Research Article

Biopolymer from *Adenanthera pavonina* L. Seeds: Characterization, Photostability, Antioxidant Activity, and Biotoxicity Evaluation

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Plant polysaccharides have been increasingly employed in the pharmaceutical, industrial, and food environments due to their versatile functional properties. In the present investigation, a heteropolysaccharide galactomannan (GAP) was extracted from *Adenanthera pavonina* L. seeds and characterized by physicochemical analyses to determine its thermal properties, photostability, antioxidant activity, and acute toxicity. GAP was characterized by FTIR, DSC, and TG. The photostability of GAP submitted to artificial UV irradiation was analyzed. Antioxidant activity was evaluated by the DPPH (2,2-diphenyl-1-1-picrylhydrazyl) free radical-scavenging method, while a bioassay method was carried out to study acute toxicity in *Artemia salina* L. Physical-chemical and functional characteristics of GAP support its potential role in the food and pharmaceutical industries. GAP was photostable under UV irradiation. *In vitro* GAP antioxidant evaluation showed that it bears free radical-scavenging activity for DPPH radicals. The median lethal concentration (LC₅₀) of GAP was 239.4 mg•mL⁻¹, indicating that this biopolymer is nontoxic. Such results indicate that this biopolymer presents characteristics of neutrality, photostability, and nontoxicity that are commercially attractive.

1. Introduction

In the past years, sustainable, ecological, and environmental materials, including natural polysaccharides, have received more attention [1]. In this context, galactomannans are the second largest group of storage polysaccharides in the kingdom plantae [2]. They consist of energy reserve polysaccharides present mainly in legume seeds, which usually have in their chemical structure linear mannose chains bound by

beta-glycosidic bonds and galactose unit branches with alpha-glycosidic bonds [3]. Moreover, galactomannans are biopolymers widely used in paper, textile, pharmaceutical, and cosmetic industries due to their viscosity, gelling, and emulsifier properties [2].

Adenanthera pavonina belongs to the family Leguminosae and subfamily Mimosoideae. In Brazil, this species is widely used in reforestation. Its seeds are popularly known as “carolina” or dragon eye and draw attention due to their

FIGURE 1: Illustration of the *A. pavonina* plant, its seeds, and the galactomannan extracted from its endosperm.

bright red color. Galactomannans extracted from the *A. pavonina* seed endosperm has attractive properties for applications in the food and biomedical industries [4]. The *A. pavonina* plant, its seeds, and the biopolymer extracted from the same can be seen in Figure 1.

Several studies have reported the *in vitro* antioxidant activities of polysaccharides. The underlying mechanism is, however, uncertain, since relationships among antioxidant activities and physical-chemical properties or structural characteristics have not been elucidated and confirmed [5].

In the present work, a galactomannan contained in *A. pavonina* seeds was first extracted and purified. Subsequently, it was analyzed by physical-chemical characterization, infrared absorption spectroscopy, thermal analyses, and photostability studies. Afterwards, the antioxidant effect and the biopolymer toxic potential in *Artemia salina* L. were investigated.

2. Material and Methods

2.1. Material. *Adenanthera pavonina* seeds were collected in Fortaleza, CE, Brazil, between July and October of 2015. A sample (voucher number 58426) was deposited at the Prisco Bezerra Herbarium of the Federal University of Ceará. 99.5% ethyl alcohol was purchased from Dynamics. 77% ethyl alcohol was purchased from Rioquímica. Methanol P.A. was purchased from Vetec. 2,2-Diphenyl-1-1-picrylhydrazil (DPPH) and potassium dichromate were purchased from Sigma-Aldrich. Trolox was purchased from Coyman Chemical. All the other chemicals employed in this study were of analytical grade and used as indicated by their manufacturers.

