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ANALYTICAL DATA OF *ACACIA NILOTICA* VAR. *NILOTICA* GUM

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ABSTRACT. This study aimed to characterize the exudate gum from *Acacia nilotica* var. *nilotica* in Sudan and compare its physicochemical properties to *Acacia seyal* var. *seyal* and *Acacia senegal* var. *senegal* (gum Arabic). Samples were collected from six different states in Sudan over three seasons. The gum had a moisture content of 10.50%, ash content of 1.86%, pH value of 5.19, specific optical rotation of +94.70, intrinsic viscosity of 10.44 $cm^3\ g^{-1}$, nitrogen content of 0.024%, protein content of 0.16%, acid equivalent weight of 1907.82, and total uronic acid content of 10.18%. Sugar content analysis revealed arabinose (41.20%), galactose (17.43%), and rhamnose (10.68%). Potassium was the predominant cation, followed by calcium, magnesium, sodium, lead, and iron. *Acacia nilotica* was classified as part of the *Gummeferae* series and exhibited a positive specific optical rotation. The Number average molecular weight (Mn) was estimated using osmometric measurements and gel permeation chromatography. The gum had a higher molecular weight and lower intrinsic viscosity compared to gum Arabic, suggesting a spheroidal shape of molecule. Amino acid analysis showed similarities with gum Arabic, with hydroxyproline and serine as principal amino acids. Variations in cationic composition were attributed to differences in soil type among collection locations.

KEY WORDS: Physicochemical characterization, *Acacia senegal* var. *senegal* gum, *Acacia seyal* var. *seyal* gum, *Acacia nilotica* var. *nilotica* gum, *gammiferae*

INTRODUCTION

Acacias are plants that belong to the family *Leguminosae's* subfamily *Mimosoideae*. With over 1350 species currently recognized, it is the second-largest genus in the family. The world's tropical and mild temperate regions are home to these species, with Australia having the highest concentration (955 species), followed by the Americas (185 species), Africa (144 species), and Asia (89 species). The categorization and phylogeny of Acacia have been clarified in recent years due to the collection of new information from morphological and molecular genetics investigations [1]. More than 30 different *Acacia* species can be found in Sudan [2], the majority of which are gum-producing species with the widest distribution. These include *Acacia senegal* (Hashab), *Acacia seyal* (Talha), *Acacia mellifera* (Kitir), *Acacia polyacantha* (Kakamut), *Acacia nilotica* (sunt), *Acacia laeta* (Shubahi), *Acacia nubica* (Lao't) and *Acacia sieberiana* (kuk) [3]. In Sudan, *Acacia nilotica* (sunt) is commonly found growing along the banks of the Nile and its tributaries on light, silty soils as well as in seasonal rivers and valleys on similar soil types. It is found in Western Sudan, Blue Nile, Central, and Southern Sudan, and the White Nile from Jebelein northward [4]. *Acacia nilotica* is classified by Bentham as being in series 4 of the *Gammiferae* family. According to Anderson, the gums in this series have strong positive optical rotation, high molecular weight, moderate acidity and viscosity values, and low rhamnose proportions. *Acacia nilotica* is fed to the cattle to enhance the quality of their milk. It's importance also comes from woods and medicinal usage of seed, pods, park, and gum also used to reforest

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the flood-prone areas next to riverbanks. *Acacia nilotica* produces a clear gum highly soluble in water. There have been very few reports of analytical data regarding the structural characteristics of the polysaccharide in *Acacia nilotica* gum [5-9]. Physicochemical characterization of Sudanese *Acacia nilotica* var *nilotica* gum shows good resemblance with gum Arabic and can be substituted for numerous applications in food and pharmaceutical industry.

EXPERIMENTAL

Chemicals and reagents

Sodium chloride (>99.9%), hydrochloric acid (37%), sulfuric acid (98%), cupper sulfate-potassium sulfate catalyst tablets and boric acid (≥99.5%) were purchased from Fisher Scientific UK. Amberlite IR (120 H+) resin and dodecanethiol (>98%) were obtained from Sigma–Aldrich UK. The distilled water used for all experiments was prepared by Merit Water Still W4000 Distillation Machine (Model No. W 4000; Bibby Scientific Company).

Gum samples

Authentic sixty-six samples of *Acacia nilotica* var. *nilotica gum* were collected from six different states of Sudan (Khartoum, Northern, White Nile, Blue Nile, Sinnar and Northern Kordofan state) representing a wide range of the natural distribution of the species in the country. Five samples were collected from each state for first and second seasons, the number was sixty samples, thirty samples of each season. Third season, a composite samples were made for analysis. Cationic composition, sugar analysis and number average molecular weight were analyzed using one composite sample for each location and three seasons. One whole composite sample was made from six locations and three seasons for amino acid analysis**.** Composite samples were prepared by mixing equal weights from each sample, taken from each location, considering the moisture content of gum samples. Sample collection was made during three consecutive seasons, by one of the authors (Amira A.E. Satti).

Instruments and apparatus

Heraeus Function line T6- Kendro oven and muffle furnace (Netherlands) were used to determine moisture and ash content respectively. To determine the pH; CORNING- Pinnale-555 pH meter (UK) was used. AA- 5 Optical Activity Ltd. polarimeter with a D-line of Na (589.3 nm) (UK) was used for measuring the specific optical rotation. Viscometric measurements were carried out using a Cannon-Ubbelohde Semi-Micro Dilution Viscometer No. 75, and temperature-controlled water bath, Cannon CT-500 series II (UK). To measure the amino acid content; Biochrom 30 amino acid analyzer (UK) was used. The elements were determined using an atomic absorption spectrometer (SensAA-Dual GBC Scientific equipment) (Australia). Determination of sugars was performed using HPLC system comprised an LC-9A pump (Shimadzu, Japan), Supelcosil NH2 4.6x250 cm column (Suleco USA), and differntial refractometer detector (IOTA Instrument, France). The number average molecular weight was determined using Osmostat 50 colloidal osmometer (Germany) and gel permeation chromatography coupled with multi-angle laser light scattering system (Wyatt Technology Corporation, USA).

