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RESEARCH ARTICLE

CHEMICAL CHARACTERISTICS OF ACEMANNAN POLYSACCHARIDES OF *ALOE FEROX SPECIES* UNDER STRESSES OF SOIL pH AND IRRIGATION

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ABSTRACT

Aloe is made up of a vast range of compounds which can be divided into two groups for convenience of study viz., minor composition and major composition. The group of major composition includes complex sugars in *Aloe* leaf gel exhibiting immune stimulating action. From the various studies found that acemannan is considered a major and main active ingredient in *Aloe* gel. Acemannan stands out as a significant component in the fraction of major components, Therefore, knowledge of its chemical composition and physical properties are quite necessary for preparation of medicinal drugs. *Aloe ferox* is among the tallest of the more than 400 aloe species. Compared to the more widely known *Aloe Vera*, *Aloe ferox* produces 20 times more bitter sap and has higher nutrient concentrations. But concentration of polysaccharide, composition of gel, yield and growth attributes of *Aloe* plants are in considerable amount varied with species, climate, and exposure to sunlight, harvesting method and soil environment Thus in this investigation, studied the production of gel aloin, and acemannan polysaccharides and its physicochemical properties under various soil pH along with desiccation level in *Aloe ferox* plant species. In order to evaluate the response of physiological and yield attributes along with the chemical composition of gel, the experiment was laid in completely randomized block design with three replications under pot culture study. The results suggested that high soil pH 7.5 along with moderate moisture condition (crop coefficient k_c=0.3 and 0.4) of the soil were favorable for vegetative growth and yield parameters of *Aloe ferox* plants. Aloin, dry solid gel content acemannan polysaccharides and was found maximum in dry soil stress condition. Nutritional value and mineral content also produced higher into higher soil pH and moderate moisture stress. The various treatments of soil pH and moisture supplements during cultivation of *Aloe* species appreciably affected the physical properties viz; density, solubility, viscosity and thermal stability of acemannan.

INTRODUCTION

Aloe ferox is among the tallest of the more than 400 aloe species and is native to southeastern and western regions of South Africa. Compared to the more widely known *Aloe Vera*, *Aloe ferox* produces 20 times more bitter sap and has higher nutrient concentrations. Two distinct parts of the *Aloe ferox* plant are used medicinally. Firstly the aloe exudates (bitter sap) and secondly the mucilaginous gel from the remainder of the leaf. The *Aloe ferox* bitter is best known for its use as a laxative. However, in addition to the purgative effect the anthraquinone (bitter) substance is also an antioxidant, antiviral and effective for cancer prevention. Numerous scientific studies on aloe gel are demonstrating its analgesic, anti-inflammatory, wound healing, immune modulating and anti-tumor activities as well as antiviral, anti-bacterial, antifungal and antiviral properties.

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The *Aloe ferox* juice has been shown to lower cholesterol and triglycerides while demonstrating anti-diabetic activity. *Aloe ferox* medicinal properties can be attributed to the synergistic effect of the combined nutritional elements producing a more powerful effect than the individual components. Aloes are members of the Liliaceae family and are mainly succulents. The nearly 420 species of Aloe are confined into world with *Aloe ferox* among the tallest. *Aloe ferox* occurs naturally in a broad belt along the southern and eastern coast of South Africa. *Aloe ferox* is a robust; single stemmed, plant usually 2m (80") high, but up to 5m (200") tall in older specimens.

Over 130 biological active compounds of the *Aloe ferox* have so far been reported. With so many components, aloe can be described as a pharmacy. The *Aloe ferox* leaf contains substances such as amino acids, minerals, vitamins, polysaccharides, glycoproteins, anthraquinones, enzymes, lignin, chlorophyll, saponins, sterols and other plant chemicals with numerous medicinal activities. Therefore *Aloe ferox species* is also commercially cultivated in India as medicinally herbal crop. Concentration of polysaccharide (Acemannan),

composition of gel, yield and growth attributes of *Aloe* plants are in considerable amount varied with species, climate, and exposure to sunlight, harvesting method and soil environment (Grindlay *et al*, 1986). Temperature, rainfall leaf age and salinity of soil affect the level of polysaccharide within a species (Beppu *et al*, 2004). Chemical compositions of gel are also varying from *Aloe vera* and *Aloe ferox* (Femenia *et al*, 1999). Therefore, need to study the effect on composition and concentration of gel and polysaccharide under various soil stress environment during cultivation practices. Thus in this investigation, studied the production of gel, Acemannan polysaccharides and its chemical composition under various soil pH along with desiccation level in *Aloe ferox* plant species.

