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ORIGINAL ARTICLE

Polysaccharide from Cumaru (*Amburana cearensis*) exudate and its potential for biotechnological applications

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Abstract

Amburana cearensis tree is used in various applications, from artisanal to pharmaceutical use. However, the gum extracted from its exudate has not yet been investigated. This study aimed to the physicochemical and structural characterization of *Amburana cearensis* Gum (AcG) by elemental, rheological, and thermal analyses, X-ray diffraction (XRD), high-Performance Liquid Chromatography (HPLC), Gel Permeation Chromatography (GPC), Infrared Spectroscopy (FTIR), UV-Vis spectroscopy and nuclear magnetic resonance (NMR). Additionally, a hemolytic assay was performed to evaluate the biocompatibility of AcG using human erythrocytes. The results showed that AcG consists of β-D-Galactopyranose monomers linked by glycosidic bonds (1→3). At the same time, the side chains exhibit β-Galactopyranose (1→6) and α-L-Arabinofuranoside (1→3,6) monomers as non-reducing terminals, whose biocompatibility was excellent in the model used. AcG was described for the first time as a biopolymer that could have broad applications in the pharmaceutical and cosmetic industries, justifying the interest in further studies about AcG applications.

Keywords: *gum of tree exudate, biopolymer, arabinogalactan, hemolytic activity.*

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1. Introduction

The exudation process is an adaptive strategy of higher plant species in response to environmental stresses or specific interactions with other organisms. The exudates of these plants are produced by specialized epithelial cells[1]. In general, exudates are chemically made up of substances of low molar mass (flavonoids and alkaloids), sugars, organic acids, proteins, inorganic salts, and lignins. Therefore, plant exudates can produce biopolymers called natural gums after isolation and purification procedures[2].

Polysaccharides from exudates have been widely studied because of their availability from renewable sources, low cost, biodegradability, and application versatility. Arabic gum (*Acacia senegal*) is traditionally used in both the food industry[3] and industrial sectors[4]. Cashew gum (*Anacardium occidentale*) is a good substitute for Arabic gum in various applications, such as emulsifiers[5]. In addition, the use of this gum in more sophisticated applications, such as encapsulation matrix for extracts[6] and essential oils[7]. Besides, cashew gum has been applied in biotechnology, such as, as a composite with larvicidal activity or a reducing agent in the green synthesis of nanoparticles with antimicrobial activity[8]. From this perspective, investigating new polysaccharides is necessary because physicochemical, structural, and rheological properties may vary according to the production source[9,10].

Amburana cearensis AC Smith, belonging to the Fabaceae family, is popularly known as amburana-de-cheiro, cherry tree, and cumaru. Its natural presence occurs in several states in Brazil, specifically in the Northeast, Southeast, and Center-West. Furthermore, this species is also found in other South American countries, such as Argentina, Paraguay, and Bolivia[11]. This tree, up to 15 meters tall, has large black seeds, white flowers, and a reddish-brown bark with thin layers that come off during drought, emitting a characteristic coumarin aroma[12].

In addition to its wide geographic distribution, *A. cearensis* stands out economically due to the versatility of its components.

The wood is widely used in carpentry and manufacturing barrels to store cachaça[13]. Its seeds are marketed as flavorings, and the seed extract or leaf powder offers a natural option as an insect repellent[14]. In folk medicine, cooking the bark of the trunk is used to produce homemade syrups, known as lickers, used to treat respiratory diseases[12].

Beyond traditional uses, the study of *A. cearensis* led to the discovery and characterization of new molecules, such as coumarin, amburosin A, and amburosin B, which have inhibitory activity against the malaria parasite[15] and other effects against Leishmaniasis[16]. Some constituents found in the exudate extracted from the trunk of *A. cearensis* were also identified. The new compound called 3',4'-dimethoxy-1'-(7-methoxy-4-oxo-4H-chromen-3-yl) benzo-2',5'-quinone was isolated from ethanolic fractions of its exudate[17]. Other constituents, such as Dilmin and Lulin, were isolated from its exudate[18].