Moisture content determination

Using the AOAC method, the moisture content of the gum samples was measured [10]. The loss on drying was determined using Heraeus Function line T6-Kendro oven and calculated as follows:

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

W_1 = original weight of sample (g). W_2 = weight of sample after drying (g).

Ash content determination

The total ash % was determined following FAO method [11], where Heraeus Function line T6-Kendro muffle furnace was used. Calculation of the total ash % was as follows:

$$\text{Ash (\%)} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

W_1 = weight of the empty crucible (g). W_2 = weight of the crucible + the sample (g). W_3 = weight of the crucible + ash (g).

pH measurements

A CORNING-Pinnale-555 pH meter inserted into a 1% aqueous solution was used to calculate the pH of gum samples.

Specific optical rotation $[\alpha]_D^T$ *determination*

At room temperature (25 °C), the optical rotation of a 1.0% aqueous gum solution was measured using an AA-5 Optical Activity Ltd. Polarimeter with a D-line of Na (589.3 nm) and a cell with a 20 cm path length. Using the mathematical relationship bellow, a specific optical rotation was computed [9]:

$$\text{Specific optical rotation } [\alpha]_D^T = \frac{\alpha \ x \ 100}{L \ x \ C}$$

α = observed angle of rotation. L = the length of sample holder in decimeters (dm). C = concentration in g/100 mL

Intrinsic viscosity determination

The Cannon-Ubbelohde semi-micro dilution viscometer, type (75 N94), and temperature-controlled water bath, Cannon CT-500 series II, set at 25 ± 0.1 °C, were used to measure intrinsic viscosity. A 4% gum solution was made by dissolving a gum sample in a 0.2 M NaCl solution. The efflux time of the diluted solution was determined after the test solution in the viscometer was gradually diluted by adding varying volumes of solvent. The intrinsic viscosity was then estimated using Huggin's equation.

Nitrogen and protein determination

Nitrogen content was determined following Kjeldahl Method [12]. 0.5 g of each sample was digested in nitrogen-free conc. Sulphuric acid, employing cupper sulfate-potassium sulfate catalyst. Digestion step was followed by steam distillation step where the released ammonia was recivied in boric acid and back titrated with HCl using methyl red indicator. The same procedure was used for blank titration and the N% was caculated as follows:

$$\% \text{ N} = \frac{14.01 \text{ x M x (volume of HCl} - \text{volume of blank)x } 100}{\text{weight of sample (grams)}}$$

where M is the molarity of HCl.

Applying the nitrogen conversion factor of 6.51 derived from gum's amino acids analysis, protein content was estimated as follows: $\%\ protein = \%\ N\ x\ 6.51$

Amino acid analysis

The amino acid analysis was carried out by Chembiotech Laboratories, University of Birmingham.

Sample hydrolysis

A sample tube containing a precisely measured amount of sample (5 mg) was filled with hydrochloric acid ((6 N), 40 mL), phenol (saturated solution in water, 0.6 mL), and dodecanethiol (Sigma, 3 mL). The tube was sealed and heated for one hour at 160 °C in a heating block. The tube was sealed and placed in a heating block at 160 °C for 1 hour. The tube was allowed to cool, then opend and placed in a dessicator under vacuum over NaOH for an hour. The residue was dissolved in sodium citrate buffer, filtered and subjected to chromatographic analysis.

Chromatographic analysis

A portion of the filtrate was injected into Biochrom 30 amino acid analyzer and separated using a cation exchange sulphonic acid high performance sodium column after elution with a series of buffers ranging in pH from 3.2 to 6.45 (buffer flow rate 35 mL/hour). By utilizing post-column derivatization with ninhydrin (flow rate 25 mL/hour) at 135 °C and monitoring the absorbance (at 570 and 440 nm), the peak detection was obtained. Using Chromeleon software and calibration curves for each relevant amino acid, quantitation is carried out.

Acid equivalent weight and uronic acid determination

The procedure described in Encyclopedia of Chemical Technology was modified slightly to determine acid equivalen weight [13]. Amberlite IR (120 H+) resin was placed into a cation exchange column. The column was repeatedly run through with sulphuric acid (2M) until the resin had been properly cleaned. The column was washed several times with distilled water until it was sulfate-free. A volume of 250 mL of the eluent and washing were collected, then they were titrated against 0.1 N NaOH after 50 mL of a 3% w/v gum solution was passed through the column by gravity. The following equation was used to compute the acid's apparent equivalent weight [14]:

$$Acid\ equivalent\ weight\ = \frac{weight\ of\ sample\ x\ 1000}{volume\ of\ titrant\ x\ normality\ of\ alkali}$$

$$\%\ Uronic\ acid\ anhydride\ = \frac{194\ x\ 100}{Acid\ equivalent\ weight}$$

194 = Molecular weight of uronic acid.

Cationic composition determination

Two grams of gum sample were placed on a porcelain dish using the dry ash. The temperature was maintained at 550 °C for 4 hours after starting in a cold furnace. After cooling the sample, 10 mL of 3 N HCl was added. The sample was gently boiled for 10 min in a dish covered with a watch glass. Cooled, filtered into a 100 mL volumetric flask and diluted to volume with deionized water. The elements were determined using an atomic absorption spectrometer (SensAA-Dual GBC Scientific equipment).

Sugar composition determination

The constituents of monosaccharides namely rhamnose (Rha), arabinose (Ara), galactose (Gal), and glucuronic acid (GlcA) present in the gum sample was measured by HPLC analysis after acid hydrolysis of gum sample. About 100 mg of gum sample was add to 4% H_2SO_4 and incubated for six hours at 100 ºC. After cooling to room temperatue one gram of barium carbonate was added and the solution was left to stand over night to complete nutralizations. The solution was centrifuged at 2500 rpm and the supernatant was removed, filtered through 0.45 µm Whatmann nylon fliter and then diluted with equal volume of acetonitrile/buffer (70:30). Out of this final solution 1 mL was taken in a vial and sbujected to HPLC analysis. The HPLC sysytem comprised an LC-9A pump (Shimadzu, Japan), a Rheodyne injection valve fitted with 50 µL smple loop, Supelcosil NH_2 4.6x250 cm column (Suleco USA), and differntial refractometer detector (IOTA instrument, France). Acetonitrile/buffere was used as mobile phase. The concentration of sugare was calcualted as peak hight and expressed as percentage of toatal sugar content [14].