MATERIALS AND METHODS

Pot experiment was conducted in research Polly house of college of Agriculture JNKVV, Jabalpur during kharif 2007. *Aloe ferox* sucker were planted in randomized block design in 16x32 cm plastic pot filled with sandy soil. Plants were treated of stress of soil pH along with desiccation during two years. Treatment was viz:

T_1=*Aloe ferox* + pH 6.0+ k_c0.2
T_2 = *Aloe ferox* + pH 6.0+ k_c0.3
T_3 =*Aloe ferox* + pH 6.0+ k_c0.4
T_4 = *Aloe ferox*+ pH 6.0+ k_c0.5
T_5=*Aloe ferox* + pH 6.5+ k_c0.2
T_6 = *Aloe ferox* + pH 6.5+ k_c0.3
T_7=*Aloe ferox* + pH 6.5+ k_c0.4
T_8 =*Aloe ferox* + pH 6.5+ k_c0.5
T_9=*Aloe ferox* + pH 7.0+ k_c0.2
T_{10} = *Aloe ferox* + pH 7.0+ k_c0.3
T_{11}=*Aloe ferox* + pH 7.0+ k_c0.4
T_{12} = *Aloe ferox* + pH 7.0+ k_c0.5
T_{13}=*Aloe ferox* + pH 7.5+ k_c0.2
T_{14} = *Aloe ferox* + pH 7.5+ k_c0.3
T_{15}=*Aloe ferox* + pH 7.5+k_c0.4
T_{16} = *Aloe ferox* + pH 7.5+k_c0.5

During treatment soil desiccation were maintain up to given crop coefficient level (k_c) through irrigation of required water to the *Aloe 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).

$$\text{Requiredwater} = \frac{\text{Evapotranspiration (loss of water ml/cm}^2\text{/day) X Crop coefficient } (\mathbf{k_c})}{\text{Soil water holding capacit}}$$

After every six months morphological growth and yield data were observed and after each year letter biochemical (protein and carbohydrate) and mineral content were evaluated. After harvested, the *Aloe* leaves have two distinct parts to be used for commercial purposes: the *Aloe* bitter (Aloin) and the *Aloe* gel (Mebusela 1990). Quantitatively, Aloin in yellow bitter sap was determined by using a Shimadzu LC-10A reverse phase HPLC system equipped with Shodex C18 column and Shimadzu PDA detector (SPD-10A). *Aloe* gel was prepared from *Aloe* leaf by 50 % IPA method according to McAnalley 1990. Chemical and biochemical composition of *Aloe* gel was analyzed in various treatments under stress of salinity and water content. *Aloe* gel solid % in gel was determined by freeze-drying technique (Waller *et al.,* 2004). Total carbohydrate % determined by phenol sulphuric acid method. Nitrogen content and Protein content (%) in gel is estimated by Kjeldhal method. Phosphorus content was determined by UV-spectrophotometer method. Potassium, calcium and sodium were estimated by Flame photometer. Other nutritionally mineral content viz: magnesium, iron copper and zinc were determined by atomic absorption spectrophotometer using diacid digestion in dry gel. The qualitative and quantitative (%) estimation of acemannan was done by gas liquid chromatographic (GLC, fig 3.5) method (t'Hart *et al.,* 1989). Prior to step up into GLC technique, the polysaccharide acemannan was hydrolyzed into monosaccharide (Morrison, 1988) and derivatized to alditol acetate form (Hoebler *et al.,* 1989). Physical properties of Acemannan polysaccharides like density, solubility, viscosity and thermal stability were determined by various prescribed method of AOAC. (Gerardo Daniel Sierra-García, 2014)

RESULTS AND DISCUSSION

Table 1 represents the results of yield parameters of *A.ferox* plants under different treatment of pH and moisture application. Yield attributes like Maximum *Aloe* gel solid (1.18%) and Aloin (61.47%) were observed in T_{14} treatment. Both treatments were found significantly higher than other treatment application.

