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Artemisia absinthium and *Artemisia vulgaris*: A comparative study of infusion polysaccharides

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ABSTRACT

The aerial parts of *Artemisia absinthium* and *Artemisia vulgaris* are used in infusions for the treatment of several diseases. Besides secondary metabolites, carbohydrates are also extracted with hot water and are present in the infusions. The plant carbohydrates exhibit several of therapeutic properties and their biological functions are related to chemical structure. In this study, the polysaccharides from infusions of the aerial parts of *A. absinthium* and *A. vulgaris* were isolated and characterized. In the *A. absinthium* infusion, a type II arabinogalactan was isolated. The polysaccharide had a Gal:Ara ratio of 2.3:1, and most of the galactose was (1→3)- and (1→6)-linked, as typically found in type II arabinogalactans. In the *A. vulgaris* infusion, an inulin-type fructan was the main polysaccharide. NMR analysis confirmed the structure of the polymer, which is composed of a chain of fructosyl units β-(2←1) linked to a starting α-D-glucose unit.

1. Introduction

Artemisia absinthium and *Artemisia vulgaris* belong to the Asteraceae family and are known and marketed throughout the world for their medicinal properties. The aerial parts of these plants are used in traditional medicine as infusions, to which anthelmintic, antibacterial, antipyretic, cytostatic, stomachic and antitumor actions have been attributed (Blagojevic, Radulovic, Palic, & Stojanovic, 2006; Kordali, Cakir, Mavi, Kilic, & Yildirim, 2005; Lorenzi & Matos, 2008).

Several low molar mass compounds have been identified in *A. absinthium* and *A. vulgaris*, such as sesquiterpene lactones, lignans, flavonoids and monoterpenes (Aberham, Cicek, Schneider, & Stuppner, 2010; Govindaraj, Kumari, Cioni, & Flamini, 2008; Lopes-Lutz, Alviano, Alviano, & Kolodziejczyk, 2008) which are considered the main active compounds of these plants (Gilani & Janbaz, 1995; Khan & Gilani, 2009; Lee et al., 2004). However, when an infusion is prepared, numerous types of low molar mass products are extracted along with macromolecular compounds. Polysaccharides are one of the main macromolecular components of infusion extracts because they are the predominant components of the plant cell wall and are also present as reserve compounds in plant tissues (Reid, 1997). The infusion is prepared with hot water which allows the extraction of reserve and structural polysaccharides present in the herbs. Structural polysaccharides from the cell wall are usually categorized as pectins, hemicelluloses and cellulose, based on their extractability. Pectins and arabinogalactans-proteins (AGPs) are water-soluble polysaccharides and can be extracted using aqueous solutions (Fincher, Stone, & Clarke, 1983; Reid, 1997).

Starch is the most abundant reserve carbohydrate in plants (Jobling, 2004) and one of the most widespread alternatives to starch as reserve carbohydrate are the oligomers and polymers of fructose, the fructans (Hendry, 1993). These polymers are also readily soluble in water (Edelman & Jefford, 1968), and can also be extracted by the infusion process.

Recently, plant polysaccharides have attracted a great deal of attention for their industrial and biological applications because of their structural variability, broad spectrum of properties and relatively low toxicity (Schepetkin & Quinn, 2006).

Artemisia species used in traditional medicine have shown to contain water-soluble polysaccharides with a wide variety of biological properties. It was demonstrated that a water-soluble carbohydrate fraction isolated from *Artemisia iwayomogi* can modulate the functional differentiation of bone marrow-derived dendritic cells (Lee et al., 2008), and showed immunomodulating and antitumor activities in mice (Koo et al., 1994). Polysaccharide fractions from *Artemisia tripartita* exhibited potent phagocyte immunomodulatory activity, ROS scavenging and complement-fixing activity (Xie et al., 2008).

No reports were found describing the composition of the polysaccharides from the aerial parts of *A. absinthium L.* and

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A. vulgaris L. In the present study, the polysaccharides present in the infusions from aerial parts of *A. absinthium* and *A. vulgaris* were isolated and characterized.

2. Experimental

2.1. Materials

Dried aerial parts of *A. vulgaris* were kindly supplied by Laboratório Santos Flora Comércio de Ervas Ltda, São Paulo, Brazil (lot number ARTER01/0310) and those of *A. absinthium* were purchased (Hubert Comércio de Produtos Alimentícios Ltda, São Paulo, lot number LOSNR01/0109). According to these companies, the plants were cultivated in southern Brazil.

2.2. Isolation of polysaccharides

The infusion of aerial parts from *A. vulgaris* and *A. absinthium* was prepared according to the traditional medicine method: one cup (200 ml) of boiling water was added to one teaspoon of herb (1.0 g for *A. absinthium* and 1.4 g for *A. vulgaris*) (Lorenzi & Matos, 2008). The material was infused until it reached 40 °C and then filtered. Each extract was concentrated and treated with ethanol (4:1 v/v). The material was kept at 4 °C overnight and then the polysaccharide pellets were isolated by centrifugation (8000 rpm, 20 min), washed two times with ethanol and dried under vacuum. The *A. absinthium* and *A. vulgaris* infusions were performed several times to obtain enough material for chemical characterization.

