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Isolation of an arabinogalactan from *Endopleura uchi* bark decoction and its effect on HeLa cells

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ABSTRACT

Endopleura uchi is a native plant from the Amazon used in popular medicine to treat myomas. A crude polysaccharide (AGb) obtained from *E. uchi* bark decoction was purified, yielding a type II arabinogalactan (AG) that was characterized by chemical and spectroscopic methods. AG was evaluated for its cytotoxic effects on HeLa cells. AG (5–500 μg/ml) reduced cell viability at 48 and 72 h (approximately 20%) but not in a dose-dependent manner. Cell proliferation was also reduced by AG, with a 25% inhibition (100 μg/ml) at 72 h. The results suggest that the cytotoxicity exhibited by AG does not involve pathways related to the cell cycle.

1. Introduction

Throughout history, natural products have been a rich source of compounds that have found many applications in the fields of medicine, pharmacy and biology. Approximately three-quarters of plant-derived drugs in clinical use came to the attention of pharmaceutical companies due to their use in traditional medicine (Newman & Cragg, 2007; Nobili et al., 2009).

Medicinal plants are mainly consumed orally and produced either by infusion or decoction (Halberstein, 2005). Plant aqueous extract components include secondary metabolites (mostly polyphenols and alkaloids) and primary metabolites, such as polysaccharides (Monobe, Ema, Kato, & Yamamoto, 2008). Polysaccharides have attracted attention due their therapeutic properties and low toxicity (Schepetkin & Quinn, 2006). Several medicinal plants have been reported to contain polysaccharides having different biological properties, such as antioxidant (Kardosová & Machová, 2006), anti-ulcer (Cipriani et al., 2006), immunostimulating (Schepetkin, Faulkner, Nelson-Overton, Willey, & Quinn, 2005) and anti-tumor (Sampedro et al., 2004).

Arabinogalactans are structural polysaccharides that are widely spread throughout the plant kingdom and have been shown to possess biological activities (Thakur et al., 2012). Arabinogalactans occur in two structurally different forms, described as type I and type II. Type I arabinogalactans have a linear (1 → 4)-β-D-Gal*p* backbone, bearing 20–40% of α-L-Ara*f* residues (1→5)-linked in short chains, generally at position 3. Type I arabinogalactans are usually found to be associated with pectins (Carpita & McCann, 2000). Type II arabinogalactan constitute a broad group of short (1 → 3)- and (1 → 6)-β-D-galactan chains connected to each other by (1 → 3, 1 → 6)-linked branch point residues. Other monosaccharides that may be present are L-Rha*p*, D-Man*p*, D-Glc*p*, D-GlcA*p* and D-GalA*p* or its 4-*O*-methyl derivative. Type II arabinogalactans are more widespread than type I and often occur linked to proteins (known as arabinogalactan proteins) (Fincher, Stone, & Clarke, 1983). Arabinogalactans isolated from *Larix occidentalis*, *Panax ginseng* were capable of activating macrophages *in vitro* by augmenting nitrite oxide, superoxide anion and/or cytokine production (Hauer & Anderer, 1993; Shin et al., 2002). An arabinogalactan protein from *Coffea arabica* also enhanced cytokine production by splenic cells at 100 μg/ml (Nosál'ová et al., 2011). Arabinogalactans also inhibited Sarcoma-180 tumor growth *in vivo* once injected into mice (100 mg/kg) (Moretão, Zampronio, Gorin, Iacomini, & Oliveira, 2004). The authors suggested that the mechanism of anti-tumor activity was due to the immunostimulatory effects of the arabinogalactans through a cell-mediated response. However, some polysaccharides exert anti-proliferative effects on particular cancer cell lines via the induction of apoptosis *in vitro*

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(Cao et al., 2010; Hung, Hsu, Chang, & Chen, 2012; Zeng et al., 2012).

Endopleura uchi (Huber) Cuatrec. (Humeriaceae) is a native plant from the Amazon basin popularly known as "uxi-amarelo" (Schultes, 1979). A bark decoction of this plant is used in traditional medicine as an anti-inflammatory agent and to treat arthritis, cholesterol, diabetes, uterine infections and myomas (Nunomura, Oliveira, Silva, & Nunomura, 2009). The present study isolated a type II arabinogalactan from an *E. uchi* bark decoction and evaluated the anti-proliferative effect of this polysaccharide against human epithelial cervical cancer (HeLa cells).

