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Main Polysaccharides Isolated and Quantified of *Aloe vera* Gel in Different Seasons of the Year

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Abstract

We developed and implemented a methodology that allowed extracting and evaluating high molecular weight polysaccharides present in the gel of *Aloe barbadensis* Miller. One of the fractions evaluated revealed the presence of high molecular weight carbohydrates (200 kDa) with a behavior similar to that of acemannan and another fraction with compounds of molecular weights between 17 and 47 kDa. We quantified the concentration of acemannan for two different growing periods. The concentration of acemannan in the high molecular weight fraction was 99.97 ppm in the rainy season and 106.03 ppm in the dry season. The concentration of acemannan in the fraction of low molecular weight was 9.364 ppm during the season of greatest rainfall and 26.939 ppm in the dry season.

Keywords

Acemannan, *Aloe vera*, Gel, Polysaccharides, Rainy Season, Dry Season

1. Introduction

The genus *Aloe* has four known species with medicinal properties: *Aloe barbadensis* Miller, *Aloe perryi* Baker,

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Aloe ferox and *Aloe arborecens* [1] [2]. There is particular interest in studying the species *A. barbadensis* Miller because it is the most widely cultivated in the world and the one that is more easily managed; it is also known that this species has a more potent medicinal effect. This species is commonly known as *Aloe vera* [2] [3].

Aloe gel contains a wide composition of polysaccharides, which have been reported as agents that stimulate the immune system. Most have been identified as glucomannan, mannan (acetylated) and pectin, all with different molecular weights [4]. The pharmacological activities of acemannans include antiviral effects, induction of the production of nitric oxide, stimulation of T cells, and macrophage activation. Some acemannans can exert their therapeutic properties through macrophages [5].

Aloe has attracted interest as a crop due to its adaptability and properties; it requires limited irrigation, depending on the ability of the soil to retain moisture, being a plant species with crassulacean acid metabolism (CAM) adapted to dry conditions and high temperatures. *Aloe vera* shows variations in the efficiency of water use, biomass production and gel production when subjected to different concentrations of water [6]. In the Yucatan Peninsula, Mexico, there are 1019 hectares of plantations perfectly suited to the soil and climatic conditions of the area. The total production of *Aloe vera* in the state of Yucatan in 2007 was of 3944.06 tonnes. However, it is necessary to promote the virtues of this plant in order to give it greater added value; thus, in this work we develop and implement a methodology that allows to extract and evaluate high molecular weight polysaccharides present in the gel of *Aloe barbadensis* Miller, and also perform a characterization of the polysaccharide fractions present in the gel both in conditions of excess and scarcity of water, using acemannan as reference.

2. Materials and Methods

2.1. Reagents and Samples

Pyridine (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA); L-Butanol (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA); Sephacryl S-300-H (Sigma-Aldrich Co., St. Louis, MO); Standard Acemannan from Verapol Premium® (donated by Natural *Aloe* of Costa Rica S.A., 4 km Liberia-Guanacaste). H_2SO_4 (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA); 3,5-Dinitrosalicylic acid (Sigma Co., St. Louis, MO); Silica gel 60 G (Merck, Street 5, No.7 Industrial Park Alce Blanco, 53370, Mexico).

Freshly cut *Aloe vera* leaves were obtained from "Sabilerosdel Mayab" plantations located in Yucatan, Mexico. The leaves were harvested in two different seasons, the first in the months of August-September (rainy season) and the second in March-April (dry season), to evaluate the effect of water on the concentration of the polysaccharides.

2.2. Extraction of *Aloe vera* Gel

The leaves of *Aloe vera* were washed and disinfected in chlorinated water for 20 min and allowed to dry. Two cross sections of 15 cm and 5 cm were performed at the apex and the base respectively, and allowed to drain to remove the bitterness. The side edges were cut to loosen the epidermis on both sides. The fillet obtained was homogenized with a blender to obtain a juice.