Number average molecular weight determination

The number average molecular weight (M_n) of the gum samples was determined by two different methods. The first method was osmometric method, using Osmostat 50 (Germany) colloidal osmometer. The second method was performed using gel permeation chromatography coupled with multi-angle laser light scattering system (GPC-MALLS).

The osmotic pressure of gum's solutions of different concentration was measured and fitted into Van Hoff's verial equation of the form: $\left(\frac{\pi}{C}\right)^{\frac{1}{2}} = \left(\frac{RT}{M_n}\right)^{\frac{1}{2}} + \left(\frac{RT}{2M_n}\Gamma_2 C\right)^{\frac{1}{2}}$ where π the osmotic pressure , C the concentration of the gum solution, R is the gas constant, T is the temperature and Γ_2 is the second verial coefficient. M_n was calculated graphically from the intercept of the above relation [15].

GPC-MALLS measurements were performed using Superose 6 10/300GL GPC column and a DAWN EOS multi-angle light scattering detector (Wyatt Technology Corporation, USA). Obtained data was processed using ASTRA Version 4.9 (Wyatt Technology) softWare [9].

Statistical analysis

All the experiments were done in triplicate and statistical analysis was carried out using Microsoft Excel (2007) computer software.

RESULTS AND DISCUSSION

Results of physicochemical properties showed insignificant differences within samples collected from different locations and different consecutive seasons as shown in Table 1, 2 and 3. The mean value for different consecutive seasons was calculated. Table 4 summarize the results obtained from different seasons as mean values.

Table 1. Physicochemical properties of *Acacia nilotica* var. *nilotica* gum samples colledcted at first season.

State	Sample code	Moisture %	Ash%	pH	Specific ptical rotation $[\alpha]_D^T$ (+)	Intrinsic viscosity cm^3g^{-1}	Nitrogen %	Protein%	Acid equivalent weight	Glucuronic acid%
Khartoum	1A1	10.39	1.82	5.39	93	10.96	0.020	0.130	2006.67	9.67
	1A2	10.18	1.68	5.29	88	9.86	0.034	0.221	2002.13	9.69
	1A3	9.12	1.76	5.28	90.5	10.01	0.019	0.124	1955.97	9.92
	1A4	8.74	1.94	5.25	82	9.81	0.024	0.156	1979.87	9.8

	1A5	9.96	2.01	5.01	83.5	10.22	0.018	0.117	1832.56	10.59
Northern State	1B1	11.02	1.71	5.21	92	11.07	0.025	0.163	1958.57	9.91
	1B2	9.57	1.36	5.13	94.5	10.66	0.027	0.176	1840.37	10.54
	1B3	8.04	1.4	5.29	83.5	10.56	0.022	0.143	1899.49	10.21
	1B4	9.2	2.23	5.32	90.5	10.98	0.026	0.169	1869.51	10.38
	1B5	11.09	1.46	5.32	83	10.74	0.027	0.176	1952.21	9.94
White Nile	1C1	10.41	1.92	5.28	100	10.07	0.036	0.234	1934.87	10.03
	1C2	10.87	1.74	5.42	90	10.19	0.029	0.189	1720.8	11.27
	1C3	10.68	1.8	5.22	92	10.58	0.021	0.137	1866.17	10.4
	1C4	11.01	1.93	5.22	83.5	9.98	0.016	0.104	1914.56	10.13
	1C5	10.41	2.36	5.17	83.5	10.74	0.022	0.143	1952.21	9.94
	1D2	11.25	1.78	5.23	99.5	9.19	0.030	0.195	1871.73	10.36
	1D3	11.14	2.25	5.16	99	10.48	0.027	0.176	1866.79	10.39
	1D4	10.81	1.98	5.4	105	10.86	0.022	0.143	1941.03	9.99
	1D5	11.72	1.88	5.29	101.5	10.13	0.027	0.176	1852.22	10.47
Sinnar	1E1	10.75	1.36	5.26	84	9.88	0.018	0.117	1691.01	11.47
	1E2	11.51	1.63	5.22	93.5	10.89	0.028	0.182	1907.72	10.17
	1E3	11.17	1.58	5.42	84	11.11	0.023	0.150	1766.59	10.98
	1E4	11.22	1.37	5.31	99.5	10.75	0.031	0.202	1807.47	10.73
	1E5	11.89	2.28	5.21	89.5	11.38	0.022	0.143	1919.37	10.11
Northern Kordofan	1F1	9.87	2.02	5.12	90	10.72	0.024	0.156	2123.66	9.14
	1F2	9.86	1.9	5.05	83.5	10.21	0.034	0.221	1955.06	9.92
	1F3	9.79	1.83	5.12	98	10.90	0.017	0.111	1881.63	10.31
	1F4	9.59	1.81	5.25	88.5	9.86	0.02	0.130	2012.53	9.64
	1F5	9	1.6	5.21	83.5	10.13	0.026	0.169	1992.24	9.74
Mean		10.37	1.82	5.24	90.92	10.49	0.02	0.16	1904.48	10.20
SD		0.91	0.27	0.09	6.47	0.54	0.00	0.03	85.36	0.46

Table 2. Physicochemical properties of *Acacia nilotica* var. *nilotica* gum samples colledcted at second season.