2. Material and methods

2.1. Material and cell line

The wood bark of *E. uchi* (1.5 kg) was purchased from Chá & Cia Ervas Medicinais, Jacarei-São Paulo, Brazil in June 2010, lot number uxi 19/05.

Minimum Essential Medium (MEM) and fetal bovine serum were obtained from Cultilab (Campinas, Brazil). Crystal violet, trypan blue and iodide propidium were obtained from Sigma–Aldrich (St. Louis, MO, USA). Annexin-V-FITC was purchased from BD Pharmingen™ (San Jose, CA, USA). All other reagents were commercial products of the highest available purity grade.

The human cervix adenocarcinoma cell line (HeLa) was obtained from Institute Adolfo Lutz (São Paulo). The HeLa cells were maintained in MEM supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin, at 37 °C in a humidified incubator containing 5% CO_2.

2.2. Determination of total carbohydrate, protein and uronic acid content

Total carbohydrates were measured by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), using glucose as standard. Uronic acid was estimated using galacturonic acid as a standard as described by Blumenkrantz and Asboe-Hansen (1973). Total proteins were determined according to Bradford (1976), using BSA as a standard.

2.3. Isolation and purification of polysaccharides from E. uchi bark decoction

According to its popular use (1 g to 100 ml of water), 500 ml of water was added to 5 g of *E. uchi* bark and boiled for 5 min. Then, the extract was concentrated under reduced pressure (50 °C) treated with ethanol (3:1, v/v) to precipitate the crude polysaccharides (AGb), which were washed three times with ethanol and dried under vacuum. This process was repeated until 5 g of crude polysaccharides was obtained. The resulting fraction was submitted to a freeze-thawing process, until no more precipitate appeared. The water soluble fraction was treated with Fehling solution (Jones & Stoodley, 1965) and the insoluble Cu^{2+} complex was isolated by centrifugation. The Fehling supernatant was then submitted to an ultra filtration process using 300 kDa cut-off membranes, resulting in a purified polysaccharide (AG).

2.4. Monosaccharide composition

The polysaccharides fractions were hydrolyzed with 2 M trifluoroacetic acid (5 h, 100 °C), and after concentration to dryness, the residues were reduced with $NaBH_4$ (Wolfrom & Thompson, 1963a) and acetylated with pyridine-acetic anhydride (1:1, v/v, 16 h, at 25 °C) (Wolfrom & Thompson, 1963b). The resulting alditol acetates were analyzed by gas–liquid chromatography (GLC) 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).

2.5. Carboxyreduction

AG was carboxy-reduced by the carbodiimide method (Taylor & Conrad, 1972). $NaBD_4$ and $NaBH_4$ were used as the reducing agents for uronic acid identity and methylation analysis, respectively. The reduction of the uronic acids was followed by the colorimetric method (Blumenkrantz & Asboe-Hansen, 1973). The monosaccharide composition of AG after the carboxyreduction was examined by gas chromatography–mass spectrometry (GLC–MS) in the form of alditol acetates using a VARIAN 3800 chromatograph coupled to a 2000 R-12 VARIAN Ion-Trap mass spectrometer using a DB-225 capillary column (30 m × 0.25 mm internal diameter), which was programmed from 50 to 220 °C at 40 °C/min and used helium as the carrier gas. The products were identified by their typical retention times and electron ionization spectra.

2.6. High pressure size exclusion chromatography

Homogeneity and average molar mass (M_w) were determined by high pressure size exclusion chromatography (HPSEC) coupled to a refractive index (RI), ultra-violet (UV) light and a Wyatt Technology Dawn-F multi-angle laser light scattering (MALLS) detector. Four Waters Ultrahydrogel 2000/500/250/120 columns were connected in series and coupled to the multidetection equipment. The eluent was 0.1 M $NaNO_2$, containing 200 ppm NaN_3 and the flow was 0.6 ml/min. The HPSEC data were collected and analyzed using the Wyatt Technology ASTRA program. All samples were filtered through a 0.22 μm pore membrane.