2.2. Extraction and Purification of Galactomannan from the *Adenanthera pavonina* Seeds. For the galactomannan

extraction, a total of 100 grams of seed mass of *Adenanthera pavonina* was placed in distilled water and heated (100°C for 20 minutes) to neutralize the enzyme activity that degrades the endosperm. After this heating, the water was discarded, and a further volume of distilled water was added at room temperature (25°C) to swell the seeds and facilitate endosperm removal. After this swelling period, the endosperms were obtained manually through separation of the seed structures and weighed. The obtained endosperms were subjected to exhaustive extraction with distilled water in a blender until complete homogenization. The galactomannan gum was filtered twice by vacuum filtration and then was precipitated twice with 70% ethanol in a ratio of 1:3 (w/w). The precipitated polysaccharide was pressed to remove the surplus ethanol. The resulting precipitation mass was solubilized in distilled water and dried using spray drying equipment (Büchi model B-290) [6]. The obtained material was packed in a glass vial and stored in a desiccator at room temperature.

2.3. pH and Zeta Potential. A solution with 1% (w/w) of *A. pavonina* galactomannan (GAP) was measured in triplicate using a Marte MB-11 pH meter, previously calibrated with buffer solution pH 4.0 and pH 7.0 [2]. A 1% (w/w) solution of GAP was prepared and the zeta potential was determined at 25°C using a Zetasizer Nano Series device (Malvern Instruments) in a capillary cell (DTS 1070) [2]. The GAP solution was prepared in MilliQ water and the zeta potential was measured in triplicate.

2.4. Fourier Transform Infrared (FTIR) Spectroscopy. The spectral properties of the polysaccharide extracted from the *A. pavonina* seeds were analyzed using Fourier transform infrared absorption spectroscopy (Agilent Technologies,

Cary 630) equipment at a room temperature of 25°C. The powder was mixed with KBr and a pellet was compressed using a KBr pellet press. The sample was scanned over a frequency range of 4000 and 650 cm⁻¹ [7].

2.5. Thermogravimetric (TG) and Exploratory Differential Calorimetry (DSC). SA409 equipment (Polyma) was used to perform the thermal analysis. The sample weight loss was recorded while the temperature was increased from 30 to 900°C, at a rate of 20°C/min, under a synthetic nitrogen air atmosphere maintained at a flow of 5 to 40 mL•min⁻¹ and under oxygen flow, maintained at 4.8 to 16 mL•min⁻¹. An uncovered alumina crucible was tared before adding a small amount of the sample with a mass of approximately 5.17 mg [8]. The GAP's thermal attributes were determined by a DSC 214 differential scanning calorimeter equipment (Polyma). The sample was heated from -10°C to 105°C, followed by cooling to -10°C, and followed by heating to 500°C in an aluminum crucible with a perforated cap with ±3.3 mg of sample, under a dynamic nitrogen atmosphere [2].

2.6. Photostability of the Polymer. A polymer solution was prepared at a concentration of 0.25% (w/v) using purified water by reverse osmosis as solvent immediately before the test and under light protection. After preparation, the solution was irradiated for 120 minutes using as a radiation source a 125 W mercury lamp bulb without the bulb and magnetic stirring of 700 rpm. The radiation intensity was measured by a Hanna HI 97500 radiometer. The solution was irradiated in a borosilicate glass reactor with temperature control (25 ± 5°C). 3.0 mL aliquots were removed using a graduated syringe interconnected to a silicone cannula at preestablished times (0, 10, 15, 30, 45, 60, 90, and 120 minutes). The UV irradiation effects were evaluated by the molecular weight determined by a falling ball viscometer (Gilmont Instruments) and calculated using the following equation:

$$\eta = \frac{\sqrt{2}}{C(\eta_{sp} - \ln \eta_{rel})^{1/2}}, \quad (1)$$

where η_{sp} and η_{rel} [9] correspond to specific viscosity and relative viscosity, respectively, and c corresponds to the polymer concentration in g•dL⁻¹.