State	Sample code	Moisture%	Ash%	pH	Specific optical rotation $[\alpha]_D^T$ (+)	Intrinsic viscosity cm^3g^{-1}	Nitrogen %	Protein %	Acid equivalent weight	Glucuronic acid %
Khartoum	2A1	10.62	1.52	5.08	92	10.79	0.02	0.130	1879.63	10.32
	2A2	10.66	1.67	5.36	94.5	10.98	0.027	0.176	1820.6	10.66
	2A3	10.73	1.4	5.01	98.5	9.96	0.022	0.143	1938.85	10.01
	2A4	10.77	1.52	5.11	94	10.81	0.022	0.143	1907.97	10.17
	2A5	10.31	1.49	5.09	83	10.74	0.021	0.137	1887.63	10.28
Northern State	2B1	10.41	1.65	5.31	107	9.86	0.02	0.130	1926.67	10.07
	2B2	9.46	1.49	5.32	87	10.22	0.027	0.176	2084.17	9.31
	2B3	7.77	2.04	5	106	10.55	0.032	0.208	1982.37	9.79
	2B4	7.39	1.39	5.36	99.5	10.29	0.034	0.221	2031.76	9.55
	2B5	9.22	2.22	5.06	92	10.98	0.025	0.163	1899.87	10.21
White Nile	2C1	10.42	2.22	5.13	103.5	10.98	0.02	0.130	1785.76	10.86
	2C2	10.49	2.3	5.26	99.5	10.79	0.026	0.169	1812.65	10.7
	2C3	10.68	1.53	5.11	83.5	11.31	0.022	0.143	1908.35	10.17
	2C4	10.99	1.46	5.37	85	12.05	0.032	0.208	1799.05	10.78
	2C5	10.15	1.88	5.03	83	11.38	0.023	0.150	1826.02	10.62
Blue Nile	2D1	11.14	2.28	5.21	99.5	10.86	0.023	0.150	1860.25	10.43
	2D2	11.25	1.78	5.23	99.5	10.43	0.030	0.195	1871.73	10.36
	2D3	11.14	2.25	5.16	99	10.15	0.027	0.176	1866.79	10.39
	2D4	10.81	1.98	5.4	105	9.96	0.022	0.143	1941.03	9.99
	2D5	11.72	1.88	5.29	101.5	10.27	0.027	0.176	1852.22	10.47
Sinnar	2E1	11.63	2.34	5.17	93	9.61	0.024	0.156	1974.61	9.82

	2E2	11.11	1.55	5.2	90.5	10.19	0.021	0.137	1826.63	10.62
	2E3	11.13	1.84	5.23	83.5	10.04	0.02	0.130	1871.73	10.36
	2E4	10.94	2.35	5.37	94.5	10.66	0.029	0.189	1899.87	10.21
	2E5	11.45	2.29	5.19	90.5	9.37	0.022	0.143	1839.51	10.55
Northern Kordofan	2F1	9.88	2	5.07	90.5	10.97	0.032	0.208	2114.65	9.17
	2F2	9.7	1.98	5.13	94	10.90	0.024	0.156	2066.71	9.39
	2F3	9.45	1.46	5.11	93	11.31	0.024	0.156	1962.73	9.88
	2F4	9.37	1.85	5.03	92	11.66	0.02	0.130	1934.49	10.03
	2F5	9.07	1.6	5.16	84.5	10.38	0.022	0.143	1900.25	10.21
Mean		10.33	1.84	5.18	93.92	10.65	0.02	0.16	1910.61	10.17
SD		1.01	0.31	0.11	6.74	0.61	0.00	0.02	79.14	0.41

Table 3. Physicochemical properties of *Acacia nilotica* var. *nilotica* gum samples colledcted at third season.

State	Sample code	Moisture %	Ash%	pH	Specific optical rotation $[\alpha]_D^T$ (+)	Intrinsic viscosity cm^3g^{-1}	Nitrogen %	Protein %	Acid equivalent weight	Glucuronic acid%
Khartoum	Comp 3A	10.83	1.89	5.1	105	10.79	0.023	0.150	1866.79	10.39
Northern State	Comp 3B	10.02	1.92	5.37	105	9.56	0.025	0.163	1826.02	10.62
White Nile	Comp 3C	10.94	1.98	5.32	107.5	10.07	0.025	0.163	1882.47	10.31
Blue Nile	Comp 3D	11.51	1.87	5.02	105	8.81	0.025	0.163	1900.25	10.21
Sinnar	Comp 3E	11.69	1.79	5.0	100	9.22	0.024	0.156	2066.71	9.39
Northern Kordofan	Comp 3F	9.85	1.99	5.09	72.5	12.7	0.024	0.156	1907.97	10.17
Mean		10.81	1.91	5.15	99.17	10.19	0.02	0.16	1908.37	10.18
SD		0.75	0.07	0.16	13.29	1.41	0.00	0.01	82.85	0.42

Table 4. The average results of physicochemical properties of the *Acacia nilotica* var. *nilotica* gum samples colledcted at different seasons.

Saeson	Moisture (%)	Ash (%)	pH	Specific optical rotation $[\alpha]_D^T$ (+)	Intrinsic viscosity cm^3g^{-1}	Nitrogen (%)	Protein (%)	Acid equivalent weight	Glucuronic acid (%)
First season	10.37	1.82	5.24	+ 90.90	10.49	0.024	0.16	1904.48	10.2
Second season	10.33	1.84	5.18	+ 93.90	10.65	0.024	0.16	1910.61	10.17
Third season	10.81	1.91	5.15	+ 99.20	10.19	0.024	0.16	1908.37	10.18
Mean value	10.50	1.86	5.19	+ 94.70	10.44	0.024	0.16	1907.82	10.18

Moisture content

The moisture content of the gum is usually affected by the season of collection, the prevailing climate conditions and the storage condition. The results in Table 4 of moisture content of *Acacia nilotica* var. *nilotica* gum show, in average, higher moisture content values compared to results reported in previous studies [8, 16].

Ash content

The mean values of ash content as shown in Table 4 is almost similar to those results obtained in a comparable investigation [6, 16], which ranged from 1.98 to 2.48%, but were significantly lower than those observed in other studies [5, 8], which were reported as 0.02% and 0.03%, respectively. These results are significantly larger to those reported by Hassan *et al*. [17], who mentioned that the average ash content was 0.21%.

pH value

As shown in Table 4, the average pH for *Acacia nilotica* var. *nilotica* gum is considerably higher than that previously obtained [8], which was found to be 4.1. Furthermore, it is greater than what has been reported for *Acacia senegal* var. *senegal* and *Acacia seyal* var. *seyal* [8].