2.7. Fourier transform-infrared analysis

Fourier transform-infrared (FT-IR) spectra were collected in the absorbance mode in the frequency range of 4000–400 cm^{-1} using a Bomem MB-100 spectrophotometer (Hartman and Braun, Canada) at a 4 cm^{-1} resolution. Spectroscopic grade KBr powder was used and discs were prepared using a salt:sample proportion of 99:1. Prior to the FT-IR analysis, the KBr was dehydrated at 120 °C for 24 h and the sample was desiccated under vacuum in an Abderhalden equipment containing P_2O_5.

2.8. Nuclear magnetic resonance spectroscopy (NMR)

The ^{13}C NMR spectra in D_2O at 70 °C were obtained using a Bruker Avance DRX400 spectrometer. The chemical shifts were expressed in δ (ppm) relative to acetone (δ 30.2).

2.9. Methylation analysis

Methylation was conducted according to Kvernheim (1987) with modifications. Briefly, 5 mg from carboxyreduced AG was solubilized in DMSO at 50 °C. Under a N_2 atmosphere, butyllithium in hexane was added with a syringe and the mixture was stirred for 1 h. After cooling in an ice bath (until frozen), methyl iodide was added and the sample was stirred for 1 h at 25 °C. The per-*O*-methylated derivatives were hydrolyzed first with formic acid (90%, 1 h, 100 °C) and then with trifluoroacetic acid (2 M, 3 h, 100 °C), reduced and acetylated. The partially *O*-methylated alditol acetates were analyzed by GLC–MS using a VARIAN 3300 chromatograph coupled to a 2000 R-12 VARIAN Ion-Trap mass

Table 1
Yield, total sugar, protein and monosaccharide composition of AGb and AG.

Fraction	Yield[a] (%)	Total sugar[b] (%)	Protein[b] (%)	Monosaccharide[c] (%)						
				Rha	Ara	Xyl	Man	Gal	Glc	UA
AGb	0.76	57	21	21.0	6.8	2.5	5.2	16.3	34.0	14.2
AG	0.04	60	14	10.4	30.6	tr	2.0	37.8	7.4	11.8
AG*	–	–	–	11.0	31.8	1.4	2.1	45.5	6.2	2.0

tr, trace.
[a] Based on bark weight.
[b] Determined by colorimetric method.
[c] Neutral sugar were determined by GC and uronic acid (UA) was determined by colorimetric method.
* After carboxyreduction.

spectrometer with helium as the carrier gas (2.0 ml/min). A capillary column (30 m × 0.25 mm internal diameter) of DB-225 was held at 50 °C during the injection and then programmed to increase by 40 °C/min to 215 °C. The partially *O*-methylated alditol acetates were identified by their retention times and electron ionization spectra.

2.10. Cell viability and proliferation assay

For the cytotoxicity assay, HeLa cells were plated at a density of 1.0×10^4 cells/well in a 96-well culture plate. After 24 h of incubation, the cells were treated with varying concentrations of AG (5, 10, 25, 50, 100, 250 and 500 μg/ml) for 48 and 72 h. Cell viability was evaluated by the crystal violet staining assay (Kueng, Silber, & Eppenberger, 1982).

For the proliferation assay, HeLa cells were plated at a density of 5.0×10^4 cells/well in a 24-well culture plate. After 24 h of incubation, the cells were treated with 25, 50 and 100 μg/ml AG for 24, 48 and 72 h. The cells were harvested by trypsinization and viable cells were counted by the trypan blue exclusion test using a phase-contrast microscope (Olympus) (Philips, 1973).

2.11. Optical microscopy analysis

HeLa cells (5.0×10^4 cells/well) were plated in 24-well culture plates containing glass coverslips. After 24 h incubation, cells were treated with AG (50, 100 and 250 μg/ml) for 72 h. Next, the coverslips were removed, and the cells were fixed in ethanol and stained with hematoxylin–eosin.