2.3. Purification of polysaccharide from A. absinthium infusion

The crude polysaccharide from *A. absinthium* infusion (named AIP) was dissolved in an appropriate volume of distilled water and dialyzed for two days against distilled water (cut-off M_w 12,000 Da). The retained portion was concentrated and freeze-dried. The product was dissolved in water and submitted to freeze-thawing until no more precipitate appeared. The soluble fraction was treated with amylase, and the starch-free fraction was submitted to ultrafiltration using a 30 kDa membrane followed by filtration through a 0.1 μm membrane. The eluate yielded a purified polysaccharide (AIP-F1).

2.4. Colorimetric methods

Total carbohydrate was assayed by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith 1956) using galactose as standard and protein by the Bradford method (1976), using BSA as standard. Uronic acid was estimated by the m-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973) using glucuronic acid as standard. The amounts of fructose and fructose-yielding carbohydrates were estimated by a ketose-specific modification of the anthrone method described by Pollock (1982) based on the method of Jermyn (1956) using inulin as a standard.

2.5. Neutral monosaccharide composition

Polysaccharides were hydrolyzed with trifluoroacetic acid (2 mol/l) in boiling water for 5 h. The hydrolyzate was evaporated to dryness and the residues were reduced with $NaBH_4$ (Wolfrom & Thompson, 1963b) and acetylated with pyridine–acetic anhydride (1:1, v/v, 1 h, at 100 °C) (Wolfrom & Thompson, 1963a). The resulting alditol acetates were examined by gas chromatography (GC) using a THERMO Trace GC Ultra gas chromatograph equipped with a Ross injector and a DB-225 capillary column (0.25 mm internal diameter × 30 m). The flame ionization detector and injector temperatures were 300 °C and 250 °C, respectively. The oven temperature was programmed from 100 to 220 °C at a rate of 40 °C/min with helium as the carrier gas (1.0 ml/min).

2.6. High-performance size-exclusion chromatography (HPSEC)

The isolated polysaccharides were analyzed by HPSEC using a Waters unit coupled to a refractive index (RI), a Wyatt Technology Dawn-F multi-angle laser light scattering (MALLS) detector and a Pharmacia LKB Uvicord VW 2251 ultraviolet (UV) detector used in 280 nm. Four Waters Ultrahydrogel columns (2000; 500; 250; 120) were connected in series and coupled to the multidetection instrument. A solution of 0.1 mol/l $NaNO_2$ and 0.02% NaN_3 was used as eluent at a flux of 0.6 ml/min. Prior to the analyses, the samples (1.0 mg/ml) were filtered through a 0.22 μm cellulose acetate membrane. The data were collected and analyzed by a Wyatt Technology ASTRA program. All the analyses were carried out at 25 °C. The refractive index increment of the solvent–solute solution with respect to a change in solute concentration (dn/dc) was determined using a Waters 2410 differential refractometer. The average molecular mass (M_w) was calculated using Wyatt Technology ASTRA software.

2.7. Nuclear magnetic resonance spectroscopy (NMR)

The ^{13}C NMR spectra were obtained from samples in D_2O at 50 °C using a Bruker DRX 400 Avance spectrometer. Chemical shifts are expressed in δ (ppm) relative to acetone δ (30.2).

2.8. Fourier transform infrared (FT-IR)

The FT-IR spectra of purified fraction AIP-F1 was recorded on a BOMEM MB-100 FT-IR spectrometer. The dried sample was ground with potassium bromide powder and pressed into a pellet for spectrometric measurement in the frequency range of 4000–400 cm^{-1}.

2.9. Carboxy-reduction of fraction AIP-F1

The carboxyl groups of the uronic acid residues of AIP-F1 were reduced to their corresponding alcohols by $NaBH_4$, using the carboxydiimide method (Anderson and Stone, 1985; Taylor & Conrad, 1972), to give a reduced polysaccharide fraction (API-CX). The reduction of uronic acid residues was measured by a colorimetric assay (Blumenkrantz & Asboe-Hansen, 1973). API-CX was hydrolyzed in 2 mol/l trifluoroacetic acid in boiling water for 5 h. The solution was evaporated and the monosaccharides were reduced and acetylated as above. The resulting alditol acetates were analyzed by gas chromatography–mass spectrometry (GC–MS).

2.10. Methylation analysis

The AGP linkage analysis was done on carboxyl-reduced polysaccharide (API-CX). The fraction was methylated according to the method of Kvernheim (1987), using butyllithium (15% in hexane) in DMSO-MeI, under a nitrogen atmosphere. The per-O-methylated product was first hydrolyzed with formic acid (90%) in boiling water for 1 h and then with trifluoroacetic acid (2 mol/l) for an additional hour. The hydrolyzate was evaporated to dryness, and the residues were reduced and acetylated to give a mixture of partially O-methylated alditol acetates.

