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Full Length Research Paper

Physicochemical study of acemannan polysaccharide in *Aloe species* under the influence of soil reaction (pH) and moisture application

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Two Aloe plant species were subjected to different treatment of pH (6.0, 6.5, 7.0, and 7.5) and moisture supplements at crop coefficient (kc 0.2, 0.3, 0.4, 0.5) under cultivation practices. Aloe polysaccharide (acemannan) was found to be a major component in the leaves of Aloe plant. Acemannan is considered a major and the main active ingredient in Aloe gel. Therefore, knowledge of its chemical composition and physical properties are quite necessary for preparation of medicinal drugs. Results of experiments revealed that for both the plant species, lower soil pH (6.0), and moisture supplements (k_c > 0.3) were not suitable as their acemannan exhibit lower density, less solubility in the main solvent water, poor thermal stability, and less viscosity when compared to the control. But acemannan of higher pH (7.5) and moderate moisture (k_c 0.3) showed statically better physical properties than the other one. Acemannan of *Aloe ferox* showed higher density and viscosity, higher solubility in water and thermal stability than *Aloe vera* acemannan. In hydrolytes composition of Aloe polysaccharide, mannose saccharide was found to be the major concentration in *A. vera* acemannan and glucose in *A. ferox* acemannan. They were found statically and marginally different in their concentration under treatment of pH and moisture supplements.

Key words: Aloe polysaccharide, acemannan, physicochemical properties, Aloe gel.

INTRODUCTION

Many compounds with diverse structures have been isolated from both the central parenchyma tissue of Aloe leaves and the exudates arising from the cells adjacent to the vascular bundles. Aloe is made up of a vast range of compounds which can be divided into two groups for the convenience of this study, viz., minor composition and major composition. The group of major composition includes complex sugars in Aloe leaf gel exhibiting immune stimulating action. Acemannan stands out as a significant component in the fraction of major components (Bassetti and Sala, 2005). The saccharide group makes up most of the organic substances existing in the world; it is the significant component of nutrition, and serves as energy-producing base. It is also known as carbohydrates. In plants, its synthesis is associated with photosynthesis mediated by chlorophyll. Aloe has two categories of sugars, glucose and mannose as monosaccharides (simple molecules), and acemannan and cellulose as polysaccharides (complex molecules).

In Aloe gel, the precursor beta-(1,4)-acetyl-polymannose is better known as the acemannan. In glucomannan it is made up of 97% water and 0.7% solids, a mix of simple sugars and polysaccharides with varied chain lengths and of varying molecular weight. The longer chain polysaccharides which range from 10,000 to 20,000 monomer units of glucose and mannose are called mucopolysaccharides. Because of their distinct characteristics, the mucopolysaccharides are water

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bound in nature and develop a viscous form similar to mucilage. Upon immersion in water, acemannan becomes viscous in the same way as mucilage. The chemical nomenclature of acemannan is beta-(1,4)-acetylpolymannose, because it contains a long chain polymer made up of glucose and mannose and reaches a molecular weight of about 18,000 to 20,000 units of molecular mass.

From the previous mentioned study, acemannan is considered to be the major and the main active ingredient in Aloe gel. Therefore, knowledge of its chemical composition and physical properties are quite necessary for preparation of medicinal drugs.

Physical properties of acemannan like density, solubility, viscosity and thermal stability are quite essential in the process of making drugs. These properties were found to be greatly affected by different soil environment such as the physical and chemical properties of soil which are accountable for extraneous control, like soil pH and moisture status.

MATERIALS AND METHODS

Two Aloe plant species (*A. vera* and *A. ferox*) were cultivated under different treatment of pH (6.0, 6.5, 7.0, 7.5) and moisture application at crop coefficient (k_c 0.2, 0.3, 0.4, 0.5) with one set of control (no irrigation nor control of soil pH).