2.12. Cell cycle analysis

Cells (1.0×10^5) were plated in a 60 mm plate. After 24 h incubation, the cells were treated with AG (50 and 100 μg/ml) for 72 h. The cells were harvested by trypsinization and centrifuged, and the pellet was washed twice with PBS and stained with a solution consisting of 50 μg/ml propidium iodide, 0.1% Triton-X, 0.1% RNase A and 0.1% sodium citrate in a total volume of 300 μl. After a 30 min incubation at 4 °C in the dark, the proportion of cells in the different phases of the cell cycle was analyzed using a FC500 flow cytometer (Beckman Counter) and the results were calculated using the MXP software.

2.13. Statistical analysis

The statistical analysis was conducted by analysis of variance (ANOVA) followed by a Tukey test. The values for the means ± SD were used and differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. E. uchi bark decoction contains an arabinogalactan (AG)

The *E. uchi* bark decoction was treated with excess EtOH to obtain the AGb fraction (0.76% yield based on barks weight). This fraction exhibited a multimodal elution profile by HPSEC and the monosaccharide composition revealed that AGb contained glucose as major component (34%), followed by rhamnose, galactose and uronic acid (Table 1). AGb was submitted to the freeze-thaw process, which significantly lowered the glucose content, followed by treatment with the Fehling solution to remove the xylose and mannose contaminants and ultrafiltration through the 300 kDa membrane, resulting in a homogenous polysaccharide (AG) with an average molar mass (M_w) of 1.09×10^5 g/mol (dn/dc 0.136), as shown by the HPSEC elution profile (Fig. 1). This polysaccharide contained arabinose and galactose as major monosaccharides, with smaller proportions of rhamnose, uronic acid and glucose (Table 1). The molar ratio of arabinose:galactose:rhamnose:uronic acid was 2.6:3.2:0.9:1, indicating the presence of arabinogalactans. The detection of a single peak simultaneously by RI, MALLS and UV (280 nm) suggests that proteins present in the AG (14%) are linked to the polysaccharide, as is usually found for type II arabinogalactans.

The ^{13}C NMR spectrum of AG (Fig. 2) showed several signals in the C-1 region (δ 97.7–109.1). Signals at δ 106.9, 107.6 and 109.1 were assigned to C-1 of α-L-Ara*f* units, and those at δ 103.2 and 102.7 to C-1 of the β-D-Gal*p* units. The signal for the carboxyl group from uronic acid was observed at δ 174.3. The signals at δ 16.8 and 99.1 were attributed to CH_3-6 and C-1 of the α-L-Rha*p* units, respectively, and the signal at δ 97.7 may be attributed to α-D-Glc*p* residues. The signals at δ 82.2 and 71.2 correspond to O-3 and O-6 linked β-D-Gal*p* units, respectively, corroborating the suggestion of the presence of a type II arabinogalactan. The signals were

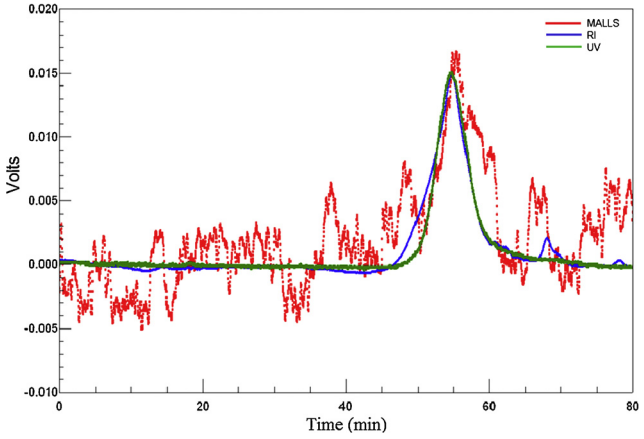

Fig. 1. HPSEC elution profile of AG.