2.3. Determination of Total Sugars

The composition of total carbohydrates was assessed using a colorimetric assay with phenol red (5% w/v in water) and concentrated sulfuric acid. The absorbance reading was 490 nm [7].

2.4. Determination of Reducing Sugars

The analysis of reducing sugars was performed following the DNS method [8].

2.5. Isolation by Ultrafiltration

One liter of *Aloe vera* gel was filtered through four layers of gauze and then with Whatmanfilter paper no. 1 to remove coarse solids. The filtrate was passed through a membrane of 0.2 μm in order to remove smaller impurities.

Using an ultrafiltration device Amicon® Ultra-0.5 (Millipore, Sigma-Aldrich Co., St. Louis, MO), we ob-

tained two fractions of *A. vera* gel with a nominal molecular weight limit (NMWL) of 100 kDa. One fraction contained components smaller than 100 kDa (concentrate), and the other compounds larger than 100 kDa (filtrate).

2.6. Molecular Exclusion Chromatography

The 100 kDa fraction was used to extract polysaccharides by size-exclusion chromatography (SEC). We used a chromatographic column of 95 cm of height and 1 cm of diameter, and a Sephacryl S-300 (Sigma-Aldrich Co., St. Louis, MO). The elution was performed with sterile bi-deionized water, and 80 fractions of 1 mL were collected. We used molecular weight markers (dextran blue) to estimate the size of the polysaccharides contained in the fractions. The 80 fractions were evaluated with thin-layer chromatography (TLC), using n-butanol: pyridine. water as solvent (6:4:3). The development was done with H_2SO_4 and H_2O (2:1), with subsequent heating at 105˚C.

2.7. Colorimetric Tests for Polysaccharides Present in the *Aloe* Extract

To determine the content of polysaccharides in the *Aloe* extract, we quantified acemannan using a colorimetric assay [9].

3. Results and Discussion

Table 1 shows the recovery percentages of polysaccharides obtained by filtration and ultrafiltration. It is noted that for every 100 g of *Aloe vera* gel, 4.27 g of gel with polysaccharides of molecular weight higher than 100 kDa are obtained.

3.1. Total Sugars

Samples of *Aloe* juice collected during times of drought and rain filtered with filter paper showed values of total sugars of 5.944 mg/mL and 2.988 mg/mL respectively, while the values for samples filtered with a 0.22 µm membrane were 4.348 mg/mL and 1,839 mg/mL, respectively (**Figure 1(a)**).

3.2. Reducing Sugars

Similarly, it was observed that the content of reducing sugars in samples of *Aloe* juice collected during the periods evaluated was 2.71 mg/mL and 1.659 mg/mL in samples filtered with paper, and 2.695 mg/mL and 1.437 mg/mL in samples filtered with a 0.22 µm membrane during times of drought and rain, respectively (**Figure 1(b)**).

The dry season led to an increase in the concentration of total and reducing sugars in both fractions. Studies found that *Aloe vera* plants underwent osmotic adjustment due to the increase in total sugars, proline and other components when subjected to different amounts of water [10]. Likewise, previous studies have indicated concluded that the presence of these compounds can vary according to the different stages of growth [11]. It is possible that the increase of sugars is related to the amount of water during the rainy season.

3.3. Analysis of the Fractions by Thin-Layer Chromatography

The fractions were analyzed by thin-layer chromatography (TLC): concentrate (≤100 kDa) and filtrate (>100 kDa); we used glucose, galactose, arabinose and acemannan standards (**Figure 2**). **Table 2** shows the retardation

Table 1. Recovery percentages of the extraction of the polysaccharides (P/P).

Process	Filtrate (%)	Retained (%)
Gel Extraction	22.69	77.31
Filter Paper (Whatman No.1)	22.41	0.28
Membrane of 0.22 µm	17.09	5.32
Microcon® 100	12.82	4.27

Table 2. Retardation factor (Rf) obtained in *Aloe vera*.

