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Abir EM Ajab
Department of Chemistry, Kordofan University, Sudan

Mohammed E Osman
Department of Chemistry, College of Science, Sudan University of Science and Technology, Khartoum P.O. Box 407, Sudan

Elfatih A Hassan
Department of Chemistry, College of Science, Sudan University of Science and Technology, Khartoum P.O. Box 407, Sudan

Seifeldawla A Elhag
Department of Chemistry, Sinnar University, Sudan

Eid I Brima
Department of Chemistry, Faculty of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia

Correspondence
Abir EM Ajab
Department of Chemistry, Kordofan University, Sudan

Characterization and molecular weight distribution of gum from *Acacia nilotica* var. *adstringens*

Abir EM Ajab, Mohammed E Osman, Elfatih A Hassan, Seifeldawla A Elhag and Eid I Brima

Abstract
Physical and chemical properties of gum exudates from *Acacia nioltica* var. *adstringens* from N. Kordofan State were determined. Average values of moisture and ash content of *Acacia nioltica* var. *adstringens* gum were 11.01% and 2.06% respectively, average of pH was 5.06. Average value of specific optical rotation and intrinsic viscosity were (+96.4) and (11.06cm^3g^{-1}) respectively. The average acid equivalent weight and total glucouronic acid were (1869) and (10.4%) respectively. Acid hydrolysis of the gum revealed a sugar content of arabinose (52%), galactose (24%) and traces of rhamnose (>1). The average value of nitrogen was (0.06%) and hence, protein content was (0.44%). The calorific value was (4.06 Kcal/g). The Cationic composition of gum is in order Ca > Mg >K > Na Fe, Zn, Sr, Mn, Sn, Zn,Cu and Ni, but As, Cd, Co, Cr and Pb are present as trace.
Gel permeation chromatography results show that three main components designated arabinoglactan protein (AGP), arabinoglactan (AG) and glactoprotein (GP). The molecular weight of the AGP was 2.54 x10^6 Da and a radius of gyration of 36.6 nm.

Keywords: Characterization, molecular weight distribution, gum, *Acacia nilotica* var. *adstringens*

1. Introduction
Acacia nilotica is an *Acacia* species, rich in bioactive secondary compounds and are important for a variety of functions (Gupta 1970, Mahgoub 1979) [8, 11], such as source of tannins, gums, timber, fuel and fodder.
A. nilotica gum varies in colour from very pale yellowish brown to dark reddish brown depending on the quantity of tannins in the sample. The lighter, more highly valued, gums are soluble in water of low viscosity (New, 1984) [13].
It is composed of galactoaraban which gives on hydrolysis L-arabinose, D-galactose, and L-rhamnose, D-glucuronic acid and 4-O-methyl- D-glucuronic acid (Malviva, 2011) [12].
Acacia adstringens is a subspecies of *A. nilotica* which is occasionally, used for firewood and good quality charcoal in the Sahelian regions, and is a preferred firewood in Tanzania. It has a hard heavy heartwood, it is resistant to water and termites. It is a source of gum and tannin, the gum is locally used for making ink. The flowers are a source of pollen and nectar for bees (Wickens *et al.*, 1995) [15]. The gum is tasteless and almost completely soluble in water (50g/l00ml). The darker samples contain tannin and are much less soluble, and leave behind a gelatinous residue. *A. nilotica* gum is dextrorotatory. It contains 13% of moisture and on ignition; it leaves behind 1.8% of ash (CaO, 52.2; and MgO, 19.7%). The gum of *A. nilotica* has a higher molecular weight (Mw, 2.3 x 10^6) than *A. Senegal* (Mw, 600,000); (Sao, 2012) [10], high, positive specific rotation (+106°), (1.05%) methoxyl content and contained only traces of rhamnose (Anderson *et al.*, 1966) [3]. *A. nilotica* gum gave solutions of low viscosity, and it's unusually low nitrogen content (0.08%). (Anderson *et al.*, 1966, 1963) [3, 5].