Despite all the applications already proposed for *A. cearensis* tree, its gum has been little studied. Recently, the possibility of using *A. cearensis* gum in the development of biodegradable food packaging was demonstrated[19,20], but studies on the properties of this gum are still scarce.

Thus, aiming to determine the properties of this polysaccharide, this study aims to characterize the gum isolated from the exudate of Amburana cearensis (AcG), covering its physicochemical properties, chemical structure, and biocompatibility.

2. Materials and Methods

2.1 Gum extraction and purification

Amburana cearensis exudate was collected from native tree material between July and September 2019, and its species are located in Cocal da Estação-PI (Brazil), coordinates 03"28'33.00° S and 41"33'28.00° W . After collection, the material was sun-dried for two weeks until total solidification of the resin was observed, using the methodology adapted from the work of Paula et al.[9]. Initially, the solidified exudate was crushed to facilitate its solubilization in distilled water (at 5% w/v). The mixture was kept under constant stirring at room temperature (25 °C) for 2 h. Ultimately, this mixture was filtered through filter paper with a porosity of 14 µm and a glass funnel. The pH filtrate was adjusted to a neutral value with 0.05 mol/L NaOH. Then, sodium chloride at 20% w/w was added under stirring for 30 min. The polysaccharide was precipitated with ethanol 99.8% (Dynamic, Brazil) at the ratio 3:1 (v/v) ethanol: exudate solution.

The system was centrifugated at 5000 rpm for 5 min. The supernatant was discarded while the residue obtained was washed with ethanol and acetone (Dynamic, Brazil) and dried at 100 °C for 3h. Subsequently, the isolated gum (AcGIs) was crushed again and subjected to two purification steps similar to the primary extraction process, except that in the second purification, sodium chloride was not added to the system, as described in the literature[1,2,9].

The products obtained were called (AcGP1), i.e., *Amburana cearensis* gum purified once, and (AcGP2), i.e., *Amburana cearensis* gum purified after two purification steps or twice, called bipurified here. The moisture content was determined from exudate to bipurified gum. For the other analyses, only the gum of the bipurified *Amburana* (AcGP2) was used because it presented a higher degree of purity of the polysaccharide studied. Then the polysaccharide was named only *Amburana cearensis* gum (AcG). All AcG base solutions or other solutes were prepared with distilled water.

2.2 Moisture content and ash

The moisture content was determined by mass difference (in triplicate). For each material obtained from the exudate, AcGIs (isolated), AcGP1(purified), and AcGP2 (bipurified)—1.0 g were heated at 105 °C for 5 h. Afterward, each mass was measured again, and the mass difference was considered as the mass of water removed from each sample. The moisture content was determined according to Bashir and Haripriya[21], Equation 1.

$$\%MC = \left(\frac{M_H - M_d}{M_H} \right) x100 \quad (1)$$

where moisture content (MC) is expressed as a percentage of water mass per polysaccharide mass of exudate, AcGIs, AcGP1, and AcGP2; MH is the mass of the hydrated material, and Md is the mass of the dry material.

The mass difference for AcG also obtained the ash content (AS). A mass of 1.0 g of AcG was placed into a well of known mass, weighed, and calcined. A heating rate of 10 °C/min was applied up to 600 °C and maintained at that temperature for 2h. After the calcination period, the system was slowly cooled to room temperature, and the ash mass obtained was weighed. This experiment was carried out in triplicate, and the moisture content was used to determine the mass value of the dry gum. The ash content was expressed as the percentage of ash weight per mass of dehydrated gum, as calculated by Bashir and Haripriya[21] in Equation 2.

$$\%AS = \frac{A_m}{G_m} x100 \quad (2)$$

where AS is expressed as the percentage of ash mass per mass of AcG, Am is the mass of the ash, and Gm is the gum mass without moisture.

2.3 Polysaccharide composition

2.3.1 Hydrolysis of polysaccharide and determination of monomers

AcG was hydrolyzed using trifluoroacetic acid (TFA) to determine monosaccharides. A volume of 1.0 mL of water was added to 10 mg of the material and maintained under stirring for 24 h in a capped tube. Afterward, 1.0 mL of 4.0 mol/L TFA was added, and the system was sustained in a thermal bath at 100 °C for 3.5 h. Subsequently, the tubes were uncovered for the evaporation of the acid. Then, methanol was added, and the system was dried in a rotary evaporator three times.