During treatment, soil desiccation were maintain up to a given crop coefficient level (k_c) through irrigation of the required water to the *A. ferox* plants and soil pH were maintain up to given stresses through addition of HCl/NaOH. Required irrigation for maintaining moisture level up to crop coefficient was calculated by using following equation given by Hellman (2004).

$$\text{Required water} = \frac{\text{Evapotranspiration (loss of water ml/cm}^2\text{/day)}}{\text{Soil water holding capacity}} \times \text{Crop coefficient } (k_c)$$

After one year experiment of assessing identity, purity, quality and quantity of the Aloe polysaccharide (acemannan). Acemannan, the acetylated mannans in the gel of polysaccharides was extracted and isolated by alcohol precipitation method (McAnalley, 1990). Aloe gel was homogenized with ethanol (5 : 95 w/v) and stirred for 20 to 30 min. The alcoholic Aloe gel mixture was then allowed to stand for four hours. The clear supernatant liquid was decanted or siphoned off without disturbing the precipitate at the bottom of the container. The solution was then placed centrifuge buckets and centrifuged at 2000 g for 10 min. The precipitates was collected and washed with fresh ethanol. This fraction was then freeze-dried for 30 min. The dried pellets of polysaccharide were weighed. For qualitative and quantitative estimation of acemannan (%), the polysaccharide obtained from each samples' treatments with control sets of Aloe plant species was hydrolyzed to monosaccharide (Morrison, 1988), derivatized into alditol acetate (Hoebbler et al., 1989), and finally quantified by Gas liquid chromatography technique which is recent and conventional method (t'Hart et al., 1989). The monosaccharide standard was derivatized as the alditol acetate adopting the same procedure and the chromatogramme obtained with gas-liquid chromatography (GLC) was used to identify retention time and RF values. Shimadzu class GC-17 Gas Chromatograph with Capillary Glass column [DB 100 (30 m x 0.25 mm)], Flame ionised detector and He as carrier gas of flow rate 0.4 ml/min was used for determination of hydrolytes compositions of acemannan polysaccharides. Temperature setup was controlled at 270, 220 and 250°C of injection port, oven and detector respectively.

Isolated and purified acemannan of each treatment with control Aloe plant species were used for analysis of physical properties. The density was determined as mass (grams) per volume (milliliters) of the dry acemannan powder (AOAC, 1980). Solubility's in water and other media may differ. The method used for measurement was AOAC (1990). The solvents used were water, acetone, propylene glycol and 0.9% sodium chloride. 0.04 grams of acemannan powder was weighed and added into each of the sample tubes. Ten milliliters (10 ml) of the solvent was added producing a 0.4% (wt./volume) mixture. The mixture was subjected to agitation for 5 h at room temperature. The suspension was transferred to centrifuge tubes and centrifuged for 50 min at 1,500 rpm. The solvent blanks were similarly treated. The solutions were decanted from the solids. Both the solutions and the solids (deposits) were dried in an oven at low heat temperature (< 50°C) until it was completely dried. The dried solids were weighed; the solvent flasks that showed deposits were also weighed. This weight was subtracted from the weight of the samples.

Viscosity measurements were made using a Cannon-Fenske type viscometer (Model 150) at 40°C. The aqueous acemanna (0.2%) solutions were loaded into a viscometer and were allowed to warm for ten minutes in a water bath. The solution flow was then observed to the nearest 0.1 s according to the manufacturer's directions. The calibration factor was 0.04197 centistokes/s at 40°C.

Thermo gravimetric analysis (TGA) technique measures change in loss of weight with temperature. Weight loss was calculated using the following formula and the end of the maximum limit temperature (780°C) ash content was also calculated.

$$\text{Weight loss of acemannan and ash (\%)} = \frac{\text{Initial weight } (W_1) - \text{weight after 200°C (W2, W3, W4)}}{\text{Initial weight } (W_1)} \times 100$$

In order to find test of significance, the data of all the experimental parameters were statistical analyzed using ANOVA. The level of probability p = 0.05 was included to compute critical difference ($CD_{5\%}$) at the respective error degree of freedom ($SE_m \pm$).

RESULTS AND DISCUSSION

The results of physicochemical properties of acemannan of plant species viz., *A. vera* and *A. ferox* under different treatment of pH and moisture were represented in Tables 1 and 2.