2.11. Gas chromatography–mass spectrometry (GC–MS)

GC–MS was performed using a 3800 Varian gas chromatograph linked to a 2000 R-12 Varian Ion-Trap mass spectrometer, with helium as carrier gas (1 ml/min). A capillary column

(30 m × 0.25 mm internal diameter) of DB-225 was held at 50 °C during injection and then programmed at 40 °C/min to 210 °C (constant temperature).

GC was performed using a THERMO Trace GC Ultra gas chromatograph equipped with a Ross injector and a DB-225 capillary column (0.25 mm internal diameter × 30 m). The flame ionization detector and injector temperatures were 300 °C and 250 °C, respectively. The oven temperature was programmed from 100 to 220 °C at a rate of 40 °C/min with helium as the carrier gas (1.0 ml/min).

2.12. Periodate oxidation

AIP-F1 was oxidized in 0.05 mol/l $NaIO_4$ at room temperature (25 °C) and in the dark for 72 h. The reaction was stopped with 1,2-ethanediol and then the solution was dialyzed for 48 h. The resulting polyaldehydes were reduced with $NaBH_4$, neutralized with acetic acid and dialyzed for 48 h. The polyalcohol was submitted to total acid hydrolysis, and the products were analyzed as alditol acetates by GC as described above.

2.13. Fructose detection and chromatographic analyses of fructans

To verify the presence of fructose in *A. vulgaris* infusion polysaccharides, the sample was hydrolyzed in 10 mmol/l H_2SO_4 (pH 2.0) in boiling water for 15 min. The hydrolyzate was neutralized with $BaCO_3$ and the insoluble material was filtered. The monosaccharides were analyzed by high performance liquid chromatography (HPLC) and thin-layer-chromatography (TLC). After hydrolysis and neutralization, the sample from *A. vulgaris* was applied to the origin of a silica gel TLC plate (Macherey-Nagel). The plate was developed three times in 1-butanol/2-propanol/water (3:12:4, v/v/v) at room temperature. The compounds were visualized by spraying with urea-phosphoric acid reagent, a ketose-specific stain (Sims, Cairns, & Furneaux, 2001; Wise, Dimler, Davis, & Rist, 1955). The monosaccharides were also analyzed by HPLC using a Shimadzu system (Japan) equipped with a CBM-10A interface module, CTO-10A column oven, LC-10AD pump and with a RID-10A refractive index detector. A Supelcogel Pb column (30 cm × 7.8 mm) (Supelco e USA) and Supelcogel Pb pre-column (5 cm × 4.6 mm) were used. The HPLC-column was eluted with water at a flow rate of 0.5 ml/min at 80 °C.

3. Results and discussion

3.1. Polysaccharides from the infusion of the aerial parts from A. absinthium

Polysaccharides from the aerial parts of *A. absinthium* were isolated from a traditionally prepared infusion (Lorenzi & Matos, 2008). The crude polysaccharide yield from the *A. absinthium* infusion (AIP) was 5.8%, which represents 58 mg of polysaccharide in each cup of infusion. The sample displayed a polymodal elution profile by HPSEC (data not shown) and was submitted to several steps of purification as shown in Fig. 1. Initially, the sample was dialyzed (12 kDa cut off), and this was followed by a freeze-thawing step. After the removal of the insoluble material, the soluble part was subjected to treatment with α-amylase followed by ultrafiltration using 30 kDa and then 0.1 μm membranes. The fraction obtained after ultrafiltration using the 0.1 μm membrane was named AIP-F1 and had a yield of 6.0%, based on the crude polysaccharide obtained from the *A. absinthium* infusion.

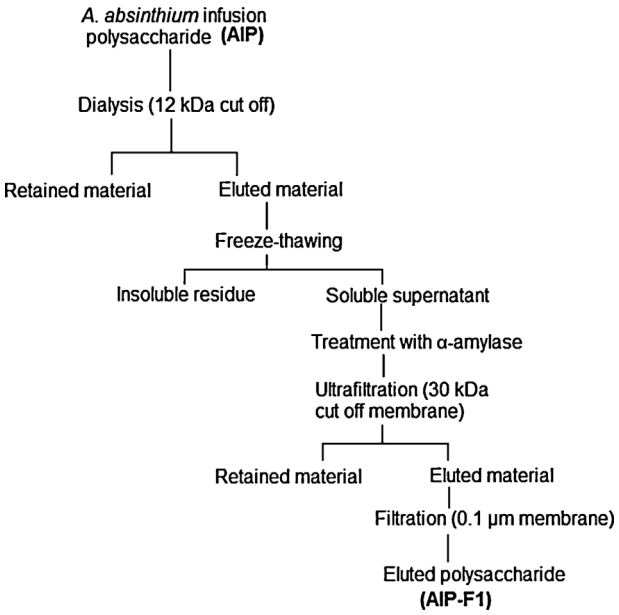

Fig. 1. Purification scheme of polysaccharides from the infusion of aerial parts from *A. absinthium*.