After drying, the sample was dissolved in water with a 1mg/mL concentration or 0.1% (m/v) and analyzed by high-performance liquid chromatography (HPLC). For this purpose, a Shimadzu LC-20AD was coupled to a refractive index detector (RID-10A). A Rezex organic acid

column measuring 300 mm × 7.8 mm was used for this analysis using 8.0 mmol/L H_2SO_4 as eluent. The measurement was performed at 55 °C with a 0.6 mL/min flow rate, and the injected volume was 50 µL[21].

2.3.2 Determination of molar mass

The molar mass distribution was determined using gel permeation chromatography (GPC) on Shimadzu LC-20AD equipment and a refractive index detector (RID-10A). A linear polysep column was used for analysis, 300 mm × 7.8 mm and 0.1 mol/L $NaNO_3$ as eluent. The measurement was carried out at 30 °C with a 1.0 mL/min flow, and the injected volume was 50 µL (sample). Concentration: 1mg/mL or 0.1% (m/v). Exclusion limit: 10^3-10^7 g/mol. The calibration curve was obtained using pullulan as a pattern (Equation 3):

$$Log\,M = 14.40967 - 1.1392 \times Ve \quad (3)$$

2.3.3 Protein content

The nitrogen content (N) was initially determined by elemental microanalysis using this Series II CHNS/O Analyzer from PerkinElmer. Then, the protein content was determined using factor 6.25.

2.4 Spectroscopic characterizations (UV-VIS, FT-IR, NMR)

The UV-Vis spectrum was obtained using a Nanometers spectrophotometer ranging from 200 nm to 800 nm. The solution concentration for each sample (exudate, AcGIs, AcGP1, and AcGP2) was prepared at 0.1% (m/v).

The characterization by NMR, all ^1H, ^{13}C-BB (Broadband) - One-dimensional ^{13}C experiments carried out under total decoupling of hydrogens. The FT-IR characterization was performed on analytical grade KBr pellets using Perkin-Elmer FT-IR Spectrum 1000. HSQC and ^{13}C-DEPT 135 NMR spectra were acquired on a Bruker Avance DRX 500 spectrometer at 70 °C. The sample was dissolved in D_2O. The chemical shift was expressed in ppm using DSS as the internal standard.

2.5 Study of hemolytic activity of AcG

The hemolytic activity of *A. cearensis* gum was performed with human erythrocytes against different gum concentrations (62.5 to 2000 µg/mL), according to Quelemes et al.[22]. Briefly, blood was collected by venipuncture in an anticoagulant tube (EDTA-K2, BD Vacutainer®), and the erythrocytes were separated from the plasma by centrifugation (3600 rpm/15 min). The erythrocytes were washed thrice with saline (0.85% w/v), and a suspension (4% erythrocytes) was prepared. This erythrocyte suspension was incubated with the gum for 30 min at 37 °C; after that incubation period, the supernatant was collected, and the absorbance read at 492 nm. Saline solution (0.85% w/v) and 0.1% (v/v) Triton-X were used as controls. The percentage of hemolysis (% H) was calculated with the following Equation 4:

$$H(\%) = \frac{\mathbf{Abs}\text{Gum} - \mathbf{Abs}\text{Saline}}{\mathbf{Abs}\text{Triton} - \mathbf{Abs}\text{Saline}} x100 \quad (4)$$

3. Results and Discussions

3.1 Polysaccharide extraction and purification

The AcG extraction was based on the method described by Rodrigues et al.[23]. In this study, AcG presented a yield of 75.9%, approximating the values obtained for other polysaccharides extracted by processes based on the same method used here, such as the gum extracted from the exudate of *Anacardium occidentale*[2] (77%) and *Prunus persica*[24] (75%).