Density of acemannan polysaccharide was recorded at 0.53 and 0.88 g/l respectively, and at low pH 6.0, to moderate the moisture supplement k_c 0.3 in *A. vera*. While in *A. ferox*, acemannan was relatively denser exhibiting the density ranging from 0.63 to 1.03 gm/l at

Table 1. Physicochemical properties of acemannan in *Aloe species* under stress conditions of soil reaction (pH) and soil moisture (k_c).

Treatment	Acemannan in *A. vera species*						Acemannan in *A. ferox species*					
	Density (g/ml)	Viscosity (centistokes/sec)	Solubility (%)				Density (g/ml)	Viscosity (centistokes/sec)	Solubility (%)			
			Water	0.9% NaCl	Acetone	Propylene glycol			Water	0.9% NaCl	Acetone	Propylene glycol
k_c 0.2	0.79	1.18	24.65	85.60	0.01	0.13	0.92	1.57	29.77	90.50	0.08	0.09
k_c 0.3	0.88	1.23	26.04	87.10	0.03	0.16	0.97	1.54	28.41	93.37	0.01	0.07
k_c 0.4	0.73	1.21	26.85	86.61	0.08	0.16	0.86	1.47	30.07	88.40	0.07	0.09
k_c 0.5	0.56	1.17	22.43	83.97	0.02	0.09	0.73	1.45	30.19	87.63	0.04	0.08
pH 6.0	0.53	1.14	21.35	77.77	0.02	0.09	0.63	1.42	29.75	84.47	0.02	0.06
pH 6.5	0.59	1.09	24.47	81.47	0.06	0.18	0.68	1.43	29.56	90.43	0.07	0.10
pH 7.0	0.74	1.24	26.53	84.57	0.02	0.11	0.88	1.51	31.48	91.53	0.09	0.13
pH 7.5	0.83	1.29	27.85	87.70	0.03	0.13	1.03	1.59	33.73	94.50	0.08	0.11
control	0.66	1.19	23.46	84.23	0.08	0.12	0.77	1.47	30.55	88.43	0.04	0.09
$CD_{5\%}$ Plant x Treatment	0.031	0.029	0.36	1.77	0.014	0.016	0.031	0.029	0.36	1.77	0.014	0.016
$SE_m \pm$	0.01111	0.01000	0.12522	0.61511	0.00510	0.00510	0.01111	0.01000	0.12522	0.61511	0.00510	0.00510

pH of 6.0 to higher pH of 7.5, respectively. For both plant species, lower soil pH (6.0) and moisture supplements ($k_c > 0.4$) were not suitable, as their acemannan exhibited lower density compared to the control one. The variation in density of acemannan depends on the content of inorganic salt which co-precipitate with the acemannan and also with the rate of hydration. The higher density of acemannan powder, the more rapid will be the lyophilization and the more ease in drugs formation.

The viscosity of the aqueous solution of acemannan is depicted in Table 1. Acemannan in *A. ferox* was more viscous ranging from 1.42 to 1.59 centistokes/s in comparison with that of *A. vera* which range from 1.09 to 1.29 centistokes/s. The viscosity of acemannan significantly varied with plant species and the soil environment during cultivation. This studied ascribed *A. ferox* polysaccharide as more useful for commercial production of herbal products.

Solubility of acemannan signifies penetrating capacity of the component in a medium which is conducive attribute in making of drugs. Therefore, pharmaceutical industries use the medium as a suitable solvent. Acemannan, by visual observation, is a white to off white amorphous powder. The data on solubility of acemannan are presented in Table 1. Acemannan powder dissolving in pure water will produce a highly viscous solution. Solubility of acemannan was significantly but not drastically affected with various source of production. McAnalley (1990) also reported that acemannan powder varied in character of solubility that depends slightly on the source of Aloe leaf but largely on the degree of processing such as filtration, ethanol precipitation and drying. From the aforementioned study, it was concluded that the solubility of acemannan was significantly affected by plant species but marginally affected with soil treatments during cultivation. Acemannan in *A. vera* at pH 7.5 was soluble in water to a great extent (27.85 to 21.35%), while in *A. ferox*, the solubility varied from 33.73 to 28.41% at pH 7.5 and pH 6.0 treatment.