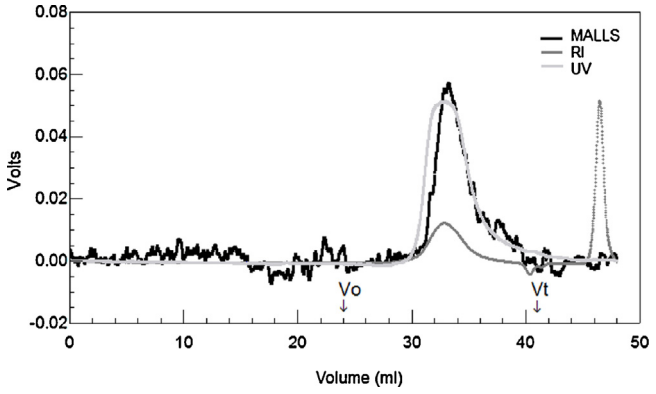

Fig. 2. Elution profile of AIP-F1 obtained by HPSEC-MALLS/RI/UV.

3.2. Characterization of fraction AIP-F1

Fraction AIP-F1 showed a monomodal elution profile when analyzed by HPSEC-MALLS/RI/UV (Fig. 2) and was investigated by chemical and spectroscopic methods. The monosaccharide composition of AIP-F1 is shown in Table 1. Galactose, followed by arabinose were the main components of AIP-F1. Minor amounts of rhamnose, xylose, mannose, glucose, uronic acids and fucose were also found. The fraction AIP-F1 contained 10.4% uronic acids which were reduced to their respective neutral sugars. Glucuronic acid was the predominant uronic acid in this fraction,

Table 1
Monosaccharide composition of fractions AIP, AIP-F1 and AIP-CX.

Fraction	Monosaccharide composition (%)[a]							
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA
AIP	5.3	tr	14.9	4.7	12.8	29.8	28.2	4.3
AIP-F1	10.9	tr	16.0	3.9	7.4	36.5	14.9	10.4
AIP-CX	10.3	3.0	11.7	3.4	10.5	38.4	21.6	1.1

tr, trace.
UA = uronic acid.
[a] Neutral monosaccharide determined by GC and uronic acid determined by colorimetric method.

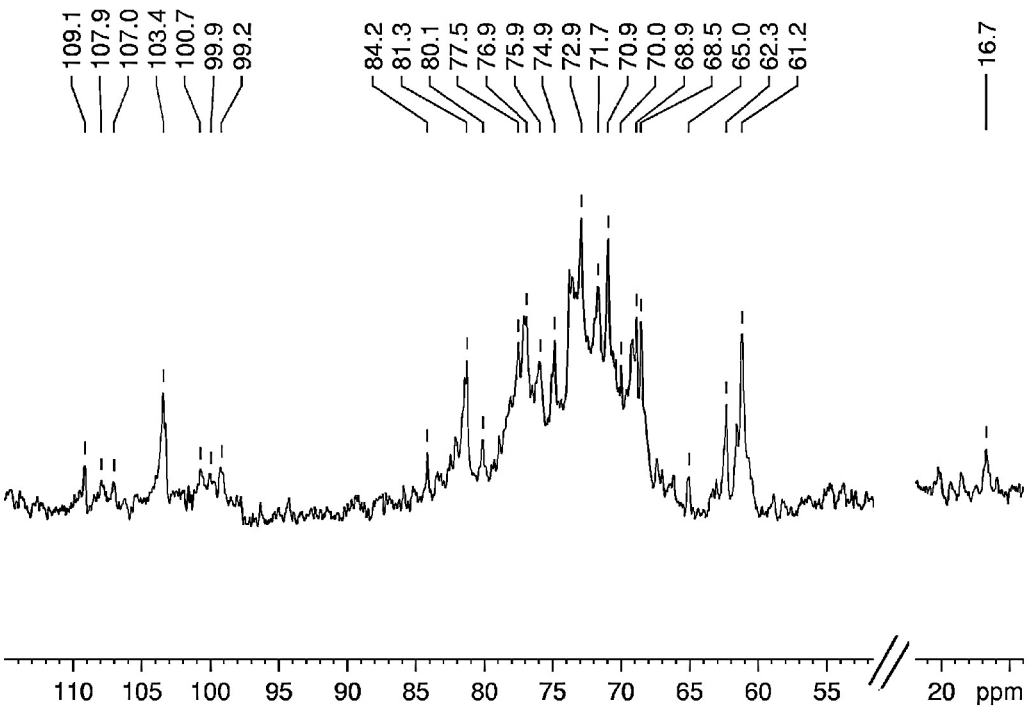

Fig. 3. ^{13}C NMR spectrum of AIP-F1. Solvent was D_2O at 70 °C. Numerical values for δ are in ppm.

due to increased glucose in the carboxyl-reduced sample (AIP-CX) compared with the native sample (AIP-F1), as shown in Table 1.