3.2 Moisture content and ash

The moisture content was evaluated for the exudate, AcGIs (isolated), AcGP1 (purified), and AcGP2 (bipurified) were respectively 11.24 ± 0.04, 10.46 ± 0.13, 8.90 ± 0.11, and 8.06 ± 0.08. The obtained values showed a gradual reduction in the hydrophilic capacity of the material as the extraction and purification processes were carried out. This is because the extract contains more carbohydrates, making it more hydrophilic. The moisture content values obtained for exudate and AcG are close to those found for other polysaccharides derived from the exudate and extracted by similar techniques, such as almond gum (12.23%) and Arabic gum (10.77%)[21].

The ash content is an important parameter, particularly for the food industry, in calculating nutritional value or as an index of food refinement[25]. The AcG ash content (5.32%) was higher when compared to other gums, such as Angico (*Anadenanthera macrocarpa)* (1.8%)[26] and banana (0.8%)[27]. This value suggests that its application as a food additive is inadequate, considering that, for this purpose, the ash content is desired to be below 2.5%[25].

3.3 Polysaccharide composition

3.3.1 UV-Vis and FTIR spectroscopies

The absorption spectra in the UV-Vis region of the exudate of *Amburana cearensis* and the polysaccharide (AcG) in their various stages of purification are illustrated in Figure 1A, where absorption bands can be observed at 251, 280, and 378 nm. The absorption bands at 251 nm and 280 nm can be attributed to the presence of proteins associated with nucleic acids that constitute the polysaccharide since absorption bands located at 260 nm and 280 nm are attributed to these chemical species[23,24].

Phenolic compounds could be identified from the occurrence of the band at 378 nm, characteristic of these compounds[25]. This is clear when considering that several quinone compounds, chalcones, and flavonoids were isolated from phytochemical studies that evaluated the constituents of the exudate of *Amburana cearensis* from ethanolic extracts[13,14].

When comparing the UV-Vis spectra of the exudate of *Amburana cearensis* with the polysaccharide (AcGIs), Figure 1A, the absence of bands at 378 nm and 251 nm, formerly attributed to phenolic compounds and amino acids, is noted in the AcGIs spectrum. However, in the same spectrum, it is possible to observe the band at 280 nm associated with the presence of protein. Interestingly, despite the successive purification steps to which the polysaccharide was subjected, the band located at 280 nm remains.

Therefore, it is possible to infer that the structure of AcG is a protein-polysaccharide complex, as observed in the polysaccharide extracted from *Acacia senegal*[26].

Thus, it is understood that the purification process to which the polysaccharide was submitted helped remove inorganic and organic compounds such as chalcones, flavonoids, and a considerable part of proteins and amino acids that were not part of the chemical structure of the polysaccharide macromolecule.

Fourier transform infrared spectroscopy (FTIR) was used to obtain spectra for Exudate, AcGIs, AcGP1, and AcGP2; however, no significant differences were observed between them, so was illustrated in Figure 1B FTIR for only AcGP2. The fact that there are no differences between the FTIR spectra of the samples evaluated (data not shown) suggests that the AcG extraction and purification process does not promote chemical changes in its structure.

The broad bands observed in the absorption region from 3170 to 3600 cm^{-1}, Figure 1B, are characteristic of the presence of O–H groups of carbohydrates[28,29]. Medium-intensity absorption bands are observed from 2840 to 3000 cm^{-1}, attributed to the symmetrical and asymmetrical vibrations of C–H of the methyl and methylene groups arising from the monosaccharide units AcG[29] and may also result from ethanol residues.

Characteristic signs confirming the presence and characterization of carbohydrates were observed at 1035 cm^{-1} assigned to the C–O group, 1050 cm^{-1} assigned to the C–O–C glycosidic bond, and 1075 cm^{-1} assigned to the C_1–H anomeric carbon[28]. The presence of the C–H bond in α and β configurations was confirmed by the deformation frequency around 712 and 780 cm^{-1}, respectively[30].

The presence of protein can be identified through the absorption frequencies at 1613 cm^{-1}, referring to type I amide carboxylate ions, at 1425 cm^{-1} (carboxylate ions), and 1300 cm^{-1} characterized by the presence of unordered protein and type III amide group[31,32]. The N–H stretch, which often absorbs at 3400 cm^{-1}[29], overlaps the wide O–H band.