The data in Table 1 reveals that the various solvents attained different solubility ratio of acemannan polysaccharide for both the plant species. Acemannan was particularly insoluble in common organic solvents such as acetone and propylene glycol, but fully soluble in inorganic solvent such as 0.9% NaCl.

Table 1 presents the solubility of acemannan in 0.9% NaCl solution as 88% in the case of *A. vera* and 94% in case of *A. ferox*. Water exhibited medium range of solubility. Therefore, acemannan formed a thick gel in propylene glycol and used in pharmaceutical industries.

Thermo gravimetric analysis gives an idea in characteristic weight loss profile with temperature. Effect of temperature on acemannan weight loss is presented in Table 2. The data revealed that

Table 2. Physico-chemical as thermal stability properties of acemannan (% of weight loss) in Aloe species under stress conditions of soil reaction (pH) and soil moisture (k_c).

Treatment	*A. vera*								*A. ferox*							
	Thermal stability (% of weight loss)				Chemical composition (%)				Thermal stability (% of weight loss)				Chemical composition (%)			
	200°C	400°C	600°C	>600°C (residue)	Mannos	Glucose	Galactose	Xylose	200°C	400°C	600°C	>600°C (residue)	Mannos	Glucose	Galactose	Xylose
k_c 0.2	40.30	45.23	78.70	25.17	60.9	15.9	3.9	1.9	38.08	42.48	72.23	30.63	25.4	50.3	5.1	6.1
k_c 0.3	40.41	45.38	78.36	26.71	64.2	22.7	6.6	3.0	38.38	42.66	71.76	32.79	31.0	71.2	8.2	5.6
k_c 0.4	40.50	45.67	77.93	26.34	73.7	24.0	6.0	1.2	38.53	43.06	72.26	32.45	34.9	82.0	6.3	4.0
k_c 0.5	40.39	45.54	78.25	23.15	56.0	19.1	3.2	0.6	38.43	42.95	71.84	30.66	29.7	45.3	5.3	2.6
pH 6.0	39.74	44.81	78.16	21.67	33.1	6.6	2.0	0.5	38.38	43.15	72.09	28.79	14.8	48.7	2.7	2.8
pH 6.5	40.77	44.58	77.88	22.58	56.8	10.8	1.8	1.6	38.44	43.27	72.46	29.22	18.3	58.8	3.5	3.6
pH 7.0	41.81	45.22	78.27	24.99	66.7	20.4	2.9	2.2	38.44	42.77	72.35	31.67	21.4	75.5	4.5	4.2
pH 7.5	39.99	45.08	78.14	25.71	85.4	32.5	5.0	2.4	38.32	42.36	71.65	32.93	23.4	79.9	8.9	4.7
Control	38.20	44.84	78.36	22.34	49.0	20.6	2.3	1.9	38.09	43.16	71.55	29.46	14.4	56.5	3.2	3.8
$CD_{5\%}$ Plant x Treatment	0.080	0.087	0.087	0.085	3.10	2.50	0.78	0.67	0.080	0.087	0.087	0.085	2.59	4.04	0.86	0.71
SE_m ±	0.02811	0.03000	0.03000	0.03000	1.064	0.857	0.268	0.228	0.02811	0.03000	0.03000	0.03000	0.887	1.385	0.296	0.242

acemannan decomposition pattern was different from those of other polysaccharides. Under identical operating conditions, the pattern of thermal decomposition was different from those of cellulose, dextran or amylan. Significant weight loss of acemannan was identified at temperature ranging from 200 to 600°C. The acemannan fractionation was controlled by these two temperature levels. However, the various treatments of soil pH and moisture supplements during cultivation of *A. species* marginally affected (not dramatic change) the thermal stability of acemannan.

Data in Table 2 represent the mean hydrolyte composition of acemannan polysaccharide under influence of treatment of pH and moisture supplements. The data revealed that several monosaccharides like mannose, glucose, gulactose, xylose and arabinose in trace amount were found in acemannan polysaccharides of Aloe plants (Figure 1a and b). The quantity was significantly affected by the different treatments of soil pH and moisture supplements of individually *Aloe species*.