Table 1. Growth and Yield parameter of *Aloe ferox* under soil pH along with desiccation stresses.

S.No	Treatments	Gel (%)	Aloe gel soild (%)	Acemannan %
1	T1	24.64	0.45	26.8
2	T2	26.83	0.43	37.5
3	T3	25.64	0.38	34.3
4	T4	15.62	0.31	24.4
5	T5	31.07	0.49	29.0
6	T6	33.04	0.60	47.1
7	T7	32.71	0.54	42.1
8	T8	30.80	0.37	28.0
9	T9	37.36	0.68	40.3
10	T10	60.84	1.02	65.0
11	T11	65.44	0.88	58.9
12	T12	40.16	0.66	38.9
13	T13	38.36	0.75	52.8
14	T14	68.87	1.18	87.8
15	T15	75.77	0.92	79.7
16	T16	41.49	0.79	55.7
	$CD_{(5\%)}$	2.6788	0.0519	3.47

Mebusela *et al.,* (1990) supported this study and also similar ranged of yield attributes of *A. ferox* plant species. Chemical composition of gel liquid and dry gel solid were also found significantly varied with different application of pH and moisture supplements (Table 2). Nitrogen content (1.60%) and protein (9.98%) observed maximum in T_{14} in gel liquid. *A. ferox* gel was found in higher range of protein from 3.98 to 9.98%. Femenia *et al.* (1990) also found higher range of chemical constituents for protein and sugar in *A. ferox* gel Phosphorous (0.155%), potassium (7.05%), Calcium (9.06%), zinc (0.96%), magnesium (2.72%) were recorded maximum in T_{15} treatment. Sodium (3.24%) and iron (0.56%) were recorded higher in T_{14} and copper were found rich (0.098%) in T_{12}. This finding was analogous to those reported by Rajasekaran *et al.,* 2005. Mineral contents in *Aloe* plants plays

Table 2. Biochemical and mineral content (% of dry gel) of *Aloe ferox* under soil pH along with desiccation stresses

S.No	Treatments	(%) of liquid gel			% of dry gel									
		Carbohydrate	N	Protein	P	K	Na	Ca	Zn	Cu	Mg	Fe	Galactose	Mannose	Glucose
1	T1	33.27	0.73	4.54	0.003	4.12	1.93	6.72	0.33	0.014	1.34	0.12	3.25	29.10	31.73
2	T2	38.62	0.88	5.52	0.002	4.26	1.72	7.52	0.43	0.020	1.61	0.18	3.85	30.63	31.92
3	T3	31.42	0.86	5.38	0.002	3.82	1.10	7.20	0.38	0.017	1.50	0.22	3.64	29.33	31.58
4	T4	28.44	0.64	3.98	0.002	3.55	2.24	6.82	0.30	0.013	1.44	0.14	3.13	27.40	30.45
5	T5	43.23	1.03	6.44	0.003	4.42	2.30	7.63	0.44	0.022	1.67	0.20	4.12	31.35	34.31
6	T6	48.25	1.11	6.96	0.005	4.71	2.35	7.53	0.57	0.025	1.90	0.23	4.33	32.61	35.37
7	T7	44.38	1.21	7.58	0.008	4.86	2.42	7.65	0.52	0.029	1.76	0.27	4.15	32.55	34.69
8	T8	40.37	0.93	5.82	0.006	4.36	2.38	7.33	0.46	0.018	1.51	0.21	3.65	30.37	33.51
9	T9	63.36	1.26	7.88	0.009	5.28	2.62	8.47	0.73	0.023	2.14	0.29	4.37	34.44	40.61
10	T10	73.39	1.41	8.79	0.103	6.06	2.71	8.65	0.92	0.037	2.69	0.41	5.29	34.90	44.43
11	T11	69.60	1.46	9.17	0.105	5.78	2.78	8.66	0.85	0.042	2.52	0.46	5.65	35.93	41.16
12	T12	54.39	1.18	7.38	0.015	5.49	2.61	8.19	0.56	0.098	2.34	0.29	5.01	31.50	38.68
13	T13	68.32	1.30	8.15	0.107	6.68	3.12	8.52	0.74	0.036	2.11	0.35	4.65	32.82	39.83
14	T14	76.30	1.60	9.98	0.155	6.83	3.24	8.82	0.84	0.046	2.57	0.56	5.12	36.13	46.55
15	T15	71.57	1.51	9.42	0.135	7.05	3.15	9.06	0.96	0.050	2.72	0.52	5.81	37.79	43.76
16	T16	56.37	1.34	8.38	0.116	6.17	3.12	8.08	0.76	0.042	2.34	0.38	4.51	34.86	43.39
	$CD_{(5\%)}$	0.3842	0.0201	0.1278	---	0.1136	0.1431	0.2779	0.0368	0.0482	0.1578	0.0234	0.2227	0.7812	0.8880