3.4 Main monomers and molar mass distribution

AcGP2 after the hydrolysis process indicated the predominant presence of arabinose and galactose monomers, with a molar ratio of arabinose and galactose of 1.32:1, Figure 2. The majority presence of these monomers suggesting that the polysaccharide can be a heteropolysaccharide arabinogalactan type[3,33,34].

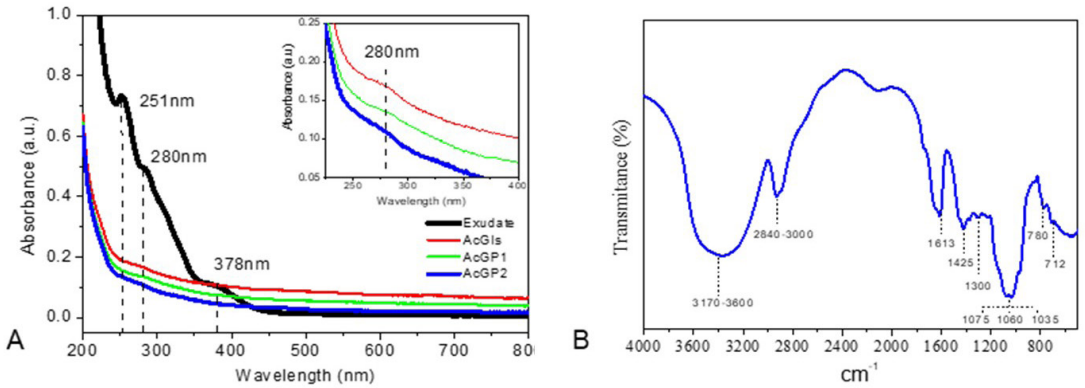

Figure 1. (A) Absorbance spectra in the Ultraviolet-Visible region of a 0.1% w/v aqueous solution of the exudate of *Amburana cearensis*, AcGIs, AcGP1, and AcGP2; (B) FTIR spectrum obtained for AcGP2 in KBr.

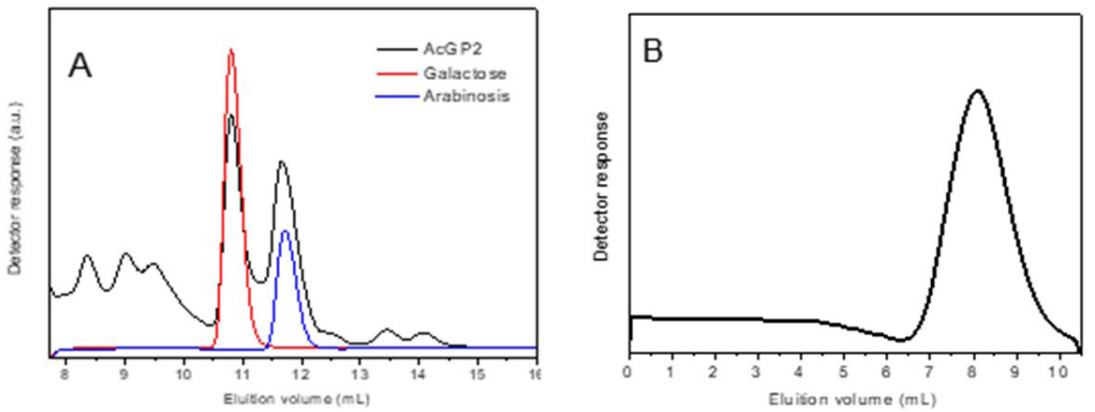

Figure 2. (A) HPLC graph to determine the main monomers of AcGP2; (B) GPC graph to determine the molar mass distribution of AcGP2.

The multiplication of the nitrogen content (N) by 6.25 is traditionally used to estimate the value of the protein content in percentage (P%)[23]. Arabinogalactans present a wide range of P% from 0.4% to 38%[3,35].

The *Amburana cearensis* gum, after the successive solubilization-precipitation stages in the extraction and purification processes, presented N equal to 0.79%, according to elemental analysis. Its estimated protein content was 4.93% ± 0.88%. This value was higher than that found in cashew gum (1.1%)[23] and Arabic gum (2.0%)[35], while well below the gum content of Acacia Senegal gum (27%)[3].