2. Materials and Methods
2.1 Materials
Forty five samples of the gum were collected from Elain forest south east of Elobied, North Kordofan state, Sudan during seasons 2016-2017. The gum samples were dried under shade and cleaned to remove impurities such as wood pieces and sand particles. Each sample was reduced to a fine powder using a mortar and pestle and kept in labeled self-sealing polyethylene bags.

2.2 Methods

2.2.1 Physicochemical properties of the gum

Gum samples were analyzed for moisture%, pH, ash%, intrinsic viscosity, specific optical rotation, nitrogen and protein content, Equivalent weight and total Uronic Acid according to AOAC, 1990 method. The cations were determined using ICP-MS Brima (2016) [7].

2.2.2 The calorific value

0.5g (taking into account the moisture content) of gum sample were weighted and placed into a plastic bag, big bag or small bag which have cross cal. Val. 46383, and 46463 respectively, the bag was covered by rolling it and placed into a decomposition vessel which is surrounded by a water jacket. Then the sample was combusted in an oxygen atmosphere (The calorimeter IKA® C1), and the calorific value of the sample was calculated from the resulting increase in temperature.

2.2.3 Molecular weight and molecular weight distribution

Gel permeation chromatography coupled to a multi-angle laser light scattering detector (GPC-MALLS) system was used to determine the molecular weight and molecular weight distribution. Loading sample injector equipped with 100 ml sample loop. The system utilizes Waters (Division of Millipore, USA) Solvent Delivery System Model 6000A connected to a column containing Superose 6 (Amersharm Biosciences) (10 x 300mm), manual Rheodyne Model 7125 syringe.

The column eluent was monitored by three detectors, refractive index (RI) Wyatt Optilab DSP interferometric refractomter operated at 633 nm (Wyatt Technology Corporation, USA), multi-angle laser light scattering photometer DAWN EOS using He–Ne laser at 690 nm (Wyatt Technology Corporation, USA), and an Agilent 1100 series G1314A UV detector (214 nm, Agilent Technologies) (Al-Assaf *et al.*, 2005) [1]. RI provides an accurate concentration profile, MALLS enables absolute molecular mass and radius of gyration (Rg), and the UV detects the proteinaceous components of the gum (Katayama *et al.*, 2006) [9]. The data was processed by the Astra for Windows software (version 4.90.07, Wyatt Technology Corporation).

1.0 mg/ml gum samples was prepared (based on dry weight) in 1mM phosphate buffer at pH 7 containing 0.2M NaCl, and hydrated by roller (SRT9. Stuart Scientific, UK) mixing the solution overnight to ensure complete sample fully dissolution. The solutions were centrifuged for 10 minutes at speed of 3000 rpm using Megafuge 1.0R (Heraeus SEPATECH, Germany) centrifuge, filtered using 0.45-µm nylon filter (Whatman, 13 mm) prior to injection into the GPC-MALLS system.

3. Results and discussion

3.1 Physicochemical properties

Table 1 shows the percentage moisture and ash content of *A. nilotica* var. *adstringens* samples ranged between 10.18% and 11.95% with average value of 11.01%, while Ash percentage ranged between 1.17% and 2% with average of 2.06% and pH ranged between 5.00 and 5.14 with average of 5.06. The results agree with those reported for *A. nilotica* var. *nilotica* gum (Satti 2011 and Alobied, 2015) [14, 2]. The acid equivalent weight of *A. nilotica* var. *adstringens* samples ranged between 1807.23 and 2000 with a corresponding glucouronic acid content within the range of 9.70% and 10.73% with average values of 1869.25 and 10.4%, respectively. Acid equivalent weight and the calculated glucouronic acid values are in a good agreement with the values reported for *A. nilotica* var. *nilotica* gum (Anderson *et al*., 1963, Anderson, 1977, Satti, 2011and Alobied, 2015) [5, 4, 14, 2].

