Carbohydrate Polymers 78 (2009) 361–363

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Polygalacturonic acid: Another anti-ulcer polysaccharide from the medicinal plant *Maytenus ilicifolia*

Thales R. Cipriani [a], Caroline G. Mellinger [a], Lauro M. de Souza [a], Cristiane H. Baggio [b], Cristina S. Freitas [b], Maria Consuelo A. Marques [b], Philip A.J. Gorin [a], Guilherme L. Sassaki [a], Marcello Iacomini [a,*]

[a] Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, CEP 81531-980, CP 19046, Curitiba, PR, Brazil
[b] Departamento de Farmacologia, Universidade Federal do Paraná, CEP 81531-980, CP 19031, Curitiba, PR, Brazil

ARTICLE INFO

Article history:
Received 19 February 2009
Received in revised form 11 March 2009
Accepted 23 March 2009
Available online 28 March 2009

Keywords:
Maytenus ilicifolia
Polysaccharide
Anti-ulcer

ABSTRACT

Maytenus ilicifolia is one of frequently used medicinal plants in Brazil. Its leaves are used in homemade and industrial medicines for effective treatment of stomach ulcers. A polygalacturonic acid (PGA) was obtained from its leaves by aqueous extraction, followed by fractionation via a freezing–thawing process and Fehling precipitation. Methylation analysis and ^{13}C NMR spectroscopy showed PGA to consist of (1 → 4)-linked α-D-Gal*p*A repeating units. It significantly inhibited ethanol-induced gastric lesions in rats, with an ED_{50} of 103 mg/kg, suggesting that it has a protective anti-ulcer effect. This polysaccharide may thus play an important role in the anti-ulcer effect of *M. ilicifolia*.

1. Introduction

Maytenus ilicifolia is a medicinal plant found in Paraguay, Uruguay, Argentina, and Southern Brazil. Its leaves are widely used as infusion for effective treatment of stomach ulcers and gastritis (Souza-Formigoni et al., 1991). Therefore, *M. ilicifolia* was approved as an herbal medicine in Brazil, and extracts obtained by maceration of its leaves in alcohol, standardized by their tannin contents, are commercialized. This process favors the extraction of secondary metabolites over primary ones (e.g. polysaccharides). However, many herbs used in popular medicine have been reported to contain polysaccharides having anti-ulcer effect, including *Panax ginseng* (Sun, Matsumoto, & Yamada, 1992), *Bupleurum falcatum* (Yamada, 1994), *Angelica sinensis* (Ye, So, Liu, Shin, & Cho, 2003), and *Cochlospermum tinctorium* (Nergard et al., 2005).

We have recently described a structural characterization of a type II arabinogalactan (Cipriani et al., 2006) and an acidic heteroxylan (Cipriani et al., 2008) obtained from infusion and alkaline extraction of *M. ilicifolia* leaves, respectively, which had a protective anti-ulcer effect. We now investigate if other polysaccharides from this plant could also have the same activity, so a pectic polysaccharide, liberated by aqueous extraction was isolated, characterized, and its anti-ulcer protective effect determined.

2. Experimental

2.1. Plant material

Leaves of *M. ilicifolia* Mart. ex Reissek (Celastraceae) were collected in Curitiba (Southern Brazil), and identified by Dr. Olavo Guimarães, Department of Botany, Federal University of Paraná (UFPR).

2.2. Obtaining PGA

Ground leaves (350 g) were defatted with $CHCl_3$–MeOH (2:1, 1 L) at 60 °C for 2 h (3×) and then extracted with water (1 L) under reflux for 3 h (3×). The aqueous extract was evaporated to a small volume, which was added to EtOH (3× vol.). The resulting precipitate was dissolved in water, dialyzed, and submitted to freeze–thawing until no more precipitate appeared. The soluble component was treated with Fehling solution (Jones & Stoodley, 1965), and the resulting insoluble Cu^{2+} complex isolated by centrifugation, neutralized with HOAc, dialyzed, and deionized with H^+ form cation-exchange resin, to give PGA. Its homogeneity and average molar mass (M_w) was determined by high-performance size-exclusion chromatography (HPSEC) coupled to refractive index and multi-angle laser light scattering detectors, as previously described (Cipriani et al., 2008).