The mass distribution profile (Figure 2B) proved monomodal for AcG, indicating no mixture of types I and II arabinogalactans[34]. The weight average molar mass (Mw), 3.83×10^5 Da, peak molar mass (MpK), 1.67×10^5 Da, and a polydispersity index, 1.06×10^1, of the AcGP2 were also determined by GPC.

The assumption that the AcGP2 structure is a complex of polysaccharide-protein is reinforced by the high value of the molar mass peak determined by GPC (Figure 2B). This complex type was also observed in other arabinogalactans, such as Angico gum (*Anadenanthera macrocarpa*)[26], *Acacia senegal*, and *Acacia seyal* gums[3].

Additionally, we also investigated the rheological behavior of AcGP2 (data not shown), where the flow curves of 1% (w/v) AcGP2 aqueous solutions at 37 °C and 50 °C were obtained, and the solutions presented non-Newtonian behavior, typical of polysaccharides extracted from exudates of higher plant species such as *Prunus persica*[24] *e Sterculia striata*[10]. Thus, the viscosity behavior of AcGP2 indicates that it can be used as a material for hydrogel or film formation.

3.5 NMR spectroscopy

Figure 3 shows the ^{13}C, DEPT 135, and HSQC NMR spectra obtained in the characterization of AcG, where the signals, as well as their correlations at 106.0/4.50 and 112.0/5.23 δ (ppm), corresponded to the anomeric carbon region and were assigned to the carbon (C1/H1) of the β-D-Galactopyranose and α-L-Arabinofuranose units respectively (Figure 3A and 3B), taking into account the latter, where the shift signal at 112.0 δ (ppm) can be assigned to C1 of non-reducing terminal units. In addition, residue-related signals referring to the O-substituted and terminal non-reducing α-L-Araf units can be seen in the region of δ 111; 110, and 109; in the chemical shifts at δ 104.2, and 100, some signs correspond to residues of the β-Galp units[36-39].

The presence of signals at 72.0 and 63.8 δ (ppm) are attributed to the $–CH_2$ carbons present in the β-D-galactopyranose (C6) and α-L-arabinofuranose (C5) units. These signals are confirmed in the ^{13}C NMR spectrum in DEPT 135[40], Figure 3E.

The signals and their correlations at 106.0/4.50 (C1/H1); 72.8/3.54 (C2/H2); 84.0/4.20 (C3/H3); 71.0/3.66 (C4/H4); 75.50/3.90 (C5/H5) and 72.0/4.03 (C6/H6) δ (ppm), confirm the presence of (β-D-Galactopyranose 1→3)–bonded units, the which suggests being the main chain (Figure 4A). All signals were attributed from a comparison with studies found in the literature for polysaccharides similar to AcG, such as those extracted from the species *Lentinus* edodes[42], *Ganoderma resinaceum*[43], *Picea abies* and *Pinus sylvestris*[44].

Typical signals and correlations suggesting the presence of (α-L-Arabinofuranose 1→3,6)–bonded units (Figure 4B) showed a paramagnetic chemical shift in the ^{13}C and ^{1}H NMR spectrum at 112.0/ 5.23 (C1/H1); 83.0/3.75 (C2/H2); 79.0/3.92 (C3/H3); 87.0/4.12 (C4/H4) and 63.8/3.45 (C5/H5) δ (ppm).