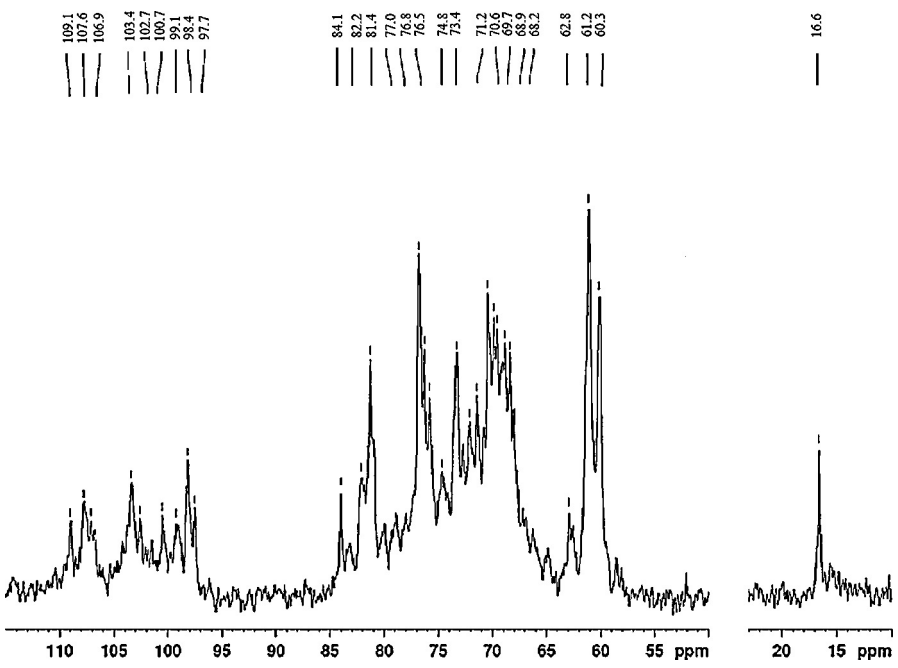

Fig. 2. ^{13}C NMR spectrum of AG.

attributed according to the literature (Pellerin, Vidal, Williams, & Brillouet, 1995; Redwell et al., 2011).

The identity of the uronic acid was determined after the reduction of its carboxyl group. The monosaccharide composition showed an increase in the galactose content from 39 to 46%, indicating the presence of a galacturonic acid. The ^{13}C NMR spectrum showed an increase in the C-6 signal at δ 61.1 and 60.3 and no signal at δ 174.3.

Result from methylation analysis of AG after carboxyreduction is showed in Table 2. The result from methylation analysis of AG after the carboxyreduction showed that AG is a highly branched polysaccharide, containing nonreducing end-units of Ara*f* (2,3,5-Me_3-Ara) (17.7%) and Gal*p* (2,3,4,6-Me_4-Gal) (7.5%). The galactopyranosyl units are 3-*O*-, 3,6-di-*O*-, and 6-*O*-substituted according to 2,4,6-Me_3-Gal (8.6%), 2,4-Me_2-Gal (30%), and 2,3,4-Me_3-Gal (4.1%) methylated derivatives, respectively. The arabinosyl units were substituted at O-5 and O-3, according to 2,3-Me_2-Ara (11.7%) and 2,5-Me_2-Ara (4.7%) derivatives, respectively. The Glc*p* units are non-reducing ends according to the presence of 2,3,4,6-Me_4-Glc (6.4%). The presence of 3,4-Me_2-Rha (9.2%) indicates that 2-*O*-Rha*p* units are also present, most likely linked to terminal Glc*p*, Ara*f* or Gal*p*A residues.

The FT-IR spectrum of AG showed bands at 1148, 1075, 1043 and 813 cm^{-1}. Kacuraková, Capek, Sasinková, Wellner, and Ebringerová (2000) analyzing a type II arabinogalactan found the following bands: 1156, 1078 and 1040 cm^{-1}. According to the authors, the IR bands of β-(1 → 6)- or β-(1 → 3)-linked galactan occur at 1078–1072 cm^{-1}, while the side chain arabinans are found at ~1044 cm^{-1}. In addition, a band at 1610 cm^{-1}, which might correspond to a protein, was found for AG, corroborating the presence of arabinogalactan-protein. This purified arabinogalactan was used in biological experiments to evaluate their effect on HeLa cells.

Table 2
Linkage analysis of polysaccharide AG isolated from *Endopleura uchi* bark decoction.

