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Carbohydrates and Ascorbic Acid Contents in the Acacia Senegal and Acacia Seyal Gum Arabic

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ABSTRACT

Gum Arabic (GA) samples had been intentionally collected, identified and confirmed by comparing to the herbaria materials of the Faculty of Agriculture in Khartoum University, and by physical appearance. The primary tests and the cold extraction processes had been carried in the Central Laboratory of Sudan University of Science and Technology at Shambat. The three GA samples were Acacia Senegal, Acacia Seyalvar.seyal and Acacia Seyalvar.fistula. The major sugars in GA samples had been investigated using HPLC techniques, and the Ascorbic acid level was measured by UV spectrophotometry technique. The study found that galactose was the most abundant sugar in all the tested varieties of GA (40.23% for A.Senegal, 35.13% for A.Seyalvar. seyal and 31.17% for A.Seyalvar. fistula). Arabinose and Rhamnose sugars and the Uronic acid were notably higher in A.Senegal than A.seyal samples. The approximate ratio of the three essential sugars {galactose: arabinose: rhamnose} in the A.Senegal and A.Seyal vars. were {9:5:2} and {13:2:1}, respectively. The level of Ascorbic acid in the A.senegal variety was 4 mg/100mg but was below detection limits in the A.Seyal varieties.

Keywords: GumArabic; Acacia; major sugars; Ascorbic acid

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INTRODUCTION

Gum Arabic (GA) (E414, acacia gum)is one of the most important commercial poly- saccharine and it is probably the oldest food hydro-colloid in current use [1,2,3].It is the gummy dried exudation obtained from various species of Acacia trees of the Leguminosae family. About 500 species of Acacia are distributed over tropical, sub-tropical and sub-Saharan (Sahel zone) areas of Africa, India, Australia, Central America and North America [3-6].AcaciaSenegal and AcaciaSeyal remain the most commercially important species [7] named after the trees Acacia Senegal and AcaciaSeyal, which produce the gum[8].Hundreds of thousands of Sudanese peoples use GumArabic for their livelihoods, and Sudan is still the world's largest producer of this gum" Gum Hashab and Gum Talha". Sudanese natural gums include many other varieties, some of them are not "true gums" as they are soft and melt when subjected to heat[9].The stem and branches of Acacia Senegal tree, when wounded (intentionally by farmers), they exudate gum (like tears) as a protection mechanism, then the exudates dried forming solid nodules [5,6].

Acacia gum had aprebiotic and hypoglycaemic effect, It is used as meal substitutes, and used as hydrocolloids and emulsifier fixing agent in cosmetics and textiles, and in biomedical/pharmaceutics industries[10,11,5,6,].The Acacia Senegal gum is highly distinct from other natural gums [12].

The similarities in some properties of an Acacia Senegal gums and some other gums (e.g.Anogeissusleiocarpus) may be due to the presence of the same six different kinds of sugars, L-arabinose, D-galactose, D-glucuronic acid, D-mannose, 4-o-methyl glucuronic acid (uronic acid), and L-rhamnose[13,14,15,16]. Particularly theGA is a source of arabinose and ribose (0.49-0.56%),both of them were first discovered and isolated from this gum, and were named after it [6,17-24].The composition of (GA) is dependent to some extent on the age of the tree, location, and other ecological factors [3,4].

Ascorbic acid (vitamin C) is easily oxidized to form dehydroascorbic acid (reversible reaction)[25].The precursors in the plant ascorbic acid biosynthesis pathway seem to be L-galactose, glucose, or glucuronic acid[26]:

D- glucose →D-glucoronic acid→ L-ascorbic acid

UV spectrophotometric method for vitamin C detection in plant had been developed by Hussain Jobalet al. [27] who founds high contents of vitamin C (from 28 Up to 161 mg/100g) in some different plants. They confirm the effect of some factors such as temperature, light, pH, and oxygen in vitamin C concentration.

The aim of this study was to measure the most abundant sugars - as these sugars represent the most important carbohydrates in GA-, particularly galactose, arabinose, rhamnose, and uronic acid, in addition to the detection of ascorbic acid in three varieties of the Gum Arabic (GA) collected from Sudan.

The Sudanese GA is considered the best gum worldwide, it has a wide range of nutritional, medicinal, and industrial uses. This study may help in better utilization of the Gum Arabic, and in distinguishing between its different types. Detection of ascorbic acid in GumArabicis rarely discussed in the published literature.

EXPERIMENTAL

Sampling & Extraction:

Different crude natural Gum Arabic samples were collected from the local market (Omdurman area – the national Capital of Sudan), representing the harvest of rainfall season 2017, imported from Western Sudan (Kordofan region). The samples had been classified according to reference herbarium material from the herbaria of the Faculty of Agriculture in Khartoum University.