Specific optical rotation

One of the analytical factors that allows one group of Acacia species gums to be differentiated from another group of *Acacia* species gums is the specific optical rotation. *Acacia nilotica* is classified as part of the *Gummeferae* series and exhibits a positive specific optical rotation, which is charaterized by dextrogyrte behavior, However, *A. senegal* var. *senegal* belongs to the *Vulgares* series and possesses a negative specific optical rotation.. The average values of optical rotation agree with the literature [5-6, 8]. It's interesting to note that they exceed the results of the FAO study on Nigerian gum [9], which showed a value of +21. *Acacia nilotica* var. *nilotica* has a larger specific optical rotation value than *Acacia seyal* var. *seyal*, as stated in the literature [6, 8, 17-20].

Intrinsic viscosity

Acacia nilotica is distinguished by its low viscosity because it belongs to the *Gummeferae* series [5, 18]. The intrinsic viscosities of *Acacia nilotica* var. *nilotica* shown in Figure 1. The results agreed with those mentioned in the literature [5, 6] which was 9.5 (cm^3g^{-1}), However, it is significantly less than the value reported by an FAO study for Nigerian gum [9], which was 35 (cm^3g^{-1}). When considering the various variations of this species, the low specific optical rotation value and exceptionally high intrinsic viscosity value mentioned in the FAO study may lead to the conclusion that the investigated gum did not belong to *Acacia nilotica* var. *nilotica*. When compared to *Acacia nilotica*, both *Acacia senegal* var. *senegal* and *Acacia seyal* var. *seyal* exhibit higher intrinsic viscosity values [6, 8, 17, 19-24].

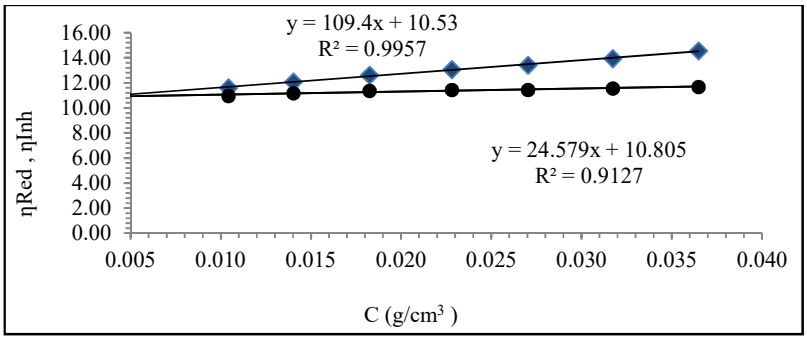

Figure 1. Intrinsic viscosity of *Acacia nilotica* var. *nilotica* gum.

Nitrogen and protein content

The Kjeldahl method was used to practically determine nitrogen and protein content. The total protein content was estimated using an amino acid analysis nitrogen conversion factor (NCF) of 6.51. According to the literature, the nitrogen conversion factor (NCF) of *Acacia senegal* var. *senegal* is 6.6 [25]. *Acacia nilotica* var. *nilotica* has a typical nitrogen content based on the results of a comparable study [5], but slightly less than the value mentioned in the literature [8] which was 0.06%. The protein content determined by this study is significantly lower than that determined by an FAO analysis on Nigerian gum [9, 16]. These percentages were 1.9% and 4.7%, respectively. *Acacia senegal* var. *senegal* and *Acacia seyal* var. *seyal* both possess larger nitrogen and protein content than *Acacia nilotica* var. *nilotica* [6, 8, 17-20, 22-25].

Amino acids composition

The resulting amino acid profiles for the analyzed samples of *Acacia nilotica* var. *nilotica* are expressed in (nmol/mg) and (µg/mg). The reported data has been corrected to account for hydrolysis losses for threonine (Thr) and serine (Ser) of 7% and 12% w/w, respectively. Methionine (Met) losses are highly variable and were not taken into account. Asparagine (Asn) and glutamine (Gln) are converted to aspartine (Asp) and glutamate (Glu), respectively, during acid hydrolysis tryptophan (Trp) is completely destroyed by acid hydrolysis and is normally determined separately by alkaline hydrolysis. Cysteine (Cys) recovery is highly variable (often undergoing complete loss during acid hydrolysis). The sum of the determined individual amino acid contents is also shown in Table 5.

Table 5. Amino acid composition of *Acacia nilotica* var. *nilotica* gum and its fractions.

Amino acids	*A. nilotica* (control)		Fraction 1		Fraction 2	
	nmol mg^{-1}	µg mg^{-1}	nmol mg^{-1}	mg mg^{-1}	nmol mg^{-1}	µg mg^{-1}
Asp	1.12	0.15	1.49	0.20	77.13	10.27
Thr	0.78	0.09	1.03	0.12	20.00	2.38
Ser	1.68	0.18	2.14	0.23	36.77	3.86
Glu	0.73	0.11	1.11	0.16	33.60	4.94
Gly	1.14	0.09	1.60	0.12	47.83	3.59
Ala	0.71	0.06	0.96	0.09	37.21	3.31
Val	0.73	0.09	1.02	0.12	45.06	5.28
Met	0.04	0.01	0.10	0.02	0.85	0.13
Ile	0.56	0.07	1.03	0.14	21.11	2.77
Leu	0.86	0.11	1.59	0.21	64.31	8.44
Tyr	0.44	0.08	0.91	0.16	3.58	0.65
Phe	0.37	0.06	0.62	0.10	11.12	1.84
His	0.46	0.07	0.65	0.10	5.12	0.79
Lys	0.68	0.10	0.77	0.11	14.78	2.16
Arg	0.23	0.04	0.33	0.06	4.37	0.76
Pro	1.20	0.14	1.35	0.16	27.30	3.14
HO-Pro	2.22	0.29	2.28	0.30	35.66	4.68
NCF*	6.51	-	6.62	-	6.85	-

*Nitrogen conversion factor.