Sample	Rf	SDs.
Glucose	0.475	0.006
Galactose	0.425	0.007
Arabinose	0.5	0.006
Acemannan	0	0
Concentrate (≤100 kDa)	0.437	0.059
Filtrate (>100 kDa)	0.437 0.625	0.0469 0.0781

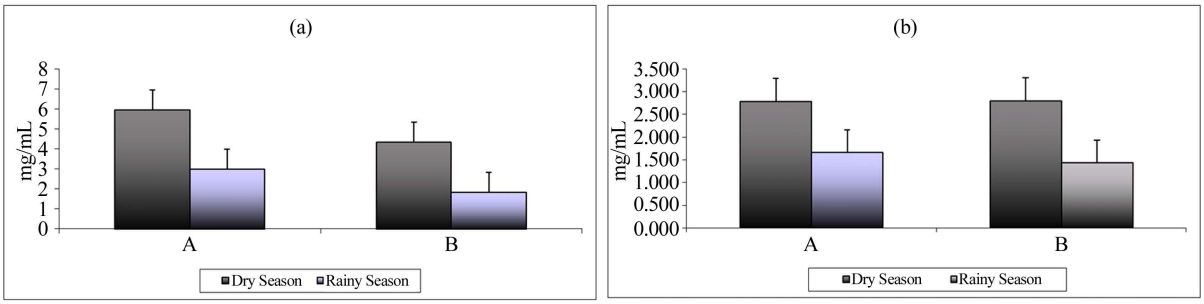


Figure 1. (a) Composition total sugars; (b) Composition of reducing sugars of *Aloe vera* in the dry and rainy seasons. (A) Filtered with filter paper; (B) Membrane filtration.

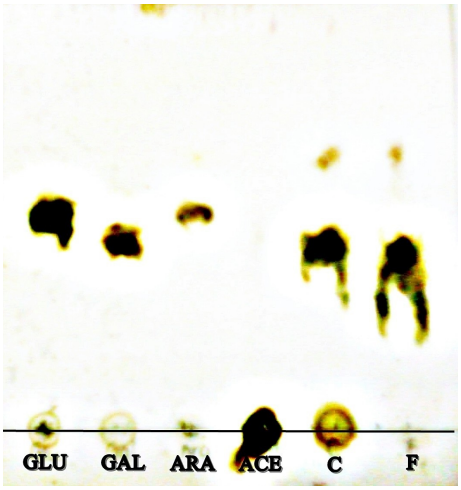
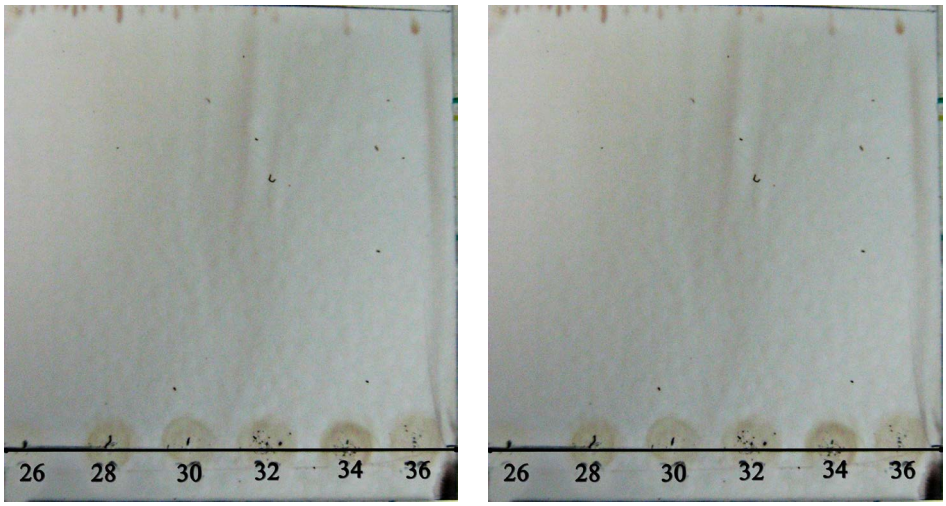