The gum shows a specific optical rotation between (+75.6 and +104), these results are agree with values obtained by (Satti 2011 and Alobied 2015) [14, 2] for *A. nilotica* var. *nilotica* gum.

Table 2 shows analysis of composite sample of the gum. The table shows that, the average values of intrinsic viscosity ($11.06cm^3g^{-1}$), nitrogen (0.06%) and hence protein (0.44%). These results are agree with the results reported by Karamalla (1999) for *A. nilotica* var. *nilotica* and *adstringens* gum, also in good agreement with values reported by (Satti, 2011 and Alobied 2015) [14, 2] for *A. nilotica* var. *nilotica* gum.

Table 3 shows that calcium, potassium, magnesium and sodium are the four most abundant elements present, and they were in order: Ca > Mg >K > Na. The samples tend to have higher average content of Fe, Zn, Sr, Mn, Sn, Zn, Cu and Ni. The results also shows that elements such as As, Cd, Co, Cr and Pb are present as trace.

Table 4 shows sugars content which were found to be 52% arabinose, 24% galactose, 10.4% and traces of rhamnose.

3.2 The calorific value

Table 5 shows that the calorific value of *A. nilotica* var. *adstringens* gum is 4kcal/g. This value is very low, so that the gum is suitable to use in application in dietary food and nutraceutical products.

3.3 Molecular weight and molecular weight distribution

Figure 1 shows the elution profile of *A. nilotica* var. *adstringens* gum. The light scattering response shows the presence of two peaks with partial separation as the second peak which appears as a shoulder. The first peak has a high narrow response corresponding to the minor peak which appeared at the elution volumes of (~7.5 ml). The second peak is broader with lower response. The RI response also shows the presence of these two peaks fig (2), the first peak has a high narrow response coincided with the light scattering response and corresponding to the minor peak which appeared at the elution volumes of (~7.6 ml) and represents about (28.54%) of total mass. The second peak is broader with lower response corresponding to the major peak and contained the majority of the gum which appeared at an elution volume of (~10.4 ml) and (~71.46%) of total mass. The UV response showed that there is protein associated with the high molecular weight materials and the second peak also appears as shoulder and follows the same LS response. The UV response shows three peaks. The first peak (peak 1) is for the (AGP) which has the protein core and the attached carbohydrate. The second peak (peak 2) appears after the (AGP) and corresponds to the arabinoglactan (AG). The UV response shows a third low molecular weight peak (peak 3) which elutes at ~15-18.5 ml before the total volume of the column (~20ml), which corresponds to glycoprotein (GP)

The weight average molecular weight (Mw) for whole gum was found to be 2.538 $x10^6$ g/mol, with mass recovery of 114.7% and the radius of gyration was 36.6 nm. The AGP component was (28.54%) of total gum with molecular weight of 4.935 $x10^6$g/mol, and radius of gyration 33nm. The (AG and GP) component was found to be 71.46% of the total gum with molecular weight of 1.571 $x10^6$ g/mol. Mass recovery of AGP component is low (28.54%), this mean a good emulsifying property.

The molecular weight parameters of *A. nilotica* var. *adstringens* gum are agreement with those reported by Satti (2011) [14] for *A. nilotica* var. *nilotica*, but it is higher compared with *A. senegal* and *A. seyal* gum.