These individual amino acid content values have been used to estimate the total theoretical nitrogen content of the samples and the ratio of the nitrogen and protein values were used to estimate the conversion factor for nitrogen to protein for each sample. Hydroxyproline and serine were the principal amino acids for *Acacia nilotica* var. *nilotica* whole gum and the majority of

fraction 1 isolated. This pattern of protein amino acids distribution is similar to that found in *Acacia senegal* var. *senegal* and *Acacia seyal* var. *seyal* [26-27]. Although *Acacia seyal* var. *seyal* has less protein than *Acacia senegal* var. *senegal*, the distribution of the amino acids overall is not significantly different [27]. Since it is thought that hydroxyproline and maybe serine are responsible for the peptide chain's attachment to the polysaccharide, the hydroxyproline level is important [28]. Fraction 2 of *Acacia nilotica* var. *nilotica* had aspartine and leucine (Leu) as principal components. The amino acid composition of fraction 2 of *Acacia senegal* var. *senegal* was significantly different, with hydroxyproline and serine as a major components in some samples and aspartine, serine, glutamate, glycine (Gly) and leucine as principal components in other samples [27, 29]. However, the amino acid ratios of *Acacia nilotica* var. *nilotica* gum were lower than those found in *Acacia senegal* var. *senegal* and *Acacia seyal* var. *seyal* [12, 18, 20, 25, 27, 30-33].

Acid equivalent weight and uronic acid

Table 4 shows that the acid equivalent weight and associated computed uronic acid content of *Acacia nilotica* var. *nilotica* gum are nearly identical for the three seasons. This range coincides with earlier results [5, 6], however uronic acid is much lower than the FAO study (21%) for Nigerian gum [9]. These findings differ from those obtained for *Acacia senegal* var. *senegal* [20, 25], which indicated a range of 1153 to 1500 for acid equivalent weight and a range of 12.93% to 16.33% for uronic acid content.

Cationic composition

The average cationic composition of *Acacia nilotica* var. *nilotica* gum samples was measured using an atomic absorption spectrophotometric method, and the results are shown in Table 6.

The following was the order of the main elements: K > Ca > Mg > Na > Pb > Fe > Cu > Zn. The high levels of potassium, calcium, and magnesium indicate that the gum is a potassium, calcium, and magnesium salt. The cationic composition ratios of *Acacia nilotica* gum, on the other hand, were lower than those reported for *Acacia senegal* var. *senegal* and *Acacia seyal var. seyal* [20]. The differences among the samples regarding cationic composition are relatively significant due to the difference in soil type.

Table 6. Cationic composition in (ppm) of *Acacia nilotica* var. *nilotica* gum.

State	Na	K	Ca	Mg	Fe	Zn	Cu	Pb
Khartoum	377	688	1288	225	18	1.63	6.15	26
Northern State	24	121	1437	217	12	1.97	5.98	33
White Nile	34	7615	918	239	19	1.09	3.52	60
Blue Nile	16	248	1239	123	27	2.54	5.23	82
Sinnar	202	442	994	133	31	1.97	6.50	19
Northern Kordofan	414	488	1447	160	14	2.44	5.11	14
Composite	183	6264	1184	162	15	2.03	6.35	48
Mean	179	2266	1215	180	19	1.96	5.55	40
SD	167	3221	203	47	7	0.49	1.04	25

Sugar composition

The sugar content of *Acacia nilotica* var. *nilotica* gum was determined using HPLC. The values of arabinose, galactose and rhamnose were shown in Table 7. According to Bentham's classification, *Acacia nilotica* var. *nilotica* gum belonged to the *gummiferae* series, which has a higher percentage of arabinose than galactose and the lowest percentage of rhamnose. The

findings are consistent with what has been written about *Acacia nilotica* gum [5-6, 8]. According to Kapoor *et al*., the level of rhamnose in Indian *nilotica* gums was insignificant [16]. Arabinose is the sugar with the highest percentage in *Acacia seyal* var. *seyal* gum, while galactose is the sugar with the lowest percentage in *Acacia senegal* var. *senegal* gum [20, 25, 34-36].

Table 7. Sugar composition (%) of *Acacia nilotica* var. *nilotica* gum.

State	Arabinose	Galactose	Rhamnose
Khartoum	34.14	14.27	5.48
Northern State	43.66	18.04	11.12
White Nile	31.09	18.53	14.51
Blue Nile	38.05	17.69	12.54
Sinnar	47.77	15.63	13.34
Northern Kordofan	49.99	19.022	7.23
Mean	41.20	17.43	10.68
SD	7.01	1.80	3.28

Number average molecular weight

As shown in Figure 2, the number average molecular weight (M_n) of *Acacia nilotica* var. *nilotica* was determined using osmotic pressure measurements and estimated from the intercept of the plot of $\sqrt{\pi}/c$ against concentration. According to Table 8, the values of number average molecular weight (M_n) obtained by osmotic pressure measurements were greater than the values obtained by gel permeation chromatography. It is likely that the discrepancies between the two procedures are what caused the results to diverge. Comparatively, *Acacia senegal* var. *senegal* and *Acacia seyal* var. *seyal* achieved lower results than *Acacia nilotica* var. *nilotica* [34]. The higher molecular weight with lower intrinsic viscosity compared with *Acacia seyal* var. *seyal* and *Acacia senega*l var. *senegal* indicates that the molecule has the spheroidal shape. The spheroidal shape of the gum together with the proteinaceous portion attached to the polysaccharides facilitate alignment and close-packing of the gum at the oil-water interface and responsible for the emulsifying and stabilizing properties of the gum [37]. The finding obtained for *Acacia nilotica* is consistent with the knowledge that the *Gummiferae* series has typically high molecular weights that can approach an order of magnitude of 6 (10^6). Additionally, it is consistent with previous results [38-40].