O-Me-alditol acetate	Linkage	Mol%
2,3,5-Me_3-Ara	Terminal	17.7
3,4-Me_2-Rha	2-	9.2
2,5-Me_2-Ara	3-	4.7
2,3,4,6-Me_4-Glc	Terminal	6.4
2,3-Me_2-Ara	5-	11.7
2,3,4,6-Me_4-Gal	Terminal	7.5
2,4,6-Me_3-Gal	3-	8.6
2,3,4-Me_3-Gal	6-	4.1
2,4-Me_2-Gal	3,6-	30.0

3.2. AG exhibit cytotoxicity against HeLa cells

To assess the cytotoxic effects of AG isolated from *E. uchi* bark decoction, the cells were exposed to different concentrations of AG (5–500 μg/ml) at 48 and 72 h. Cell viability was determined by the crystal violet protocol. Fig. 3A shows the effect of AG on HeLa cell viability. AG effect was not dose-dependent and the cell viability was reduced by ~20% at doses ranging from 25 to 500 μg/ml. Cell proliferation was accessed by the trypan blue exclusion test (Fig. 3B). AG inhibited cell growth at 72 h at all doses (approximately 25%) and also presented an effect at 48 h (inhibition of 15%).

HeLa cells exposed to 250 μg/ml AG did not show significant morphological changes, although a slightly increase in chromatin condensation was observed (data not shown). The cell cycle modulation of HeLa cells treated with AG (50 and 100 μg/ml) was analyzed after 72 h of treatment. No statistical difference was found for the percentage of cells in the G1 and G2/S phases (data not shown), indicating that there is no cell cycle arrest when the HeLa cells were treated with AG.

4. Discussion

Arabinogalactans are essential structural polymers present in plant cell walls and a main component of gum and exudates. D-Galactose and L-arabinose are the major monosaccharides found in type II arabinogalactans and AGP, with most samples containing more Gal than Ara (Fincher et al., 1983). Type II arabinogalactans have been isolated from different parts of plants, such as fruits (Qin, Yamauchi, Aizawa, Inakuma, & Katoa, 2001), flowers (Gane et al., 1995), roots (Yamada, Kiyohara, Cyong, & Otsuka, 1987), leaves (Xie et al., 2008) and seeds (Zippel, Wells, & Hensel, 2010).

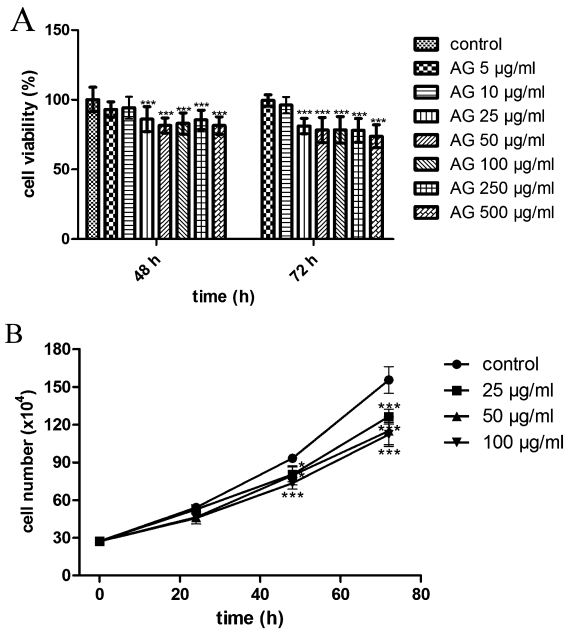

Fig. 3. Viability and proliferation of the HeLa cell line after treatment with AG (A and B, respectively). The values represent the means ± SD of percentage of viable cells compared with the control (data represent three independent experiments, each in triplicate) $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

In the present study, a type II arabinogalactan (AG) was isolated from an *E. uchi* bark decoction. The structural features of the polysaccharide from *E. uchi* bark decoction indicated a typical type II arabinogalactan, containing a (1 → 3)-linked *β*-D-Gal*p* main chain that is substituted at O-6 by (1 → 6)-linked *β*-D-Gal*p* chains.