Table 1: Physicochemical properties of *A. nilotica* var. *adstringens* gum

Sample code	Moisture%	pH	Ash%	Acid Eq.W	Uronic acid%	S.O. rotation(+)
Sample 1	10.78	5.11	2.13	1875	10.35	91.5
Sample 2	10.18	5.14	2.44	2000	9.70	86.6
Sample 3	10.74	5.01	2.44	1829.27	10.61	104
Sample 4	10.76	5.00	1.17	1948	9.96	101
Sample 5	11.8	5.01	2.04	1875	10.35	75.6
Sample 6	10.53	5.11	2.43	1807.23	10.73	98.5
Sample 7	10.74	5.12	1.47	1875	10.35	101
Sample 8	11.57	5.09	2.42	1851.85	10.48	101
Sample 9	10.96	5.02	1.99	1829.27	10.61	99
Sample 10	11.77	5.10	2.04	1829.27	10.61	96
Sample 11	10.95	5.03	1.64	1875	10.35	91
Sample 12	11.95	5.05	2.43	1807.23	10.73	101
Sample 13	10.60	5.05	1.69	2000	9.70	98
Sample 14	10.70	5.01	1.99	1829.27	10.61	99
Sample 15	11.17	5.04	2.43	1807.23	10.73	103
Average	11.01	5.06	2.05	1869	10.4	96.4

Table 2: Physicochemical properties of *A. nilotica* var. *adstringens* gum-composite samples

Sample code	Intrinsic viscosity cm^3g^{-1}	Nitrogen%	Protein %
Comp 1	11.22	0.080	0.52
Comp 2	11.76	0.054	0.36
Comp 3	10.33	0.056	0.36
Comp 4	11.78	0.081	0.53
Comp 5	10.07	0.083	0.54
Whole comp	11.17	0.039	0.25
Average	11.06	0.06	0.44

Table 3: Cationic composition of *Acacia nilotica* var. *adstringens* gum samples (ppm)

Sample Code	Ca	Mg	K	Na	Mn	Fe	Ni	Cu	Zn	Sr	Sn	Co	Cr	As	Cd	Pb
1	336308	303706	412	689	12.65	22.59	1.43	2.14	18.65	28.67	25.00	0.06	0.84	0.01	0.004	0.06
2	1124655	233372	1150	798	3.00	28.25	0.17	0.85	9.76	5.56	9.53	0.05	0.22	0.03	0.03	0.31
3	1187942	228996	601	551	40.15	135.00	0.96	4.45	102.08	46.03	18.47	0.30	1.06	0.06	0.10	1.30
4	1232663	198191	414	537	25.49	34.99	1.11	36.78	19.49	43.09	127.33	0.12	1.07	0.05	0.08	5.09
5	1301454	266914	236	457	40.34	24.38	0.95	5.80	10.42	52.17	20.96	0.24	1.00	0.14	0.19	1.64
6	1240408	249263	435	436	37.58	49.02	0.91	3.97	7.04	43.51	15.74	0.16	0.81	0.06	0.12	1.49
7	1322207	246512	504	570	39.52	64.93	0.94	4.22	7.02	54.28	17.54	0.18	0.98	0.06	0.10	2.03
8	1249075	252810	698	665	44.66	39.24	1.02	5.11	7.16	48.70	15.25	0.17	0.93	0.06	0.12	1.77
9	1280477	269230	417	448	45.26	236.63	1.46	5.08	6.48	53.51	20.27	0.35	1.76	0.10	0.10	2.54
10	1141688	252900	541	560	22.64	33.45	1.02	3.55	6.62	41.04	11.62	0.12	0.91	0.05	0.08	0.94
11	265453	312544	408	800	32.13	41.16	0.60	3.31	4.61	38.54	15.00	0.11	0.64	0.04	0.06	1.37
12	1377507	250483	431	430	33.22	28.89	0.40	2.61	4.16	34.53	20.91	0.10	0.50	0.02	0.22	0.71
13	1414990	239325	517	471	15.23	25.02	0.44	4.25	7.82	38.90	14.69	0.33	1.46	0.06	0.22	0.78
14	1507254	285510	405	451	31.77	79.01	1.44	1.88	4.81	35.27	17.24	0.13	1.17	0.03	0.20	0.70
15	1268437	290132	821	647	41.65	29.09	0.38	2.27	17.31	34.43	16.73	0.13	0.61	0.03	0.18	0.74
Mean	1150035	258659	533	567	31.02	58.11	0.88	5.75	15.56	39.88	24.42	0.17	0.93	0.05	0.12	1.43
Comp	1241240	323855	954	693	38.18	46.38	0.28	1.78	10.96	48.96	33.65	0.06	0.40	0.01	0.002	0.40