The physical appearance of the gum bulbs then authenticated and confirmed by a collective agreement of doctors and specialized technicians in the Faculty. Three Gum Arabic varieties were the subject of analysis (Acacia Senegal, Acacia Seyalvar.seyal and AcaciaSeyalvar.fistula).The extraction done in the Central laboratories of the College of Agricultural Studies, Sudan University of Science and Technology at Shambat, then a series of laboratory experiments were undertaken at the Central laboratories of the Faculty of

Agriculture in Khartoum University,with the primary objectives to measure the major sugars – as sugars represents the most important carbohydrate- and Ascorbic acid content in these different types of GumArabic. The samples were scanned for chemical and physical properties using the cold extraction methods.

Physiochemical parameters:

The determination of moisture content, ash content, and pH were done according to the methodology described by the Association of Official Analytical Chemists[28]. The moisture content was determined by using an oven (No.03-822, FN 400, Turkey) at 105 °C±1 °C. Triplicate results were obtained for each sample and the mean value was reported according to the following formula:

$$\text{Moisture content (\%)} = \frac{W1 - W2}{Wt1} \times 100$$

Where: W1= Sample weight before drying; W2 =Sample weight after drying; Wt1=initial sample weight.

The total ash content was calculated using this equation:

$$\text{Total ash \%} = \frac{\text{ash weight}}{\text{Original sample weight}} \times 100$$

Determination of sugars:

For the Determination of sugars**,** the extracted gum sample centrifuged to remove the lead oxalate which was previously added. The supernatant was filtered, and collected in vial for injection in high - performance liquid chromatography (HPLC Hewlett series), with refractive index (RI) detector, HPLC carbohydrate analysis column (300mmx7.88mm). The mobile phase was deionized water, at flow rate of 0.8ml/min temperature 35^{o}C [29].The Mass Spectrum was detected at 0.667min.

Ascorbic acid:

Ascorbic acidhad been detected by UV spectrophotometer. Total ascorbic acid (ascorbic acid + dehydroascorbic acid) has been determined according to the simple method described in Kapur(2012) study[30]. This method is based on the oxidation ascorbic acid to dehydroascorbic acid by bromine water in the presence of acetic acid. After coupling with 2,4-dinitrophenylhydrazine (DNPH) a red complex was produced and the absorbance of that complex was spectrophotometrically measured at 521 nm. A linear concentration range for standard solutions of ascorbic acid was obtained up to 10 µg ml^{-1}, with a correlation coefficient of 0.9929. The limit of detection of ascorbic acid was found to be 0.01µg ml^{-1}[30].

Statistical calculations:

The Mean and the standard deviations of triplicate trials of each parameter were calculated using Microsoft Excel 2016 version.

The ratios of sugars had been calculated manually by dividing the level of one sugar by the summation of the three sugars levels, then the result was multiplied by 100. For example:

Galactose ration = (Conc of Galactose) /(Conc of Galactose + Conc of Arabinose + Conc of Rhamnose) x 100

RESULTS AND DISCUSSION

Gum Arabic (Acacia) natural specimens had been collected from the local market and evaluated. The physical appearance of the natural gums is of great importance to determine their types. Generally, the Gum Arabic is odorless and tasteless. The A.Senegalgum(from Hashab trees)was aglobular nodule with a rough surface and small cracks, it varies from colorless through shades of yellow to light brown. A.Seyalvar.seyalgum (from Talha trees)was in the form of fragile nodules that may be crushed, its color varies from colorless to dark brown, odorless and sometimes with sweet or bitter taste. **(Fig.1)**

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Fig 1: Two Gum Arabic varieties of Acacia types, A. Senegal: almost colorless (to your left hand); A. seyal: brown to reddish color (to your right hand).

The screening chemical tests and physiochemical analysis have resulted no significant differences between A.Senegal and A.Seyl gum varieties concerning the moisture contend, ash content, and the pH value **Table 1**.

Table 1: Results of some physicochemical parameters of the three Gum Arabic varieties samples

	Gum Arabic varieties		
parameter*	Acacia Senegal	Acacia.Seyalvar.seyal	Acacia.Seyalvar. fistula
Moisture%	11.00 ±2.00	8.57 ±1.27	10.93 ±1.78
Ash%	4.73 ±0.49	4.27±0.55	4.13±0.58
pH	4.17±0.29	4.2 ±0.50	3.67±0.42

* The Mean of a triplicate trials ± SD.

These results were almost similar to what reported by Anderson (1976) [21],who used these analytical parameters, and the levels of essential components, and the ratios of the major sugars present (galactose, arabinose, and rhamnose) asa chemical fingerprint distinguishing Acacia gums from other botanical genera.