2.3. Carboxy-reduction

PGA (30 mg) was carboxy-reduced by the carbodiimide method (Taylor & Conrad, 1972), using $NaBH_4$ as the reducing agent, giving

* Corresponding author. Tel.: +55 41 3361 1655; fax: +55 41 3266 2042.
E-mail address: iacomini@ufpr.br (M. Iacomini).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.03.026

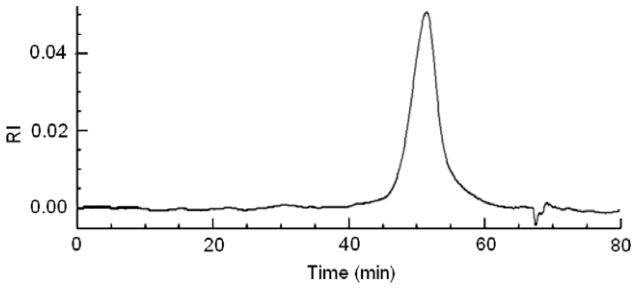

Fig. 1. Profile obtained on HPSEC of PGA.

a product (PGA-CR) with the $-CO_2H$ groups of its uronic acid residues reduced to $-CH_2OH$.

2.4. Monosaccharide analysis

PGA and PGA-CR (2 mg) were hydrolyzed in 2 M TFA (1 mL, 100 °C, 8 h), the solution then evaporated, and the residue dissolved in water (1 mL). The resulting monosaccharide was examined by silica-gel 60 TLC (Merck), the plates being developed by EtOAc:*n*-PrOH:HOAc:H_2O (4:2:2:1) and stained with orcinol–H_2SO_4 (Sassaki, Souza, Cipriani, & Iacomini, 2008). The hydrolyzate of PGA-CR was reduced ($NaBH_4$, 2 mg, 18 h), HOAc was then added, the solution evaporated to dryness and the resulting boric acid removed as trimethyl borate by co-evaporation with MeOH. Acetylation was carried out with Ac_2O–pyridine (1:1, 2 mL, 12 h), and the resulting alditol acetate extracted with $CHCl_3$. This was analyzed by GC–MS (Varian Saturn 2000R), using a DB-225 column (30 m × 0.25 mm) programmed from 50 to 220 °C at 40 °C/min, and helium as carrier gas. Component was identified by its typical retention time and electron ionization spectrum.

2.5. Methylation analysis

PGA-CR (5 mg) was dissolved in DMSO (1 mL), followed by addition of powdered NaOH (300 mg), and MeI (1 mL). The mixture was vigorously shaken for 30 min and then left for 18 h at rest. After neutralization with HOAc, the per-*O*-methylated derivative was extracted with $CHCl_3$. It was hydrolyzed with 50% v/v aq. H_2SO_4 (0.5 mL, 0 °C, 1 h), which was diluted to 5.5% and maintained at 100 °C for 17 h, then neutralized ($BaCO_3$), filtered, reduced ($NaBD_4$), and acetylated, as described above, to give partially *O*-methylated alditol acetate. It was analyzed by GC–MS, using the conditions described for alditol acetates, except the final temperature that was 215 °C. It was identified by its typical retention time and electron ionization spectrum (Sassaki, Gorin, Souza, Czelusniak, & Iacomini, 2005).

2.6. NMR spectroscopy

The ^{13}C NMR spectrum was obtained using a 400 MHz Bruker model DRX Avance spectrometer with a 5 mm inverse probe, at 50 °C in D_2O. Chemical shifts (δ) are expressed in ppm relative to acetone (δ 30.2).

2.7. Animals

Female Wistar rats (180–200 g) were maintained under standard laboratory conditions (12 h light/dark cycle, 22 ± 2 °C). Standard pellet food and water were available *ad libitum*. The animals were deprived of food 15–18 h prior to the experiment. The experimental protocol was approved by the Institutional Ethics Committee of UFPR.

2.8. Induction of gastric lesions in rats

Fasted rats ($n = 6$) were orally fed with vehicle (water, 0.1 mL/100 g body weight), omeprazole (40 mg/kg), or PGA (1, 10, 100 mg/kg), 1 h before administration of 80% EtOH (0.5 mL/200 g, *p.o.*). They were killed by cervical dislocation 1 h after treatment. The gastric lesion area (mm^2) was determined as length × width of lesion.

2.9. Statistical analysis

Results are expressed as means ± standard error of the mean (SEM) and statistical significance was determined using one-way analysis of variance (ANOVA), followed by Bonferroni's test. Data were considered different at a significance level of $p < 0.05$.

