M., Tollier, M.T., Monties, B., Catesson, A.M., 1991. Caractérisation comparée des constituants (polyosides, lignines et acides phénoliques) des parois cellulaires de trois graminées subdésertiques pérennes: *Stipa tenacissima*, *Lygeum spartum* et *Aristida pungens*. *Cell. Chem. Technol.* 25, 11–17.
- Harholt, J., Suttangkakul, A., Scheller, H.V., 2010. Biosynthesis of pectin. *Plant Physiol.* 153 (2), 384–395.
- Kaabèche, M., Benkheira, A., de Foucault, B., 2010. L'arganeraie d'Algérie: Structure, écologie, syntaxonomie, dynamique. *Acta Bot. Gallica* 157, 563–572.
- Leivas, C.L., Iacomini, M., Cordeiro, L.M.C., 2015. Structural characterization of a rhamnogalacturonan I-arabinan-type Iarabinogalactan macromolecule from starfruit (*Averrhoa carambola* L.). *Carbohydr. Polym.* 121, 224–230.
- M'sakni, N.H., Majdoub, H., Roudesli, S., Picton, L., Le Cerf, D., Rihouey, C., Morvan, C., 2006. Composition, structure and solution properties of polysaccharides extracted from leaves of *Mesembryanthemum crystallinum*. *Eur. Polym. J.* 42, 786–795.
- McNeil, M., Darvill, A.G., Albersheim, P., 1980. Structure of Plant Cell Walls: X. Rhamnogalacturonan I, a structurally complex pectic polysaccharide in the walls of suspension-cultured sycamore cells. *Plant Physiol.* 66, 1128–1134.
- Mcneil, M., Darvill, A.G., Fry, S.C., Albersheim, P., 1984. Structure and function of the primary-cell walls of plants. *Annu. Rev. Biochem.* 53, 625–663.
- Morton, J.F., Voss, G.L., 1987. The Argan Tree (*Argania sideroxylon*, Sapotaceae), A Desert Source of Edible Oil. *Econ. Bot.* 41, 221–233.

- Nothnagel, E.A., McNeil, M., Albersheim, P., Dell, A., 1983. Host-pathogen interactions XXII: A galacturonic acid oligosaccharide from plant cell walls elicits phytoalexins. *Plant Physiol.* 71, 916–929.
- O'Donoghue, E.M., Somerfield, S.D., 2008. Biochemical and rheological properties of gelling pectic isolates from buttercup squash fruit. *Food Hydrocolloid.* 22, 1326–1336.
- Paulsen, B.S., 2002. Biologically active polysaccharides as possible lead compounds. *Phytochem. Rev.* 1, 379–387.
- Paulsen, B.S., Barsett, H., 2005. Bioactive pectic polysaccharides. *Adv. Polym. Sci.* 186, 69–101.
- Peltier, J.P., 1983. Les séries de l'arganeraie steppique dans le Souss (Maroc). *Ecol. Mediterr.* 9, 77–88.
- Ridley, B.L., O'Neill, M.A., Mohnen, D., 2001. Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry* 57, 929–967.
- Robertsen, B.B., 1986. Elicitors of the production of lignin-like compounds in cucumber hypocotyls. *Physiol. Mol. Plant P.* 28, 137–148.
- Rondeau-Mouro, C., Bléon, A., Lahaye, M., 2008. Caractérisation par RMN des biopolymères d'origine végétale, de la molécule à l'organisation supramoléculaire. *C. R. Chim.* 11, 370–379.
- Ros, J.M., Schols, H.A., Voragen, A.G.J., 1998. Lemon albedo cell walls contain distinct populations of pectic hairy regions. *Carbohydr. Polym.* 37, 159–166.
- Sebaa, H.S., Kaid Harche, M., 2014. Anatomical structure and ultrastructure of the endocarp cell walls of *Argania spinosa* (L.) Skeels (Sapotaceae). *Micron* 67, 100–106.
- Selvendran, R.R., March, J.F., Ring, S.G., 1979. Determination of aldoses and uronic acid content of vegetable fiber. *Anal. Biochem.* 96, 282–292.
- Taylor, R.L., Conrad, H.E., 1972. Stoichiometric depolymerization of polyuronides and glycosaminoglycuronans to monosaccharides following reduction of their carbodiimide-activated carboxyl groups. *Biochemistry* 11, 1383–1388.
- Voragen, A.G.J., Pilnik, W., Thibault, J.F., Axelos, M.A.V., Renard, C.M.G.C., 1995. Food Polysaccharides and Their Applications. In: Stephen, A.M. (Ed.), Marcel Dekker, New York.

Voragen, A.G.J., Schols, H.A., 1996. Pectins and pectinases. Elsevier Science BV, Amsterdam, pp. 3–19.

Vriesmann, L.C., Petkowicz, C.L., 2009. Polysaccharides from the pulp of cupuassu (*Theobroma grandiflorum*): Structural characterization of a pectic fraction. *Carbohydr. Polym.* 77, 72–79.

Yapo, B.M., Robert, C., Etienne, I., Wathelet, B., Paquot, M., 2007. Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. *Food Chem.* 100, 1356–1364.

Yu, L., Zhang, X., Li, S., Liu, X., Sun, L., Liu, H., Iteku, J., Zhou, Y., Tai, G., 2010. Rhamnogalacturonan I domains from ginseng pectin. *Carbohydr. Polym.* 79, 811–817.