The monosaccharide composition and the chemical shifts in the ^{13}C NMR spectrum (Fig. 3) indicated that AIP-F1 consists of a type II arabinogalactan. Type II arabinogalactans encompasses a broad group of short (1→3) and (1→6)-β-D-galactan chains connected to each other by (1→3) and (1→6)-linked branch point residues. Most of the remaining galactose units are substituted by a terminal arabinofuranose (Fincher et al., 1983). Although the type II arabinogalactans side chains often terminate in α-L-Ara*f*, other sugars can be present, such as Fuc*p*, Rha*p* and the uronic acids GlcpA and GalpA, which are usually in terminal positions (Steinhorn, Sims, Carnachan, Carr, & Schlothauer, 2011, Thude & Classen, 2005). The type II arabinogalactans are frequently linked to protein moiety (known as arabinogalactan-proteins or AGP) and the protein content is usually between 2 and 10% (Fincher et al., 1983).

In the ^{13}C NMR spectrum of AIP-F1, the signal at δ 103.4 was attributed to C-1 of β-D-Gal*p* units and the signals at δ 81.3 and 68.5 ppm were attributed to the C-3-linked and C-6-linked β-D-Gal*p* units, respectively (Baron Maurer et al., 2010; Capek, Matulova, Navarini, & Liverani, 2009), which are typical of a type II arabinogalactan. The chemical shifts at δ 107.9 and 107.0 were attributed to C-1 of internal α-L-Ara*f* residues, respectively and the signal at 109.1 ppm was due to C-1 of terminal α-L-Ara*f* (Baron Maurer et al., 2010; Karácsonyi, Pätoprstý, & Kubačková, 1998; Steinhorn et al., 2011). The signals at δ 62.3 and 61.2 which appeared inverted in the DEPT experiment (data not shown), were attributed to the non-substituted C-5 of α-L-Ara*f* and the C-6 of β-D-Gal*p* units, respectively.

The signal of β-D-Glc*p*A can be overlapped with the signal of the C-1 of β-D-Gal*p* at 103.4 ppm (Redgwell et al., 2011; Steinhorn et al., 2011).

In addition to glucuronic acid, rhamnose was also found as a constituent of the arabinogalactan present in AIP-F1. The signal at 100.7 ppm was attributed to C-1 of α-L-Rha*p* units and their CH_3-6 was found at a lower frequency, 16.7 ppm.

Infrared spectroscopy is quite extensively applied in plant cell wall analysis (Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000). The FT-IR spectrum of AIP-F1 showed a prominent band near 3400 cm^{-1}, characteristic of polysaccharides, due to hydroxyl group of monosaccharide units (Coimbra, Barros, Rutledge, & Delgadillo, 1999). The β-arabinogalactans have two typical bands in FT-IR analysis, at 1074 and 1045 cm^{-1}, which are attributed to galactopyranose in the backbone and arabinofuranose units in side branches, respectively (Kacurakova et al., 2000). These two bands were identified in the FT-IR spectrum of AIP-F1, confirming the presence of a type II arabinogalactan. The relative IR absorption intensities of the bands of galactose and arabinose vary in the sample according to their relative amounts (Kacurakova et al., 2000). The ratio of Gal:Ara was 2.3:1 in AIP-F1 and in the FT-IR spectrum; the galactose-related band was larger than the arabinose-related band. The ratio of Gal:Ara in AIP-F1 was comparable to the arabinogalactan isolated from the stigmas and styles of *Nicotiana alata* (2.1:1) (Gane et al., 1995), but lower than that obtained for kanuka honey arabinogalactan (5.3:1) (Steinhorn et al., 2011).

While type I arabinogalactans are usually found as neutral side-chains on plant cell-wall pectic polysaccharides, type II arabinogalactans are often covalently linked to proteins being known as arabinogalactan-proteins (AGP) (Steinhorn et al., 2011). The FT-IR spectrum of AIP-F1 showed high absorbance at wavenumbers characteristic of protein: 1610 cm^{-1} (amide I) and 1400 cm^{-1} (amide III) (Boulet, Williams, & Doco, 2007). The fraction AIP-F1 contained 7% proteins, which are probably covalently linked to the polysaccharide, suggesting that AIP-F1 is an AGP. This hypothesis was also supported by the elution profile of AIP-F1 by HPSEC-MALLS/RI/UV (Fig. 2), which showed a single peak at 280 nm detected simultaneously by RI, MALLS and UV. The protein percentage in AIP-F1 is in accordance with previously studied AGPs which typically contains 1% to 10% (w/w) of proteins (Ellis, Egelund, Schultz, & Bacic, 2010). The peak at 1610 cm^{-1} in FT-IR can also be overlapped with the band of uronic acid (Gnanasambandam and Proctor, 1999), which is also present in AIP-F1.