Figure 2. TLC chromatogram of the reference standards and the first 2 fractions of *Aloe*. GLU: glucose; ARA: Arabinose; GAL: Galactose; ACE: acemannan; C: ≤100 kDa; F: >100 kDa.

factor (Rf) obtained for each applied sample. The Rf for glucose was 0.475, 0.425 for galactose and 0.5 for arabinose; these values are similar to those reported by previous studies [12]. The acemannan standard was revealed at the application point; two compounds were observed in the concentrate fraction, one at the point of application, and another with an Rf of 0.437. Two compounds were also observed in the filtrate fraction, one with an Rf of 0.437 and another with an Rf of 0.625. None of the Rf coincides with the reference patterns, indicating that these are different compounds.

The analysis by TLC of the fractions of the dry season obtained by size exclusion chromatography (SEC) yielded the record of a compound at the origin of fractions 28, 30 and 32, and another analysis yielded a fainter record of a compound in fraction 34, all at the application point of the fraction (**Figure 3(a)**). In the sample of the rainy season, a compound was observed at the origin of fractions 28 to 36 (faint spot) (**Figure 3(b)**), similar



(a) (b)

Figure 3. Chromatogram of the fractions collected from the Sephacryl S-300 column (26, 28, 30, 32, 34, 36). (a) March-April (b) August-September.

to the standard of acemannan in both seasons. Similar results were reported in TLC chromatograms of the mucilaginous polysaccharide *Basellaalba* Linn, where the compound was found near the origin [13].

The fractions with compounds larger than 200 kDa were grouped together, according to the results (**Figure 4**). The type and molecular size of the polysaccharides isolated from *Aloe* gel seem to be very diverse, as described by several authors [14] [15]. The heterogeneity of the molecular weight of the polysaccharides may be due to the techniques used to isolate them, or to their degradation by the activity of the endogenous enzymes [12]. Similarly, previous studies, collected polysaccharides of between 21 and 109 kDa from elutions of 20 to 60 mL in the neutral separation (distilled water) of carbohydrates present in *Aloe* arborescens [16].

3.4. Quantification of Acemannan

Figure 5 shows the concentration of acemannan in the fractions obtained from the size exclusion column in the two seasons evaluated. The fraction of low molecular weight showed 26.939 ppm and 9.364 ppm of acemannan in the dry and rainy seasons, respectively. As expected, the shortage of rain increased the concentration of acemannan by nearly twice (**Figure 5(A)**).

In contrast, the high molecular weight fraction showed concentrations of acemannan of 106.03 ppm and 99.97 ppm for the dry and rainy seasons, respectively (**Figure 5(B)**). The increase in the concentration of acemannan was not significant (6.06%) during the dry season, indicating that there is a higher concentration of acemannan in the fraction of molecular weight greater than 200 kDa, which is consistent with the reports of some authors that the average molecular weight of the native polysaccharides such as acemannan fluctuates around two million daltons or higher [14] [15].

Recent research estimated the presence of acemannan as a phytomarker using the colorimetric method with congo red in commercial formulations of creams containing *Aloe vera*. The developed spectrophotometric method accurately measured the acemannan sample and it was found that the method was sensitive and accurate for routine analysis of the quality of *Aloe vera* in cosmetic formulations [17].

4. Conclusion

In the present work, we succeeded in developing and implementing a methodology that allowed us to extract and evaluate high molecular weight polysaccharides present in the gel of *Aloe barbadensis* Miller in times of drought and rain. The dry season led to an increase in the concentration of total and reducing sugars. The evaluated fractions revealed the presence of high weight carbohydrates (larger than 200 kDa), with characteristics similar to those of acemannan and other compounds of lower molecular weight, between 17 and 47 kDa. The concentration of acemannan was quantified in the two fractions obtained by molecular exclusion. The high