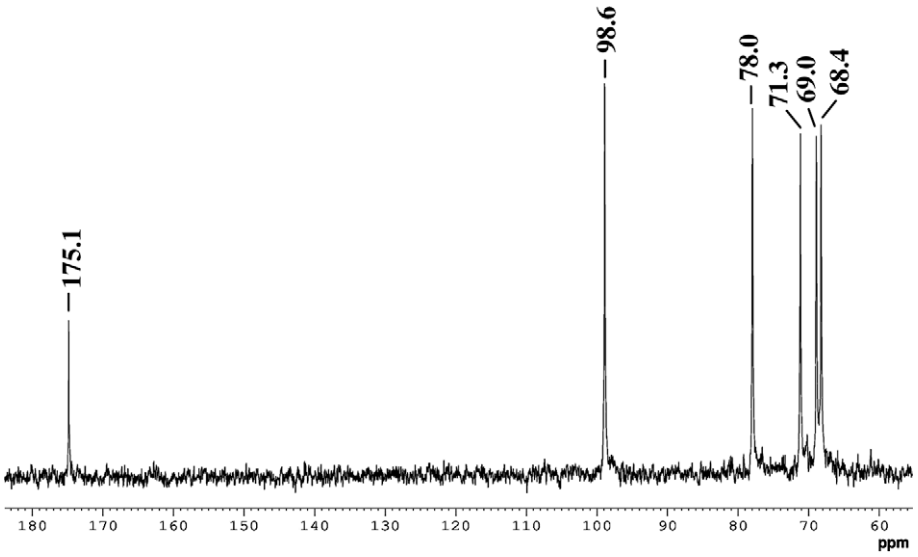

Fig. 2. ^{13}C NMR spectrum of PGA: solvent D_2O, at 50 °C, numerical values are in δ, ppm.

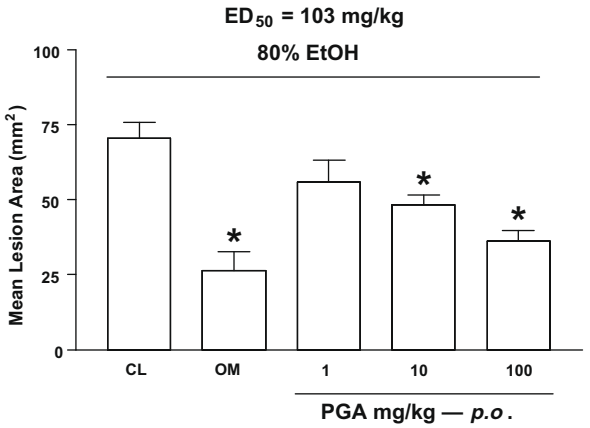

Fig. 3. Protective effect of PGA against ethanol-induced gastric lesions (CL: control, water 0.1 mL/100 g, *p.o.* and OM: omeprazole 40 mg/kg, *p.o.*). The results are expressed as mean ± SEM ($n = 6$), with $^{*}p < 0.05$ when compared to control group.

3. Results and discussion

Leaves of *M. ilicifolia* (350 g) were defatted and then extracted with water under reflux. The aqueous extract was treated with excess EtOH to obtain a crude precipitate of polysaccharides. It was submitted to freezing–thawing, and the supernatant then treated with Fehling solution, which gave rise to a precipitate component (PGA, 1.1 g). HPSEC (high-performance size-exclusion chromatography) analysis (Fig. 1) showed it to be homogeneous, with an average molar mass (M_w) of 12,200 g/mol.

TLC of a PGA hydrolyzate showed the presence of galacturonic acid only, monosaccharide analysis of carboxy-reduced PGA (PGA-CR) give rise to 100% of galactose, and methylation analysis showed that these galactosyl units were 4-*O*-substituted, in accordance with formation of 2,3,6-Me_3-galactitol acetate. The ^{13}C NMR spectrum of PGA (Fig. 2) gave rise to six signals, with one in δ 98.6 corresponding to the C-1 and another in δ 175.1 to the $-CO_2H$ group of α-D-Gal*p*A units. The substitution at O-4 was confirmed by a signal at δ 78.0, while those at δ 71.3, 69.0, and 68.4 were from C-5, C-3, and C-2, respectively (Tamaki, Konishi, Masakazu, & Masakuni, 2008). These results indicated that PGA is a polysaccharide composed of (1 → 4)-linked α-D-Gal*p*A units, namely polygalacturonic acid.