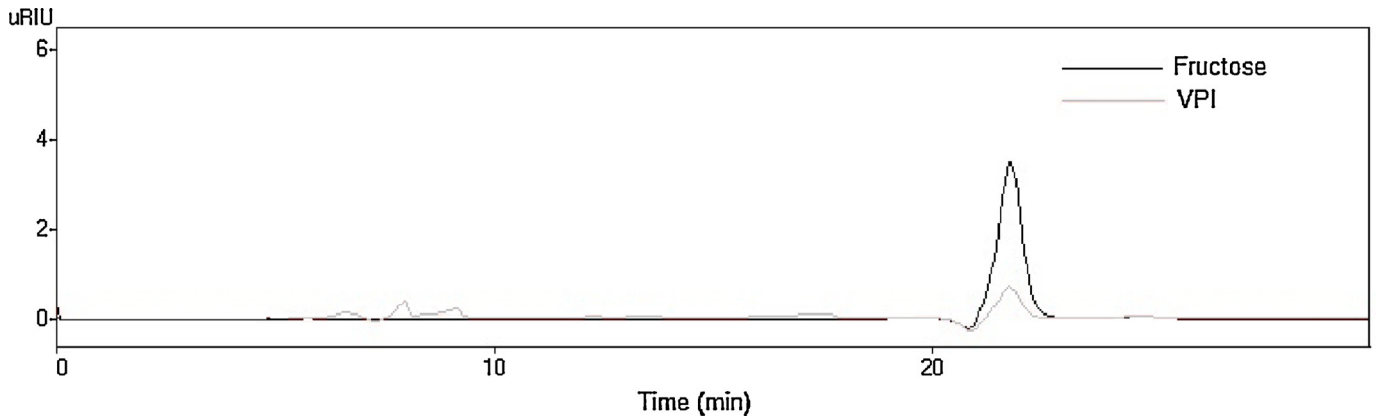

Fig. 4. HPLC chromatogram of VPI hydrolyzed sample and a fructose standard.

Table 2
Profile of partially O-methylated alditol acetates obtained by methylation analysis of AIP-F1.

O-Me-alditol acetate	Linkages
2,3,4-Me_3-Rha	Terminal
2,4-Me_2-Rha	→)3
3-Me-Rha	→)2,4
2,3,5-Me_3-Ara	Terminal
3,5-Me_2-Ara	→)2
2-Me-Ara	→)3,5
2,3-Me_2-Ara	→)5
2,6-Me_2-Gal	→)3,4
2,4-Me_2-Gal	→)3,6
2-Me-Gal	→)3,4,6
2,4,6-Me_3-Gal	→)3
2,3,4-Me_3-Gal	→)6
2,3,4,6-Me_4-Gal	Terminal
2,3,4,6-Me_4-Glc	Terminal
2,3,6-Me_3-Glc	→)4
2,3,4,6-Me_4-Man	Terminal

Results from methylation analysis of the carboxy-reduced AIP-CX sample are given in Table 2. The presence of 6-*O*-, 3-*O*- and 3,6-di-*O* substituted Gal*p* units were consistent with the presence of (1→3)-linked Gal*p* backbone with side-chains of (1→6)-linked Gal*p*. Arabinose was found to be 2-*O*-, 3-*O*-, 5-*O*- and 3,5-di-*O*-substituted and as nonreducing end-units, as described for type II arabinogalactans found in kanuka honey (Steinhorn et al., 2011) and *Lycium barbarum* (Redgwell et al., 2011).

Rhamnose, glucose and mannose were also found as nonreducing end-units in AIP-CX, consistent with other arabinogalactans structures (Redgwell et al., 2011; Thude & Classen, 2005). It is believed that these monosaccharides at the periphery of AGPs might be important for their biological activities (Göllner, Ichinose, Kaneko, Blaschek, & Classen, 2011). The presence of glucose as nonreducing end-units can also be due to glucuronic acid because the acidic units were reduced to their respective neutral sugars prior to methylation analysis. The presence of glucuronic acid as nonreducing end-units in type II arabinogalactans has been described for *L. barbarum* (Redgwell et al., 2011), and in wheat flour AGP, the nonreducing end-units of glucuronic acid was identified as linked at O-6 to the side-chains of (1→6)-linked Gal*p* (Tryfona et al., 2010).

It has also been reported that some glucuronic acid units from AGP can be substituted by terminal Rha*p* (1→4)-linked to glucuronic acid (Redgwell et al., 2011). The presence of the derivative 2,3,6-Me_3-Glc in the methylation products of AIP-CX suggest that some glucuronic acid units can also be *O*-4 substituted.

Rhamnose was found to be 3-*O*-, 2,4-di-*O* substituted and also as nonreducing end-units. The arabinogalactans from fruits of *Lycium ruthenicum* also contain (1→2,4)-linked rhamnosyl residues, which are likely in branches (Peng et al., 2012). Arabinogalactans from *Centella asiatica* (Wang, Zheng, Zuo, & Fang, 2005), *Echinacea pallida* (Thude & Classen, 2005) and *L. barbarum* (Redgwell et al., 2011) also showed rhamnose in their composition.