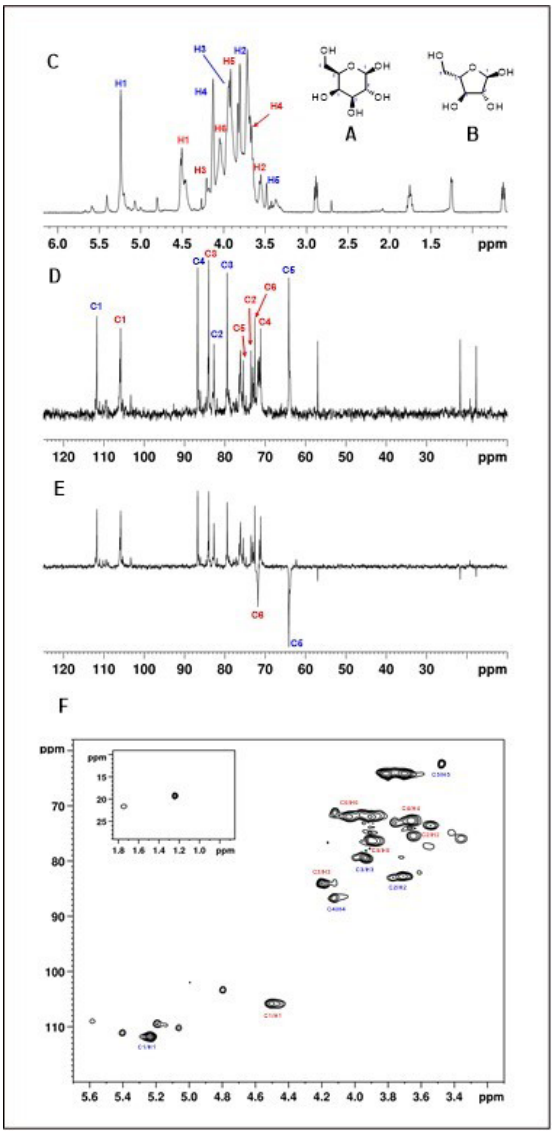

Figure 3. Unit structures (A) β-D-Galactopyranose and (B) α-L-Arabinofuranose; (C) ^{1}H NMR; (D) ^{13}C; (E) DEPT 135; and (F) HSQC. The chemical shifts in ppm of C and H refer to β-D-Galactopyranose shown in red, while to α-L-Arabinofuranose is shown in blue.

A) →3Gal*p*(1→3)Gal*p*(1→3)Gal*p*(1→3)Gal*p*(1→

B) Ara*f*(1→3)Ara*f*(1→6)Gal*p*— Ara*f*(1→3)Ara*f*(1→3)Gal*p*—

Figure 4. (A) Representation of β-D-Galactopyranose (1→3)-bonded units and (B) representation of fragments of non-reducing terminal units of arabinose and α-L-Arabinofuranose (1→3,6)-bonded chains[39-41].

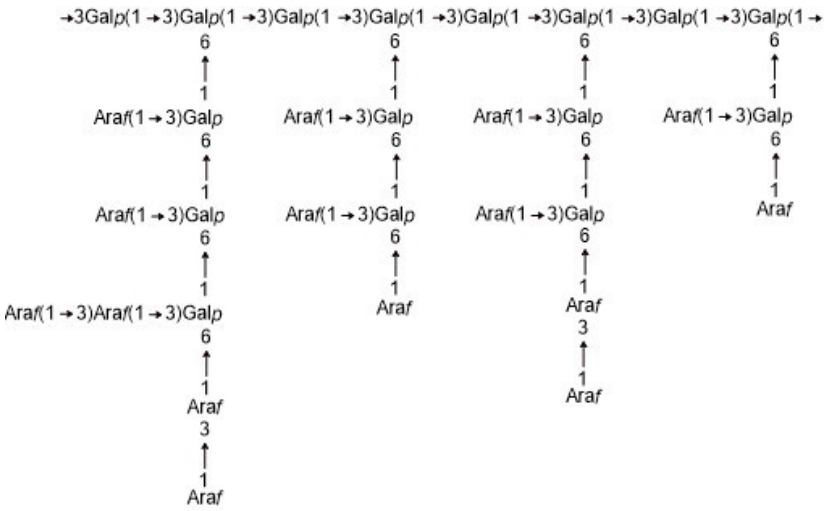

Figure 5. Suggested structure of a type II arabinogalactan found in AcG[39,41].