Table 3. Physico-chemical properties of Acemannan in aloe species under stress conditions of soil reaction (ph) and soil moisture (kc)

Treatment	Solubility				Density (g/ml)	Viscosity (centistokes/sec)	Thermal stability (TGA)			
	Water	0.9% NaCl	Acetone	Propylene glycol			200º C	400º C	600º C	>600º C (residue)
pH 6.0	28.41	84.47	0.02	0.06	0.63	1.42	38.38	43.15	72.09	28.79
pH 6.5	29.56	90.43	0.07	0.10	0.68	1.43	38.44	43.27	72.46	29.22
pH 7.0	31.48	91.53	0.09	0.13	0.88	1.51	38.44	42.77	72.35	31.67
pH 7.5	33.73	94.50	0.08	0.11	1.03	1.59	38.32	42.36	71.65	32.93
k_c 0.2	29.77	90.50	0.08	0.09	0.92	1.57	38.08	42.48	72.23	30.63
k_c 0.3	30.19	93.37	0.01	0.07	0.97	1.54	38.38	42.66	71.76	32.79
k_c 0.4	30.07	88.40	0.07	0.09	0.86	1.47	38.53	43.06	72.26	32.45
k_c 0.5	29.75	87.63	0.04	0.08	0.73	1.45	38.43	42.95	71.84	30.66
$CD_{5\%}$ Plant	0.12	0.59	0.005	0.005	0.010	0.010	0.027	0.029	0.029	0.028
$CD_{5\%}$ Treatment	0.25	1.25	0.010	0.011	0.022	0.021	0.056	0.062	0.062	0.060
$CD_{5\%}$ Plant x Treatment	0.36	1.77	0.014	0.016	0.031	0.029	0.080	0.087	0.087	0.085
$SE_m \pm$	0.12522	0.61511	0.00510	0.00510	0.01111	0.01000	0.02811	0.03000	0.03000	0.03000