Periodate oxidation of AIP-F1 was performed to confirm the presence of 1,3-Gal*p* in the backbone. After oxidation of 100 mg AIP-F1, 50 mg of oxidation-resistant polysaccharide was obtained. The ratio Gal:Ara of the oxidation-resistant polysaccharide was 5.6:1, which is much higher than that of the native polysaccharide (2.3:1). This increase in the galactose proportion suggests that most of the galactose is (1→3)-linked and resistant to periodate oxidation, as typically found in type II arabinogalactans (Fincher et al., 1983).

The AGPs are widely distributed in higher plants, where they have been found in seeds, leaves, roots and fruits. Their molar masses are very heterogeneous, presumably reflecting different extents of glycosylation (Clarke, Anderson, & Stone, 1979; Showalter, 1993). The weight-average molar mass (M_w) of AGP from the *A. absinthium* infusion (AIP-F1) was calculated to be 84,160 g/mol, similar to that found for an AGII from *Avena sativa* (83,000 g/mol; Göllner et al., 2011), but higher than those from *L. barbarum* (50,000–60,000 g/mol; Redgwell et al., 2011) and lower than those from *Echinacea sp* (1.1×10^6–1.2×10^6 g/mol; Thude & Classen, 2005).

The structure of protein and glycan moieties of AGPs is highly diverse and several studies have implicated these molecules in many important biological processes in plants, such as cell proliferation and survival, growth, resistance to stresses and plant microbe interaction (Seifert & Roberts, 2007; Pickard, 2013). In addition, some type II arabinogalactans have recently been investigated as potential immunomodulators of the human immune system (Classen, Thude, Blaschek, Wack, & Bodinet, 2006; Nosal'ova et al., 2011), and the fine structure of AGP influences its biological activity (Classen et al., 2006). Therefore, the investigation of the structural and functional properties of arabinogalactans is an opportunity for the discovery of novel therapeutic agents with immunomodulatory properties.

3.3. Polysaccharides from the infusion of aerial parts from of *A. vulgaris*

As described for *A. absinthium*, polysaccharides from the aerial parts of *A. vulgaris* were isolated from boiling water infusions prepared according to the traditional method (Lorenzi & Matos, 2008). The crude polysaccharide yield from *A. vulgaris* infusion (VPI) was 7.0%, representing 98 mg of polysaccharide in each cup of infusion. The anthrone method indicated that 85% of the carbohydrate content in the VPI consisted of fructose. Rha, Ara, Man, Gal,

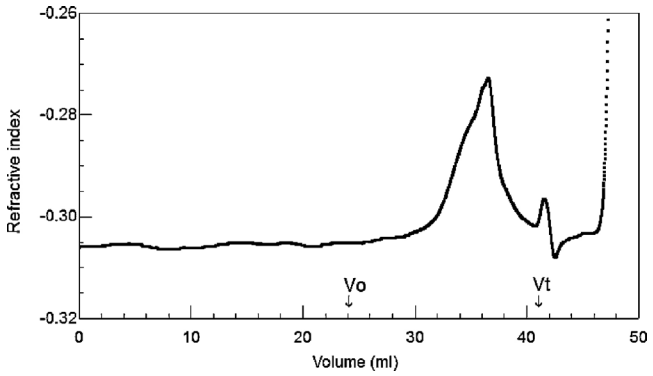

Fig. 5. Elution profile of VPI obtained by HPSEC/RI.

Glc, uronic acids and Xyl were also found in a ratio of 1.7:2.5:5.2:4.2:14.0:1.8:1.

Fructans are acid labile, therefore VPI was submitted to mild acid hydrolysis (H_2SO_4, pH 2.0, 15 min), and the resulting monosaccharides were analyzed by HPLC and TLC. The presence of fructose in this sample was confirmed by TLC using a ketose-specific stain (data not shown) and by HPLC (Fig. 4). Therefore, VPI was suggested to be a fructan-type polysaccharide.

A polymodal elution profile was observed by HPSEC for VPI using the refractive index detector (Fig. 5). The sample heterogeneity may reflect different degrees of fructan polymerization (de Oliveira et al., 2011). The main peak in the chromatogram of VPI eluted after 55 min and was not detected by MALLS, indicating that low molar mass components were predominant in this fraction. This result is in agreement with other studies, where fructans are described as polydispersed reserve carbohydrates that contain 1–70 units of fructose (Choque Delgado, Tamashiro, & Pastore, 2010).

In the ^{13}C NMR spectrum of VPI (Fig. 6), the signals at δ 103.3 and 92.5 ppm were assigned to C-2 of (2→1)-linked β-D-fructofuranose and C-1 of the starting nonreducing end-units of α-D-glucopyranose, respectively. These signals are typical of inulin-type fructans which mainly consist of β-(2←1) fructosyl-fructose linkages with a starting α-D-glucose unit (Roberfroid, 2007).