The photostability study of *A. pavonina* galactomannan was carried out after exposure of the sample to different irradiation times. The solutions were analyzed in a UV/vis spectrophotometer (Agilent Technologies, Cary 300) from a wavelength range of 200 to 800 nm. The sample absorption spectrum was determined and the degradation rate was calculated as follows [10]:

$$\% \text{ degradation} = \frac{(A_0 - A_1)}{A_0} \times 100, \quad (2)$$

where A_0 is absorbance at initial time and A_1 is absorbance at the end time.

2.7. Antioxidant Activity of GAP by the DPPH Method. The antioxidant activity of the *A. pavonina* biopolymer was

FIGURE 2: FTIR spectra of GAP.

determined according to the methodology described by Zhao et al. [11] with some modifications. One milliliter of DPPH solution (0.3 mM) was added to 2.5 mL of different concentrations of polysaccharide solution. The solutions were shaken vigorously and kept still at room temperature (25.0 ± 3°C) protected from light for approximately 30 minutes. The DPPH free radical reduction was measured by reading in an Agilent Technologies Cary 300 spectrophotometer at 515 nm. The control consisted of 1 mL of the 0.3 mM DPPH solution and 1.3 mL of methanol. Only a blank-received P.A. methanol was used. In this assay, trolox was used as an antioxidant standard to validate the assay. The percent inhibition of the DPPH moiety for the samples was calculated as follows:

$$\% \text{ inhibition of DPPH} = \frac{(\text{abs control} - \text{abs sample})}{\text{abs control}} \times 100, \quad (3)$$

where abs control is the control absorbance and abs sample is the sample absorbance.

2.8. Biototoxicity Using *A. salina* L. The brine shrimp (*Artemia salina* L.) lethality assay was used to determine whether the biopolymer was cytotoxic [12]. Brine shrimp eggs were obtained from a Maramar aquarium store in Teresina (PI, Brazil). Briefly, *A. salina* eggs were hatched in synthetic saline (NaCl 36 g•L⁻¹) at 25°C with continuous aeration. Following 48 h of hatching, nauplii were collected ($n = 10$ nauplii/sample/concentration) and added in tubes, each one with 0.5 mL of the sample (62.5 mg•mL⁻¹, 125 mg•mL⁻¹, or 250 mg•mL⁻¹) plus 4.5 mL of artificial marine water. After 24 h, the larvae were counted and the percentage of living larvae at each dose and control was determined. The median lethal concentration (LC₅₀) value of a sample below 1000 µg•mL⁻¹ was considered toxic [13, 14].

3. Results and Discussion

3.1. Obtaining and Physical-Chemical Characterizing of the Biopolymer. GAP was obtained as a white, odorless, and very

FIGURE 3: TG thermogravimetric curves for GAP in a dynamic atmosphere of nitrogen and oxygen.

FIGURE 4: DSC curves for GAP in an inert atmosphere.

light powder. The average pH of the 1% solution (w/v) was 7.1. This physical-chemical characteristic was similar to that reported by Vendrusculo et al. [15] for galactomannan extracted from *M. scabrella*. Proximity to neutral pH implies that it will not cause irritation when used in formulations and may be accepted as a neutral excipient in various pharmaceutical formulations, including emulsions and gels. The zeta potential determination of the 1% GAP solution was carried out in triplicate with a negative value of -2.75 mV. Mittal et al. [2], when characterizing solutions at 1% of galactomannan extracted from *Leucaena leucocephala* seeds, obtained a similar zeta potential of -2.58 mV. According to some

authors, the stabilization of aqueous systems occurs when the Zp is greater than 30 mV, or lower than -30 mV, because in these values the surface loads of the studied material tend to repel, thus favoring colloidal stabilization [16].