According to the literature, the arabinogalactan side chains can terminate in α-L-arabinofuranosyl (L-Ara*f*) and other sugars, such as Fuc*p*, Rha*p* and Glc*p*A (with or without 4-O-methyl) (Gaspar, Johnson, Mckenna, Bacic, & Schultz, 2001). The results showed that the arabinogalactan from *E. uchi* has Glc*p*, Ara*f* or Gal*p*A as non-reducing end units, which were also observed as terminal ends of the arabinogalactan-protein from Chinese wolfberry (*Lycium barbarum*) (Redwell et al., 2011). On the other hand, in the arabinogalactan isolated from an infusion of *Maytenus ilicifolia* leaves, only α-L-Ara*f* and 4-O-Me-Glc*p*A were found as non-reducing end units (Cipriani et al., 2006). In the arabinogalactans studied so far, the identification of the uronic acid was not always performed; however, most of them contained GlcA instead of GalA (Brecker et al., 2005; Cipriani et al., 2006; Nunes et al., 2008; Thude & Classen, 2005).

The arabinogalactan from *E. uchi* bark decoction also contained Rha*p* units. However, Rha*p* units were not found as non-reducing end units as observed for arabinogalactans from green coffee beans (Nunes et al., 2008) and *Echinacea pallida* roots (Thude & Classen, 2005), but rather they were O-2 substituted. This feature was also observed for an arabinogalactan from red wine (Pellerin et al., 1995) and *Echinacea purpurea* cell cultures (Wagner, Stuppner, Schafer, & Zenk, 1988).

Type II arabinogalactans have been found in other aqueous extracts of plants used in traditional medicine and the presence of the polysaccharide has been associated with the beneficial therapeutic effects of these extracts (Cipriani et al., 2006; Xie, Schepetkin, & Quinn, 2007; Xie et al., 2008). Over the last decades, numerous carbohydrate polymers have been shown to be responsible for biological effects, either by exhibiting the effect themselves or by inducing the effects via complex reaction cascades (Thakur et al., 2012). Arabinogalactans possess biological activities and may be partially responsible for the biological activity attributed to medicinal plants. For example, from the bark of *Mimosa tenuiflora*, an arabinogalactan protein was a stimulator of dehydrogenase activity and proliferation of skin fibroblasts showing that this polysaccharide plays an important role in wound healing (Zippel, Deters, & Hensel, 2009). A type II arabinogalactan isolated from *M. ilicifolia*, popularly used for the treatment of stomach ulcers and gastritis, significantly inhibited ethanol-induced gastric lesions in rats with an IC_{50} of 9.3 mg/kg, suggesting that the arabinogalactan released from that infusion has a protective anti-ulcer effect (Cipriani et al., 2006). Considering that, in addition to secondary metabolites, each cup of the *E. uchi* decoction prepared following its popular use (1 g to 100 ml of water) contains 7.6 mg of polysaccharides, this component was investigated. After ethanol precipitation, the crude polysaccharide was submitted to a purification process and characterized as a type II arabinogalactan (AG). In view of the use of *E. uchi* bark decoction in the traditional medicine to treat myomas (Nunomura et al., 2009), the purified (AG) polysaccharide was tested against HeLa cells to investigate whether the AG could, to some extent, be responsible for the effect of the *E. uchi*. AG showed cytotoxic and antiproliferative effects, reducing cell viability and inhibiting cell growth by approximately 20%; this effect was not dose dependent. AG did not cause any changes in the cell population at different cell cycle phases or in the annexin V positive population, indicating that the antiproliferative effect of these polysaccharides is not due to cell cycle arrest.

The anti-tumor activity of a compound can be achieved by the direct effect on the tumor cell or by the immune system activation (Ooi & Liu, 2000). In vitro evaluations have shown that some plant polysaccharides may induce apoptosis in different cancer cell lines. Cao et al. (2010) demonstrated that an arabinoglucan from *Angelica sinensis* is capable of inhibiting proliferation and inducing apoptosis in HeLa cells. According to the authors, the mechanism of action primarily involves the activation of the intrinsic mitochondrial pathway.

A polysaccharide containing Rha, Fuc, Ara, Xyl, Gal, Glc and 5.5% protein isolated from saffron corm extracts showed cytotoxic activity on HeLa cells (Escribano et al., 1999). The polysaccharide showed dose-dependent inhibition of cell growth giving half-maximal inhibition (IC50) of ~9 μg/ml. HeLa cells exposed to this polysaccharide showed swelling and local plasma membrane evaginations, suggesting that cytotoxicity is mediated by extracellular fluid uptake.