High intensity signals in the spectrum are in accordance with those expected from the fructose ring carbons, being at δ in ppm: C-1 (61.3), C-2 (103.3), C-3 (77.5), C-4 (74.9), C-5 (81.3) and C-6 (62.3). A group of low intensity signals were attributed to the α-D-glucose of the nonreducing end units of inulin: C-1 (92.5), C-3 (73.0), C-4 (69.7), C-5 (71.4) and C-6 (60.6). All of the assignments were based on previous reports (Chandrashekar, Prashanth, & Venkatesh, 2011; Fontana, Baron, Diniz, & Franco, 1994).

According to the results, the main water-soluble carbohydrate present in *A. vulgaris* infusion is an inulin-type fructan. The presence of inulin-type fructans was also reported for the leaves of *Stevia rebaudiana* and *Matricaria maritima* (Cerantola et al., 2004; de Oliveira et al., 2011), both species of the Asteraceae family (order Asterales) in which *A. vulgaris* is also included. It is known that almost all families included in the order Asterales contain fructans, at least in storage organs (Hendry, 1993).

It has been reported that *A. vulgaris* contains inulin as one of its active components (Govindaraj et al., 2008), but oligofructosides were described only for the roots of plants in *Artemisia* species (Kennedy, Stevenson, White, Lombard, & Buffa, 1988). To our knowledge, this is the first time that inulin has been reported in the leaves of an *Artemisia* specie. However, fructans were not the major carbohydrate in the infusion of *A. absinthium*, which also belongs to the Asteraceae family. Instead, apart from a starch, an arabinogalactan was extracted from the leaves of *A. absinthium*.

Inulin-type fructans have attracted a great of attention, especially in the food industry, because fructans add nutritional value to the product. Inulin is classified as a functional food because of its chemical nature and physiological and nutritional effects. Fructo-oligosaccharides and inulin are described as having prebiotic properties (Roberfroid, 2007). The regular intake of these carbohydrates modulates the composition of intestinal flora, enhances resistance against intestinal pathogens and regulates the levels of serum cholesterol and the absorption of calcium and other minerals. Fructans also seem to be involved in the positive modulation of the immune system, as well as in the reduction of the risk of several diseases, including cancer (Choque Delgado et al., 2010; Roberfroid, 2007; Taper & Roberfroid, 1999). According to the results from the present work, every cup of *A. vulgaris* infusion contains 83 mg of inulin-type fructans on average. It is possible that the fructans

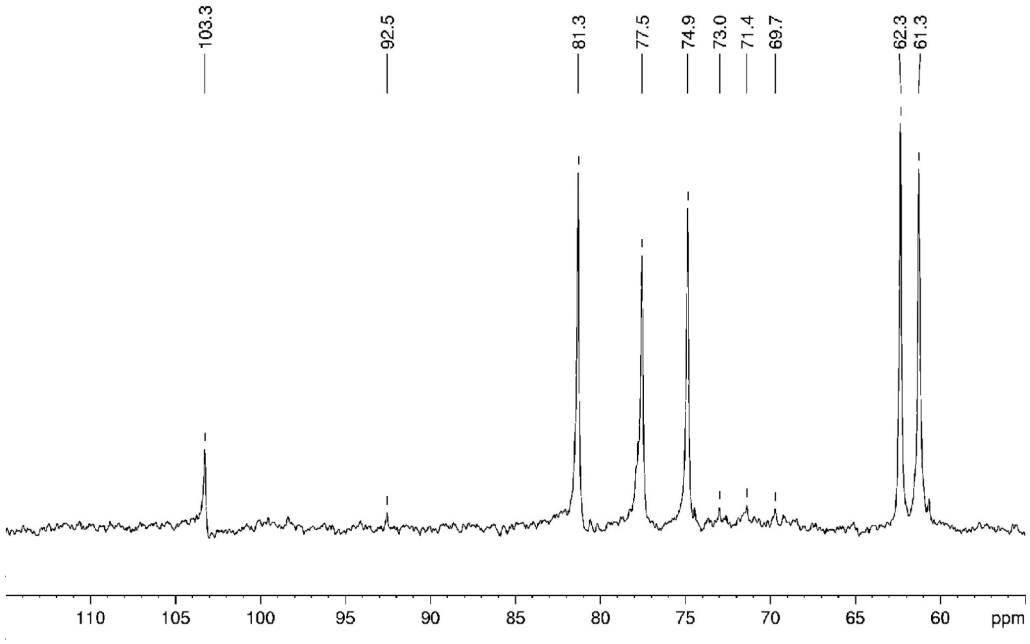

Fig. 6. ^{13}C NMR spectrum of VPI. Solvent was D_2O at 70 °C. Numerical values for δ are in ppm.

present in *A. vulgaris* infusion can contribute to the positive effects on health attributed to the infusion.

4. Conclusion

The infusions from aerial parts *A. absinthium* and *A. vulgaris*, which are used in traditional herbal medicine, contain polysaccharides. Although both species belong to the Asteraceae family, the infusion of *A. absinthium* contains a type II arabinogalactan, whereas the infusion of *A. vulgaris* contains an inulin-type fructan as the main polysaccharide.

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