Glucose was found to be maximum (79.9%) in the hydrolyte of *A. ferox* species while mannose (85.4%) was in *A. vera.* Other monosaccharide released often hydrolysis include arabinose, galactose and xylose in lesser contents (Table 2). Moreira and Filho (2008) also reported similar composition of polysaccharides.

In the *A. vera* gel, a fraction of polysaccharide acemannan was seen as being composed of different constituting monosaccharides were mannose (33.1 to 85.4%), glucose (6.6 to 32.5%), galactose (1.8 to 6.6%), xylose (0.5 to 3.0%) and arabinose remained un-detected.

In *A. ferox* species, a fraction of acemannan contained 45.3 to 79.9% glucose, 14.4 to 34.9% mannose, 2.7 to 8.9% galactose, 2.6 to 6.1% xylose and arabinose un-identified. The variation in the constituting monosaccharides is the significant result of the various treatment effects of soil pH and moisture supplements during cultivation of the two Aloe species. Table 2 shows the effect of species difference of Aloe plants as being the most significant one. It was explained by the fact that the mannosyl residues are contained in a reserve polysaccharide within the parenchyma cells which is significantly influenced by seasons and cultivation practices. Femenia et al. (1999) drew similar reasoning for the quantitative variation of acemannan composition in Aloe plants.

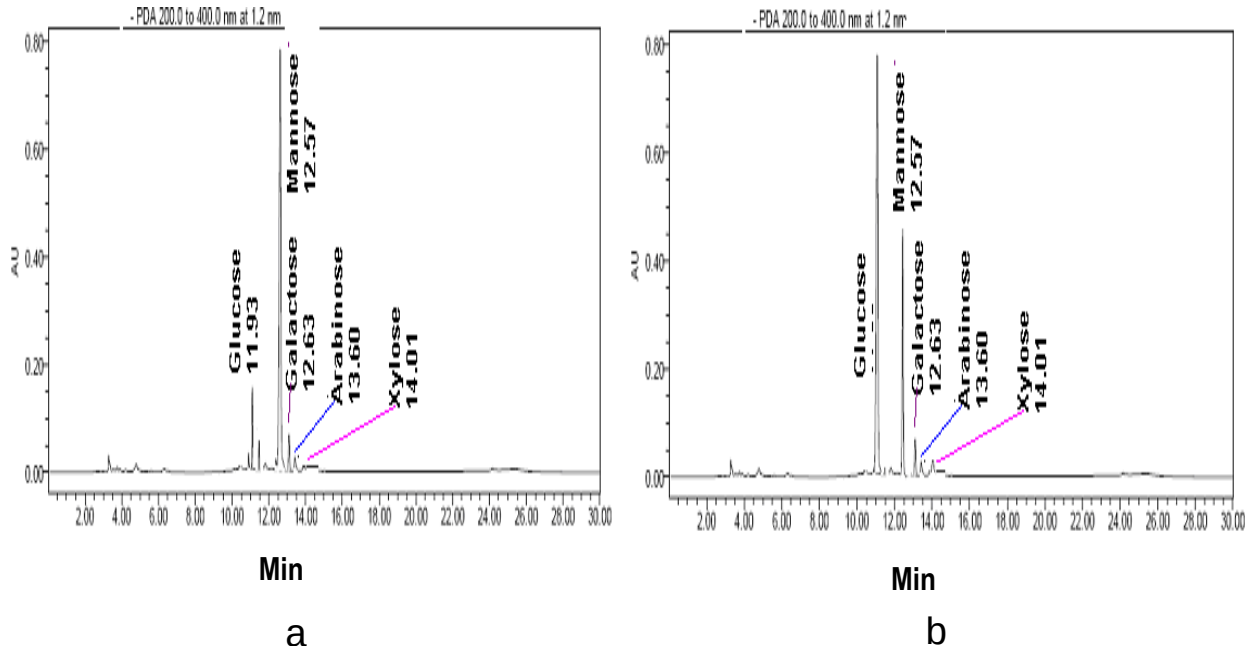

Figure 1. GLC chromatogram of various nutritive contents in gel hydrolyte of acemannan (a) *A. vera* and (b) *A. ferox* of treated the samples (pH 7.5).

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