Polysaccharides containing Fru, Xyl, Man, Glc and Gal, isolated from *Curcuma kwangsiensis*, inhibited the proliferation of nasopharyngeal carcinoma CNE-2 cells in a dose- and time-dependent manner; the first significant reduction occurred at a dose of 50 μg/ml (inhibition of 23.5%) (Zeng et al., 2012). In this case, the authors proposed that the effects were due to the induction of apoptosis mediated by attenuating Bcl-2 expression and promoting p53 expression. Hung et al. (2012) demonstrated that the polysaccharide composed of Rha, Ara, Xyl, Man, Glc and Gal at a molar ratio of 2.2:7.8:1.2:0.2:1.4:3.8 isolated from *Zizyphus jujuba* has a cytotoxic effect against melanoma cells by increasing the caspase-3 and -9 activities.

No study investigating the direct effect of arabinogalactans on cell tumor *in vitro* was found in the literature and most of them focused on immunomodulatory activities of the arabinogalactans (Wagner et al., 1988; Wang, Zheng, Zuo, & Fang, 2005; Wu, Gao, Tsim, & Tu, 2005). The arabinogalactan from *Anadenanthera colubrina* gum exhibited immunostimulating capacity *in vitro* and *in vivo* by increasing the phagocytosis and $O_2^{\bullet -}$ on the macrophages from animals treated with the polymer (Moretão, Buchi, Gorin, Iacomini, & Oliveira, 2003). TNF-α production by peritoneal macrophages from treated mice (100 mg/kg) increased 26-fold, and in this condition it showed anti-tumoral activity against sarcoma S-180, with 38% inhibition when compared to the control group (Moretão et al., 2004). The immunostimulatory

activity found for polysaccharides from *Panax ginseng* may have been responsible for macrophage-mediated cytotoxicity against B16 melanoma by macrophages previously treated with the polymer (Shin et al., 2002). An acidic arabinogalactan plant cell culture of *E. purpurea* containing (1 → 2)-linked Rha units, similar to the one obtained in this work, stimulated macrophages *in vitro* to secrete mediators like TNF-α, interleukins-1 and interferon-β2. This polymer, when injected intraperitonially, stimulated an eight-fold oxygen radical release by macrophages when compared to the control group (Wagner et al., 1988; Luettig, Steinmuller, Gifford, Wagner, & Lohmann-Matthes, 1989). From the stems of *Cistanche deserticola*, an arabinogalactan was able to stimulate the proliferation of cultured lymphocytes in a dose-dependent manner reaching an induction of over 30-fold at treatment with 100 μg/ml of arabinogalactan. Three arabinogalactans purified from roots of *Baptisia tinctoria* and *E. pallida* and suspension culture of *E. purpurea* exhibited different immunomodulatory properties. The one from *B. tinctoria* stimulated to a great extent nitrite and IL-6 production by alveolar macrophages and Ig-M production of mouse lymphocytes, while the arabinogalactan isolated from *E. purpurea* had only weak activity. The authors suggest that the presence of 5-linked Ara*f*, which is also present in the arabinogalactan from *E. uchi*, is important, since arabinogalactan of *B. tinctoria* contained 8.7% of 5-linked Ara*f* and the arabinogalactan from *E. purpurea* contained only traces of it (Classen, Thude, Blaschek, Wack, & Bodinet, 2006).

Although the arabinogalactan from *E. uchi* did not induce cell death by any mechanism investigated, its antitumor potential cannot be ruled out, since the anti-tumor activity of a polysaccharide was usually believed to be a consequence of the stimulation of the cell-mediated immune response (Ooi & Liu, 2000). In addition, the AGP from *E. uchi* bark decoction shares structural features with arabinogalactans from other sources involved in immunomodulatory effects *in vitro*. It would therefore be of interest that future studies test whether the arabinogalactan from *E. uchi* exhibits immunomodulatory properties.

5. Conclusion

The *E. uchi* bark decoction which is used in traditional medicine as an anti-inflammatory agent and to treat arthritis, cholesterol, diabetes, uterine infections and myomas, contains a type II arabinogalactan. The purified arabinogalactan from the *E. uchi* bark decoction exhibited anti-proliferative effect against HeLa cells. The results suggest that the cytotoxicity exhibited by the polysaccharide does not involve pathways related to the cell cycle.

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