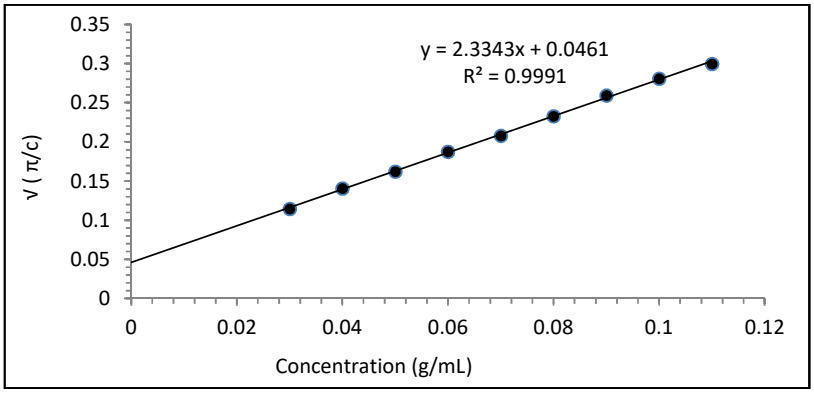

Figure 2. Osmotic pressure concentration profile of *A. nilotica* var. *nilotica* gum solutions.

Table 8. Number average molecular weight (M_n) of samples by osmotic method and GPC method of *Acacia nilotica* var. *nilotica* gum.

State	M_n	
	Osmotic pressure method	GPC method
Khartoum	$3.35x10^7$	$1.52x10^6$
Northern State	$1.06x10^7$	$2.58x10^6$
White Nile	$1.15x10^7$	$1.33x10^6$
Blue Nile	$1.79x10^7$	$1.68x10^6$
Sinnar	$3.62x10^7$	$2.37x10^6$
Northern Kordofan	$2.12x10^7$	$1.50x10^6$
Mean	$2.00x10^7$	$1.90x10^6$

The difference in composition between *Acacia nilotica* var. *nilotica* gum and gum Arabic contributes to different functional properties of gums. However, the composition of gums is within the specified range set by the FAO, WHO, Joint Expert Committee for Food Additives (JECFA) and accepted by Codex Alimentarius [41].

CONCLUSION

Characterization was undertaken using physicochemical and chemical methods. The determined properties that characterize the gum proved that *Acacia nilotica* var. *nilotica* gum belongs to *Gummiferae* series. These properties can be summarized that in comparison to *Acacia senegal* var. *senegal*, *Acacia nilotica* var. *nilotica* gum contains less nitrogen and, consequently, protein. Its optical rotation was positive as opposed to the negative optical rotation of *Acacia senegal* var. *senegal*. Additionally, the gum from *Acacia nilotica* var. *nilotica* contains more arabinose, less rhamnose, less glucuronic acid and lower viscosity than gum from *Acacia senegal* var. *senegal*. The amino acid compositions are similar to *Acacia senegal* var. *senegal* with hydroxyproline and serine being the major constituents. The higher molecular weight and a higher molecular size with lower intrinsic viscosity compared with *Acacia seyal* var. *seyal* and *Acacia senegal* var. *senegal* indicates that the molecule has the overall spheroidal shape which is thought to be typical of such proteoglycans. Determination of these parameters provided a rapid means of differentiating between the *Acacia nilotica* var. *nilotica* and *Acacia senegal* var. *senegal* species. *Acacia nilotica* var. *nilotica* gum fulfils the physicochemical regulations set by JECFA for acacia gum. It can be concluded that the properties of *Acacia nilotica* var. *nilotica*, shows good resemblance with gum Arabic and can be substituted for numerous applications in food and pharmaceutical industry

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REFERENCES

1. Maslin, B.R.; Miller, J.T.; Seigler, D.S. Overview of the generic status of Acacia (Leguminosae: Mimosoideae). *Aust. Syst. Bot.* **2003**, 16, 1-18.
2. El-Amin, H.M. *Trees, and Shrubs of the Sudan*, Ithaca Press: UK; **1990**; p 160.
3. Abdelnour, H.O. Gum Arabic in Sudan: production and socio-economic aspects. In FAO (Editor), Medicinal, culinary, and aromatic plants in the Near East. Proceedings of the

International Expert Meeting organized by the Forest Products Division FAO Forestry Department and the FAO Regional Office for the Near East, Cairo, Egypt; **1999**; p 66.
4. El-Amin, H.M. *Sudan Acacias*, 1st ed., Forest Research Institute Bulletin, Khartoum, Sudan; **1973**.
5. Anderson, D.M.W.; Stoddart, J.F. Studies on uronic acid materials: Part XV. The use of molecular-sieve chromatography in studies on acacia senegal gum (Gum Arabic). *Carbohydr. Res*. **1966**, 2, 104-114.
6. Anderson, D.M.W. Chemotaxonomic aspects of the chemistry of Acacia gum exudates. *Kew Bull*. **1978**, 32, 529-536.
7. Chalk, R.C.; Stoddart, J.F.; Szarek, W.A.; Jones, J.K.N. Isolation of two arabinobioses from *Acacia nilotica* gum. *Can. J. Chem*. **1968**, 46, 2311-2313.
8. Karamalla, K.A. *Gums Stabilizers for Food Industry*, Royal Society of Chemistry: Cambridge, UK; **2000**.
9. Al Assaf, S.; Phillips, G.O.; Williams, P.A. Studies on acacia exudate gums. Part I: the molecular weight of *Acacia senegal* var. *senegal* gum exudate. *Food Hydrocoll.* **2005**, 9, 647-660.
10. AOAC, *Official Methods of Analysis*, 13th ed., AOAC: Washington DC; **1980**.
11. FAO, *Food and Nutrition*, Paper No. 5, Rev. 2., Rome; **1991**.
12. Flindt, C.; Al-Assaf, S.; Phillips, G.O.; Williams, P.A. Studies on acacia exudate gums. Part V. Structural features of Acacia seyal. *Food Hydrocoll*. **2005**, 19, 687-701.
13. *Encyclopedia of Chemical Technology*, edited by Anthony Standen. John Wiley and Sons, Inc.: London; **1966**.
14. Price, M.L.; Van Scoyoc, S.; Butler, L.G. A critical evaluation of the vanillin reactions as an assay for tannins in sorghum grain. *J. Agric. Food Chem.* **1978**, 26, 1214-1218.
15. Flory, P.J. *Priciples of Polymer Chemistry*, Cornell University Press, Ithaca, New York, USA; **1953**.
16. Kapoor, V.P.; Farooqi, M.I.H. Studies on *Acacia nilotica* gum exudates. Structural variation due to different habitats. *Carbohydr. Res*. **1991**, 222, 289-293.
17. Hassan, E.A.; Al-Assaf, S.; Phillips, G.O.; Williams, P.A. Studies on Acacia gums: Part III molecular weight characteristics of *Acacia seyal* var. seyal and *Acacia seyal* var. fistula. *Food Hydrocoll*. **2005**, 19, 699-677.
18. Anderson, D.M.W.; Herbich, M.A. Studies on uronic acid materials. Part VI. The variation in composition and properties of gum nodules from *Acacia seyal*. *J. Chem. Soc*. **1963**, 1, 1-6.
19. Jarasek, P.; Kosik, M.; Philips, G.O. A chemometric study of Acacia (Gum Arabic) and related natural gums. *Food Hydrocoll*. **1993**, **7**, 73-85.
20. Siddig, N.E.; Osman, M.E.; Al-Assaf, S.; Phillips, G.O.; Williams, P.A. Studies on acacia exudate gums, part IV. Distribution of molecular components in *Acacia seyal* in relation to *Acacia senegal*. *Food Hydrocoll*. **2005**, 19, 679-686.
21. Duvallet, S.; Fenyo, J.C.; Vandevelde, M.C. Meaning of molecular-weight measurements of gum Arabic. *Polym. Bull*. **1989**, 21, 517-521.
22. Idris, O.H.M.; Williams, P.A.; Phillips, G.O. Characterization of gum from *Acacia senegal* trees of different age and location using multidetection gel permeation chromatography. *Food Hydrocoll*. **1998**, 12, 379-388.
23. Elmanan, M.; Al-Assaf, S.; Phillips, G.O.; Williams, P.A. Studies on acacia exudate gums: Part VI. Interfacial rheology of *Acacia senegal* and *Acacia seyal*. *Food Hydrocoll*. **2008**, 22, 682-689.
24. Karamalla, K.A.; Siddig, N.E.; Osman, M.E. Analytical data for *Acacia senegal* var. Senegal gum samples collected between 1993 and 1995 from Sudan. *Food Hydrocoll*. **1998**, 12, 373-378.