Maytenus ilicifolia is widely used for treatment of stomach ulcers and gastritis. In order to determine if PGA had anti-ulcer activity, oral treatment of 1, 10, and 100 mg/kg was performed with female Wistar rats. The gastric lesions induced by EtOH were reduced in a dose-dependent manner by 21%, 32%, and 48%, respectively, with an effective dose 50% (ED_{50}) of 103 mg/kg. Omeprazole (40 mg/kg), the positive control for the test, gave 64% reduction of the lesions (Fig. 3). Mechanisms suggested for anti-ulcer effects of polysaccharides are their ability to protect the mucosa by functioning as a protective coating, diminishing secretion of acid and pepsin, increasing mucus synthesis and/or scavenging radicals (Matsumoto, Moriguchi, & Yamada, 1993; Nergard et al., 2005; Yamada, 1994).

The results obtained with PGA and with other polysaccharides (Cipriani et al., 2006, 2008) have shown that they can contribute to the anti-ulcer property of *M. ilicifolia*. On drinking tea, water-extractable polysaccharides, as PGA, are regularly ingested. However, on medicines obtained by maceration of the leaves in alcohol, polysaccharides are not present. Thus, the residues from maceration could be used to obtain these active compounds, increasing the pharmacological value of *M. ilicifolia*.

Acknowledgments

Brazilian funding agencies CNPq and *Fundação Araucária*.

References

Cipriani, T. R., Mellinger, C. G., Souza, L. M., Baggio, C. H., Freitas, C. S., Marques, M. C. A., et al. (2006). Polysaccharide from a tea (infusion) of *Maytenus ilicifolia* leaves with anti-ulcer protective effects. *Journal of Natural Products, 69*, 1018–1021.

Cipriani, T. R., Mellinger, C. G., Souza, L. M., Baggio, C. H., Freitas, C. S., Marques, M. C. A., et al. (2008). Acidic heteroxylans from medicinal plants and their anti-ulcer activity. *Carbohydrate Polymers, 74*, 274–278.

Jones, J. K. N., & Stoodley, R. J. (1965). Fractionation using copper complexes. *Methods in Carbohydrate Chemistry, 5*, 36–38.

Matsumoto, T., Moriguchi, R., & Yamada, H. (1993). Role of polymorphonuclear leukocytes and oxygen-derived free radicals in the formation of gastric lesion induced by hydrochloric acid/ethanol, and a possible mechanism of protection by antiulcer polysaccharide. *Journal of Pharmacy and Pharmacology, 45*, 535–539.

Nergard, C. S., Diallo, D., Inngjerdingen, K., Michaelsen, T. E., Matsumoto, T., Kiyohara, H., et al. (2005). Medicinal use of *Cochlospermum tinctorium* in Mali: Anti-ulcer-, radical scavenging- and immunomodulating activities of polymers in the aqueous extract of the roots. *Journal of Ethnopharmacology, 96*, 255–269.

Sassaki, G. L., Gorin, P. A. J., Souza, L. M., Czelusniak, P. A., & Iacomini, M. (2005). Rapid synthesis of partially *O*-methylated alditol acetate standards for GC–MS: Some relative activities of hydroxyl groups of methyl glycopyranosides on Purdie methylation. *Carbohydrate Research, 340*, 731–739.

Sassaki, G. L., Souza, L. M., Cipriani, T. R., & Iacomini, M. (2008). TLC of carbohydrates. In M. Waksmundzka-Hajnos, J. Sherma, & T. Kowalska (Eds.), *Thin layer chromatography in phytochemistry* (pp. 255–276). Boca Raton, US: CRC Press.

Souza-Formigoni, M. L., Oliveira, M. G. M., Monteiro, M. G., Silveira-Filho, N. G., Braz, S., & Carlini, E. A. (1991). Antiulcerogenic effects of two *Maytenus* species in laboratory animals. *Journal of Ethnopharmacology, 34*, 21–27.

Sun, X. B., Matsumoto, M., & Yamada, H. (1992). Anti-ulcer activity and mode of action of the polysaccharide fraction from the leaves of *Panax ginseng*. *Planta Medica, 58*, 432–435.

Tamaki, Y., Konishi, T., Masakazu, F., & Masakuni, T. (2008). Isolation and structural characterisation of pectin from endocarp of *Citrus depressa*. *Food Chemistry, 107*, 352–361.

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.

Yamada, H. (1994). Pectic polysaccharides from Chinese herbs: Structural and biological activity. *Carbohydrate Polymers, 25*, 269–276.

Ye, Y. N., So, H. L., Liu, E. S. L., Shin, V. Y., & Cho, C. H. (2003). Effect of polysaccharides from *Angelica sinensis* on gastric ulcer healing. *Life Sciences, 72*, 925–932.