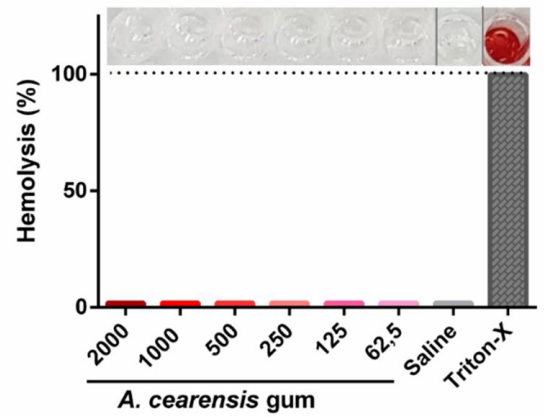

Figure 6. Hemolysis assay for biocompatibility of AcGP2.

From these results, it can be inferred that AcG can be classified as a type II arabinogalactan or arabinogalactan-protein, as they are also known (Figure 5), due to the presence of the glycosidic bond of the β-D-Galactopyranose units in its chain main of type (1→3) or (1→6)–linked with lateral branches from units of β-D-Galp(1→6) with non-reducing terminals of α-L-Araf(1→3,6), which are present in both types of arabinogalactans[41].

Because it is a polysaccharide that has not yet been studied in the literature, complementary techniques to determine exactly the arrangement of the glycosidic bonds are necessary.

3.6 Hemolytic assay

The hemolytic assay is a protocol commonly used to evaluate the toxicity of natural products against human red blood cells. When subjected to these tests, AcG did not promote hemolysis at any of the concentrations tested (≤ 2,000 μg/mL), demonstrating excellent compatibility with the cell model studied (Figure 6). Thus, apparently, the biocompatibility of AcG seems to be superior to that of other natural gums that have already been extensively studied, such as Karaya and Chichá gums (extracted from the genus *Sterculia*), which demonstrated hemolysis below 1% at the concentrations evaluated (100.0 to 0.78 μg/mL)[45]. Or even White Angico gum (*Anadenanthera colubrina*), which exhibited a hemolysis rate close to 2% at all concentrations tested (10 to 1000 μg/mL)[46]. In the work of Quelemes et al.[22], cashew gum (*Anacardium occidentale*) promoted approximately 10% hemolysis at a 1000 μg/mL concentration. These results reinforce the excellent biocompatibility of AcG and its potential for applications in biotechnology, although more studies must be carried out.

4. Conclusions

By extraction with an organic solvent, a polysaccharide was obtained from the exudate of *Amburana cearensis*. AcG was a type II arabinogalactan, constituted by a polysaccharide-protein complex. Its structure was estimated as a polymer containing the main chain composed of β-D-Galactopyranose units with glycosidic bonds (1→3), and its side chains are composed of β-D-Galactopyranose units (1→6) with non-reducing terminals of α-L-Arabinofuranose (1→3,6). Besides, hemolytic assays showed that this biopolymer has low toxicity, even when compared to other natural gums widely used in biotechnology. Thus, AcG emerges as a new product with potential applications for the industry in general.

5. Author's Contribution

- **Conceptualization** – José Regilmar Teixeira da Silva; Iranildo Costa Araújo; Eziel Cardoso da Silva; Moisés das Virgens Santana; Emanuel Airton de Oliveira Farias; Laís Ramos Monteiro de Lima; Regina Célia Monteiro de Paula; Durcilene Alves da Silva; Alyne Rodrigues Araújo; Carla Eiras.

- **Data curation** – José Regilmar Teixeira da Silva; Carla Eiras.
- **Formal analysis** – José Regilmar Teixeira da Silva; Carla Eiras.
- **Funding acquisition** – Carla Eiras.
- **Investigation** – José Regilmar Teixeira da Silva; Iranildo Costa Araújo; Eziel Cardoso da Silva; Moisés das Virgens Santana; Emanuel Airton de Oliveira Farias; Laís Ramos Monteiro de Lima; Regina Célia Monteiro de Paula; Durcilene Alves da Silva; Alyne Rodrigues Araújo; Carla Eiras.
- **Methodology** – Carla Eiras.
- **Project administration** – Carla Eiras.
- **Resources** – NA.
- **Software** – NA.
- **Supervision** – NA.
- **Validation** – NA.
- **Visualization** – NA.
- **Writing – original draft** – José Regilmar Teixeira da Silva; Emanuel Airton de Oliveira Farias; Carla Eiras.
- **Writing – review & editing** – Geanderson Emilio de Almeida.

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