important roles for physiological metabolism, vegetative growth and better sustenance under stress conditions. Despite the plants are xerophytes and halophytic, the higher contents of minerals in tissues strengthen the tolerance capacity against abiotic and biotic stresses. Flower *et al*., 1977 and Greenway and Munns (1980) stated that regulation of transport and distribution of ions within leaves cells is an important feature regarding mechanism of tolerance in *Aloe* plants. The results also depicted high contents of K and Na in the gels. These elements with high K^+/Na^+ ratio are attributed to be responsible for maintaining steady state in rate of photosynthesis conductance in leaf stomata through better water use efficiency particularly under xerophytic conditions. Wyn Jones *et al.* (1979) also suggested higher concentration of K and Na for normal growth of plants. Monosaccharide's, galactose (5.81%) and mannose (37.79%) were observed maximum in T15 while glucose (46.55%) was recorded better in T14 than other treatments in *Aloe* gel solid of *A.ferox* plant species. Moreira and Filho, 2008 also supported this ranged and composition of monosaccharide. This study concluded that higher pH (7.5 and 7.0) and moderate moisture supplements (at crop coefficient k_c 0.3 and 0.4) were required better vegetative growth yield and chemical or mineral composition of liquid and dry *Aloe ferox* gel. Genet and Van Schooter (1991) and Sheteawi *et al* (2001) were supported this studied. Study revealed that *Aloe* is made up of a vast range of compounds which can be divided into two groups for convenience of 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. From this study, acemannan is considered a major and main active ingredient in *Aloe* gel. (Supported by Gerardo Daniel Sierra-García, 2014) 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 were quite essential during processing of making drugs. These properties were found greatly affected with different soil environment on physical and chemical properties of soil liable to control extraneously viz., soil pH and moisture status (Table 3). Density of *A. ferox* was observed in the range of 0.63 to 1.03 gm/l respectively. Moisture supplement at k_c 0.3 *A. ferox* acemannan was relatively denser, the density ranging from 0.73 at k_c 0.5 to 0.97 gm/l at k_c 0.3, respectively. For the plant species lower soil pH (6.0) and moisture supplements (k_c 0.5) were significantly not suitable, as the acemannan under the soil conditions 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 rate of hydration. Higher the density of acemannan powder, more rapid will be the lyophilization and more ease in drugs formation. Table 3 depicted viscosity of acemannan aqueous solution. Acemannan in *A. ferox* was more viscous ranging from 1.42 to 1.59 centistokes/sec Moisture sulpements was found 1.57centistokes/sec at k_c 0.2 and 1.45 centistokes/sec at k_c 0.5 in *A.ferox* acemannan. In this study it was found that viscosity of acemannan was significantly affected by soil environment (pH and moisture supplements treatment) during cultivation of plant species. Solubility of acemannan signifies penetrating capacity of the component in a medium. Pharmaceutical industries use the medium as a suitable solvent. Acemannan by visual observa tion is a white to off white amorphous powder. The data on solubility of acemannan are presented in Table 3. Acemannan powder on dissolving in pure water produce a highly viscous solution. Solubility of acemannan was affected with various

sources of availability and methods of preparation (Mc Analley, 1990a). From the above study, it was found that the solubility of acemannan primarily affected with plant species and secondly with soil treatments during cultivation. Solubility of Acemannan in water solvent for *A. ferox* varied from 28.41% to 33.73% at pH 6.0 and pH 7.5 treatment respectively. Different solvents were found to have different solubility ratio to acemannan polysaccharide for the plant species. Acemannan was insoluble in common organic solvents such as acetone and propylene glycol, but completely soluble in inorganic solvent 0.9% NaCl. Solubility of *A. ferox* acemannan was reported 94.50%.in 0.9% NaCl, in propylene glycol solvent *A. ferox* acemannan was observed in the range of 0.06 to 0.13%. In acetone solubility of *acemannan* varied from 0.01 to 0.09%. Forgoing facts showed that acemannan polysaccharide was less soluble in organic solvents (propelene glycol and acetone) as compared to the water and inorganic solvent. Thermo gravimetric analysis gives an idea in characteristic weight loss profile with variation of temperature. Effect of temperature on acemannan weight loss is presented in Table 3. Significant weight loss of acemannan was identified at temperature ranging from 200ºC to 600ºC. The acemannan fractionation was controlled by these two temperature extremities. At temperature 200 ºC was found 38.53% weight loss of acemannan compound in *A. ferox* species. At temperature 400 ºC observed acemannan was reduced to 43.16% weight to original weight in *A. ferox* species. Whereas, at temperature 600 ºC the weight loss of *this polysaccharides* was observed 72.46%. Above 600 ºC plant species loosed their acemannan weigh more than 80% and form residue of ash approx 20 to 30% The results revealed that acemannan polysaccharide degraded rapidly. Results found that thermal stability of *A. ferox* acemannan was significantly affected however, the various treatments of soil pH and moisture supplements during cultivation of *Aloe* species appreciably affected the physical properties viz; density, solubility, viscosity and thermal stability of acemannan.

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