3.2. Fourier Transform Infrared (FTIR) Spectroscopy. The FTIR spectrum of galactomannan is shown in Figure 2. The FTIR spectrum shows different peaks related to functional groups. IR spectra show a 3323 cm^{-1} absorption band, characteristic of carbohydrates indicating the vibration of the hydroxyl groups ($-\text{OH}$) of polysaccharides and also water involved in the hydrogen bond formation [3]. Another band

was detected in 2909 cm^{-1} , indicating the stretching of alkane groups ($-\text{CH}_2$). According to the study of Albuquerque in 2014 [3], in galactomannan extracted from *Cassia grandis*, the region between 3800 and 2800 represents the stretching way of the group (CH). In the region comprised between 950 and 700 cm^{-1} , the polysaccharide showed characteristic absorption at 871 and 811 cm^{-1} . Such fact can be attributed to the presence of D-galactopyranose and D-mannopyranose, respectively, revealing the coexistence of glycosidic bonds [17, 18]. Other characteristics common to polysaccharides were observed in the region of 1600 to 800 cm^{-1} , such as the detection of bands of 1629, 1375, 1021, 871, and 811. This region exhibits C-C-O, C-O-C, and C-OH-coupled stretching modes of the polymer backbone [4].

3.3. Thermal Analysis. The GAP's thermal characteristics were studied with TG (Figure 3) and DSC (Figure 4).

The TG spectra, in which the first mass loss is 14.22% and occurs in 71.4°C , and is related to the water evaporation, were justified by the polysaccharides' hydrophilic nature. Similar results were found by Mittal et al. [2] in the thermal analysis of galactomannan extracted from the *Leucaena leucocephala* seeds. The second event occurred around 290.5°C and is attributed to the polysaccharides' thermal decomposition with a mass loss of 68.19%, corroborating the data found in the literature [15], where the galactomannan's thermal behavior obtained from *Mimosa scrabella* seeds evidenced a mass loss of approximately 60% in the same temperature range that was found in this work.

The TG values obtained are reinforced by the results of DSC (Figure 4), which show an exothermic peak at around 297°C , characteristic of galactomannan degradation. The DSC thermogram shows the thermal events presented by the sample, and an endothermic peak is observed at 115°C , resulting from sample dehydration, corresponding to the hydrophilic groups. A small event around 217.6°C is also observed, characteristic of glass transition temperature.

Liyanage et al. [8] observed thermal events at similar temperature ranges when analyzing the thermal stability of galactomannans extracted from *Cyamopsis tetragonolobus* L. Thermograms of another evaluated galactomannan also presented two distinct regions of weight loss with similar results to those presented in the study herein [7].

3.4. The Polymer Photostability. UV irradiation may lead to an effective molecular bond breakdown or an effective molecular chain breakdown, leading to a reduction in the intrinsic viscosity of the polymer solution [19]. The results obtained for the energy dissipation effect on the extension of the viscosity reduction were described in Figure 5.

Under UV irradiation, a water-soluble polymer chain may rupture to produce smaller chains due to the energy transfer from the incident UV irradiation, resulting in the hydroxyl radical formation that induces breakage [19]. As it can be seen in Table 1, the intrinsic viscosity after 120 minutes of irradiation was $2.034\text{ dL}\cdot\text{g}^{-1}$, which corresponds to a reduction of 7.9%. Amrutlal et al. [19] reported a decrease of 33.4% in the intrinsic viscosity of guar gum solutions after 180 minutes of UV irradiation. In another work

FIGURE 5: Effect of irradiation on GAP degradation. Each point represents an average value obtained in triplicate.

TABLE 1: Values of time of ball drop (seconds), specific viscosity (η_{sp}), relative viscosity (η_{rel}), and intrinsic viscosity (η) calculated for a GAP solution at different irradiation times.

Irradiation time	Time of ball drop (s)	η_{sp}	η_{rel}	η
0 min	59.98	5.062	4.062	2.209 ± 0.00
10 min	59.48	5.020	4.020	2.194 ± 0.02
15 min	58.68	4.952	3.952	2.169 ± 0.03
30 min	58.62	4.947	3.947	2.167 ± 0.07
45 min	57.75	4.873	3.873	2.139 ± 0.06
60 min	57.66	4.866	3.866	2.136 ± 0.07
90 min	55.55	4.687	3.687	2.070 ± 0.03
120 min	54.45	4.595	3.595	2.034 ± 0.05

Each experiment was carried out in triplicate. Average time of ball drop of the solvent: 11.85 seconds. Intrinsic viscosity (η) was expressed as mean $\pm$ relative standard deviation (SD).

performed with chitosan solutions, the intrinsic viscosity reduction was approximately 90% [20]. Analyses with solutions of *A. pavonina* galactomannan show that the solution viscosity presented a discrete reduction by UV irradiation.