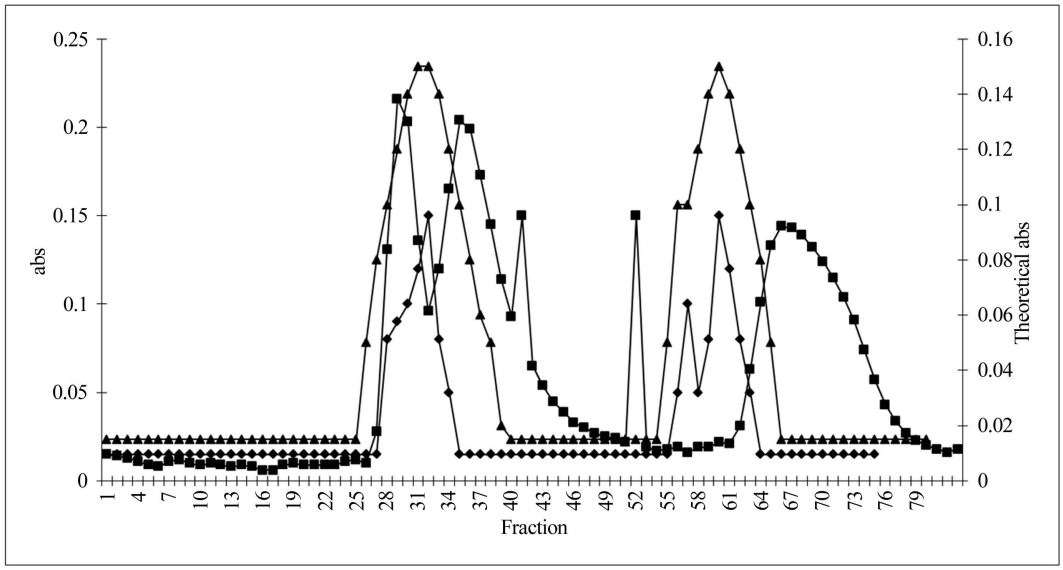


Figure 4. Comparison of *Aloe* fractions obtained by SEC on a Sephacryl S-300 column (■ Molecular weight standards; ♦ March-April season; ▲ August-September season).

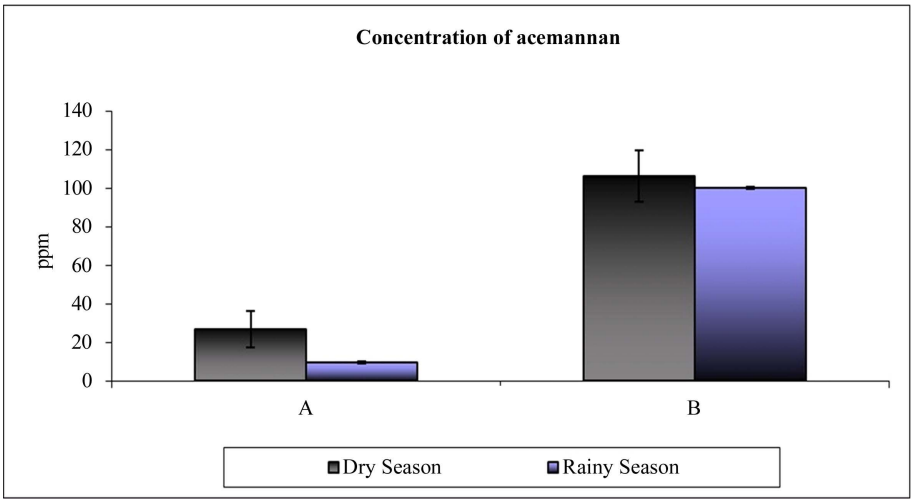


Figure 5. Concentration of acemannan in *Aloe vera* in the two seasons evaluated. (A) Low Molecular Weight Fraction (B) High Molecular Weight Fraction.

molecular weight fraction showed a higher concentration of this compound in both seasons (99.97 ppm in rainy season and 106.03 ppm in dry season) than the fraction of low molecular weight (9.364 ppm and 26.939 ppm in the rainy and dry seasons, respectively) which confirmed that the average molecular weight of acemannan was high (over two million Daltons) and that the season of drought or rain does not significantly affected the concentration of acemannan.

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