25. Osman, M.E.; Menzies, A.R.; Williams, P.A.; Phillips, G.; Baldwin, T.C. The molecular characterization of the polysaccharide gum from *Acacia senegal*. *Carbohydr. Polym.* **1993**, 246, 303-318.
26. Qi, W.; Fong, C.; Lamport, D.T.A. Gum Arabic glycoprotein is a twisted hairy rope. *Plant Physiol.* **1991**, 96, 848-855.
27. Osman, M.E.; Menzies, A.R.; Martin, B.A.; Williams, P.A.; Phillips, G.O.; Baldwin, T.C. Characterization of gum Arabic fractions obtained by anion-exchange chromatography. *Phytochemistry* **1995**, 38, 409-417.
28. Fincher, G.B.; Stone, B.A.; Clarke, A.E. Arabinogalactan-protein: Structure, biosynthesis and function. *Annu. Rev. Plant Physiol.* **1983**, 34, 47-70.
29. Randall, R.C.; Phillips, G.O.; Williams, P.A. Fractionation, and characterization of gum from *Acacia senegal*. *Food Hydrocoll*. **1989**, 3, 65-75.
30. Anderson, D.W.M.; Dea, I.C.M.; Karamalla, K.A.; Smith, J.F. Studies on uronic acid materials. Part XXIV. An analytical study of different forms of the gum from *Acacia senegal* WILLD. Carbohydr. *Res*. **1968**, 6, 97-103.
31. Ashour, M.A.; Fatima, W.; Imran, M.; Ghoneim, M.M.; Alshehri, S.; Shakeel, F. a review on the main phytoconstituents, traditional uses, inventions, and patent literature of gum arabic emphasizing *Acacia seyal*. *Molecules* **2022**, 27, 1171-1189.
32. Anderson, D.M.W.; Brown Douglas, D.M.; Morrison, N.A.; Weiping, W. Specifications for gum arabic *(Acacia senegal* var. *senegal*); analytical data for samples collected between 1904 and 1989. *Food Addit. Contam*. **1990**, 7, 303-321.
33. Islam, A.M.; Phillips, G.O.; Sljivo, A.; Snowden, M.J.; Williams, P.A. A review of recent developments on the regulatory, structural, and functional aspects of gum Arabic. *Food Hydrocoll*. **1997**, 11, 493-505.
34. Abdelrahman, M.A. Characterization and thermodynamic properties of gum of *Acacia* origin, *PhD Thesis*, Sudan University of Science and Technology, Khartoum, Sudan, **2008**.
35. Younes, A.A.O. Physicochemical Studies on some *Acacia* Gums and their Fractions, *PhD Thesis*, Sudan University of Science and Technology, Khartoum, Sudan, **2009**.
36. Bhushette, P.R.; Annapure, U.S. Comparative study of *Acacia nilotica* exudate gum and acacia gum. *Int. J. Biol. Macromol*. **2017**, 102, 266-271.
37. Gashua, I.B.; Williams, P.A.; Baldwin, T.C. Molecular characteristics, association and interfacial properties of Gum Arabic harvested from both *Acacia senegal* and *Acacia seyal*. *Food Hydrocoll*. **2016**, 61, 514-522
38. Al-Assaf, S.; Katayama, T.; Phillips, G.O.; Sasaki, Y.; Williams, P.A. Quality control of gum Arabic. *FFI J. Japan* **2003**, 208, 771-780.
39. Massey, S.; MacNaughtan, W.; Williams, H.E.L.; Wolf, B.; Iqbal, M.S. A structural study of *Acacia nilotica* and *Acacia modesta* gums. *Carbohydr. Polym*. **2017**, 175, 207-215.
40. Bhushette, P.R.; Annapure, U.S. Characterization of *Acacia nilotica* exudate gum and its flm. *J. Food Meas. Charact*. **2020**, 14, 3058-3066.
41. Phillips, G.O.; Ogasawara, T.; Ushida, K. The regulatory and scientific approach to defining gum arabic (*Acacia senegal* and *Acacia seyal*) as a dietary fibre, *Food Hydrocoll*. **2008**, 22, 24-35.