Figure 6 shows the absorption spectra of the sample irradiated at different concentrations. It can be observed that there was a decrease in the absorbance in the 260–330 nm region. The results found were better compared to studies performed with chitosan films, where the absorption spectrum was altered with the development of an absorption band at 290 nm [20].

The calculated degradation rate was 10.75%. As seen in Table 2, the absorption was discreetly reduced until 120 minutes of irradiation. This decrease is probably due to the photochemical process of the chromophores responsible for absorption in this region [20]. It can be concluded that the biopolymer extracted from *A. pavonina* is photostable, favoring its use in the pharmaceutical and cosmetic industries.

FIGURE 6: Absorption spectrum of *A. pavonina* galactomannan at a concentration of 0.25% (w/v) before and after UV irradiation.

TABLE 2: Absorbance values at maximum peaks of the UV-vis spectrum for galactomannan samples after different irradiation times.

Irradiation time	λ (nm)	$A_{\max}$
0 min	268	0.93
90 min	268	0.83
120 min	268	0.83

3.5. Antioxidant Activity. The DPPH radical elimination model is widely used as a tool to evaluate the ability to eliminate reactive species by different materials, including polysaccharides. The antioxidant mechanism of the DPPH radicals' elimination is based on the hydrogen acceptance by the DPPH radical, thus converting DPPH to a nonradical form (DPPH-H). Thus, the antioxidant activity is due to the polysaccharide's hydrogen donation capacity. The OH groups in the polysaccharide play an important role in their antioxidant activity [11]. The EC_{50} values of GAP and trolox were $75 \mu\text{g}\cdot\text{mL}^{-1}$ and $13.81 \mu\text{g}\cdot\text{mL}^{-1}$, respectively. Figure 7 shows the DPPH radical-scavenging activity of GAP.

The results indicated that the percentage of DPPH inhibition was 70.57% at a concentration of $125 \mu\text{g}\cdot\text{mL}^{-1}$ of the polysaccharide. The antioxidant activity of the biopolymer extracted from the *A. pavonina* seeds was shown to be more potent than polysaccharides obtained from *Schisandra sphenanthera*, which showed a maximum DPPH radical inhibition activity of 45% at $1500 \mu\text{g}\cdot\text{mL}^{-1}$ [11].

3.6. Estimation of the Toxic Potential. The biopolymer's median lethal concentration (LC_{50}) was $239.4 \text{ mg}\cdot\text{mL}^{-1}$

FIGURE 7: Antioxidant activities of GAP in DPPH free radical-scavenging assays.

($r^2 = 0.923$), suggesting that the biopolymer has low toxic capacity [21, 22].

4. Conclusions

Galactomannan can be easily obtained from the *A. pavonina* seed endosperm. Physicochemical evaluations of the *A. pavonina* biopolymer for FTIR showed structural similarities with galactomannans obtained from several nontraditional sources, through the observation of signals and absorption bands referring to their chemical groups.

Thermal analysis showed that GAP presented favorable thermal properties for polymer application in cosmetic

formulations. The evaluation of the *in vitro* GAP's antioxidant properties showed that it bears free radical-scavenging activity for DPPH radicals and low toxicity in the test of *Artemia salina*. GAP was shown to be photostable to UV irradiation with lower intrinsic viscosity variations than in studies using guar gum.

Given the physical, physicochemical, and toxicological characteristics, the galactomannan extracted from *A. pavonina* presented properties that contribute to the proposal of its use as an excipient of semisolid pharmaceutical forms, being able to act as an emulsion stabilizer and as the main polymeric agent of cosmetic formulations such as photoprotectors.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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