Table 4: Sugar composition of *Acacia nilotica* var. *adstringens* gum

Sugar	%
Arabinose	52
Galactose	24
Rhamnose	>1

Table 5: The calorific values of *A. nilotica* var. *adstringens* gum

Sample name	Sample location	Sample weight(g)	Bag. Cal. Value J/g	Gross cal. value	Net.cal value J/g	Net.cal value Cal/g	Cal. value Kcal/g
A. Adstringes gum	Composite	0.5741	46463	15478	17009.8	4063.7	4.064

Fig 1: *Acacia nilotica* var. *adstringens* gum

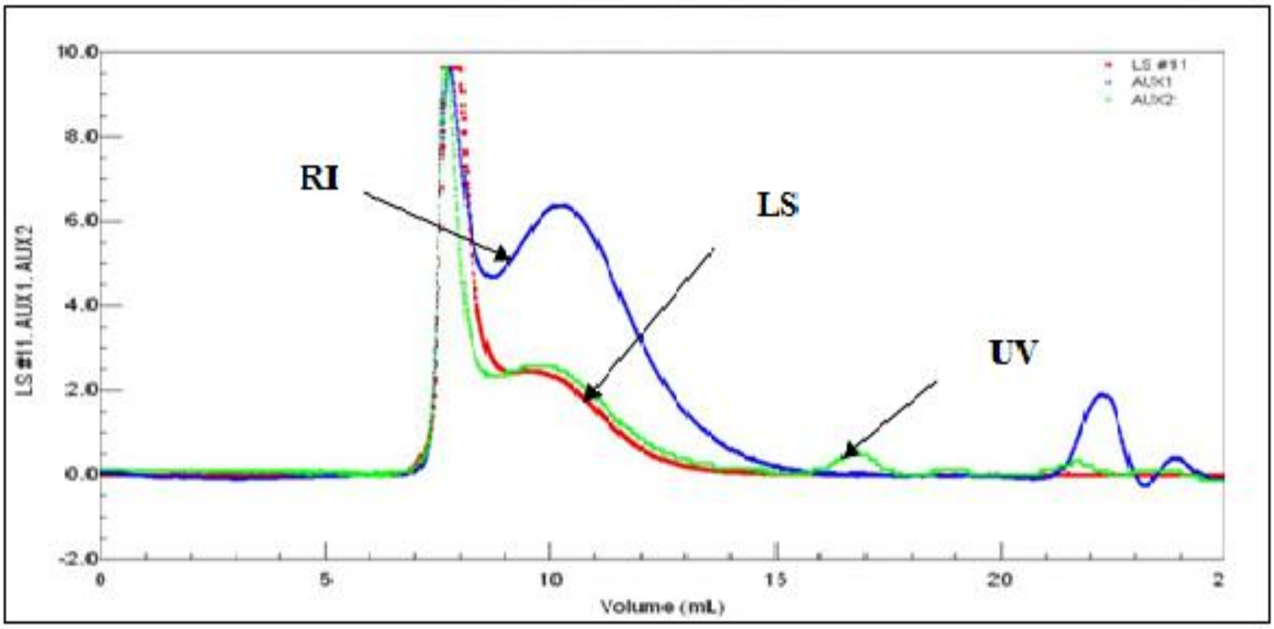

Fig 2: GPC for *Acacia nilotica* var. *adstringens* (whole gum).

4. Conclusion

- *Acacia nilotica* var. *adstringens* gum shows similar physicochemical characteristic as the same as *A. nilotica* var. *nilotica* and *tomentosa* gum.
- *Acacia nilotica* var. *adstringens* gum possess greater amount of AGP with higher molecular weight.
- Higher AGP content leads to better emulsion properties.

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