The uronic acid levels in A.Senegal; A.Seyalvar.seyal and A.Seyal var. fistula were 10.23%; 5.63% and 5.23%, respectively. Akiyama [31] and Anderson [21] found a level around 14% of glucoronic acid (uronicacid) inGA samples.

Our results of galactose sugar levels were 40.23%for A.Senegal, 35.13% for A.Seyalvar.seyal, and 31.17% for A.Seyalvar. fistula. Some previous studies reported galactose level slightly above 40% [21,31].

The arabinose sugar levels were found at21.33%for A.Senegal, 5.80% for A.Seyalvar.seyal and 5.23% for A.Seyalvar.seyal. Some previous studies reported arabinose level in the range 25-28%[21,31].

The rhamnose sugar levels were at10.20% for A.Senegal; 3.73%for A.Seyalvar.seyal and 2.37% for A.Seyalvar. fistula. Some previous studies reported rhamnose level around 10 -13% [21,31].
The levels of uronic acid, arabinose, and rhamnose in A.Senegal gum were apparently higher than in A.Seyal gum varieties.**(Table 2).**

The approximate ratio of the major sugars (galactose: arabinose: rhamnose) were{9:5:2} for A.Senegal gum, and {13:2:1} for both varieties of A.Seyal gums.**(Table 3).**

The similar or nearly similar ratio of A.Senegal gum were also found by the previous studies of Anderson [21] and Akiyama[31].The similarity of Acacia gum sugars ratios in most studies may support the idea of using these ratios as an indicator for the Acacia gums type.

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Table 2: The sugar slevels of the tested three types of Acacia Gum Arabic

	Gum Arabic varieties		
parameter*	A.Senegal	A.Seyalvar.seyal	A.Seyalvar.fistula
uronic acid%	10.23 ± 0.25	5.63 ± 0.15	5.23 ± 0.31
galactose%	40.23 ± 0.25	35.13 ± 4.22	31.17 ± 1.26
arabinose%	21.33 ± 1.33	5.80 ± 0.20	5.10 ± 0.46
rhamnose%	10.20 ± 0.20	3.73 ± 0.25	2.37 ± 0.31

* The Mean of a triplicate trials ± SD.

Table 3: The approximate ratios of the major sugars in Acacia gum types

Gum type	Sugars ratio*
A. Senegal	9:5:2
A.seyalvar.seyal	13:2:1
A.seyalvar.fistula	13:2:1

* Sugars order: (galactose: arabinose: rhamnose)

The gum exudates from A.Senegal(Hashab) tree are completely different from the gums exudates from other acacia trees [15,32]. Aline et al. (2013) [33] compared the approximate ratio of the major sugars in Acacia mearnsii de Wildgum {10:9:2} with that of the Gum Arabic {9:16:3},notifying that they used different analysis techniques (GC–MS and colorimetric methods).

Our findings of ascorbic acid (UV Spectrophotometer) in the three varieties of gum were 4 mg/100g in A.Senegal samples (Mean of triplicate trials), and no vitamin C was detected in the two varieties of A.Seyal samples. Vitamin C (ascorbic acid) is known by its fragility and liability to the physical and environmental factors, it is "broken" on oxidizing, exposure to pressure, high temperature, and other similar factors, but its resistant to freezing [34]. However, these low levels of ascorbic acid in GA samples may be due to its liability to these factors during the storing period, besides the technique chosen for detection. The literature is poor concerning studies of vitamin C levels in the GA. The low content of ascorbic acid in the GA may also be due to the low level or absence of photosynthetic activity by the Gum Arabic bulb, that activity by which the plant is usually synthesizing ascorbic acid[35].

Hussain J.et al. [27] found high contents of vitamin C (28 to 161 mg/100g) in some plants, Maysoon and Wiliam found a range from 98 to 163 mg/100g of fresh weight of some fruits [36]. Comparing these and other results of ascorbic acid levels indicates that GA is not a good source of vitamin C. The studies of Hussain[27], Sumner and Dhillon (1983) [37]concluded that medicinal plants have high vitamin C content as compared to the fruits, they concluded that the ascorbic acid is stable in solid form but oxidized in solutions by the dissolved oxygen.

Most previous studies conducted for gums types throughout the world had taken Gum Arabic as a reference on doing comparisons. For example, the previous studies on Brea gum (BG) (exudates from the Cercidium praecox tree) that grows in Argentina, have shown physicochemical characteristics and functional features similar to those of Gum Arabic, this analysis showed that the bulk of the gum composed approx. 84% of the polysaccharides [33]. Another example was the Acacia mearnsii de Wild gum exudates, collected from trees growing in the south of Brazil, was also compared with commercial Gum Arabic, the Acacia mearnsii de Wildgum had a higher protein content than Gum Arabic, this gum contains (4%)uronic acid content, compared with (17%) of the Gum Arabic[33].That study of Alineproposed mearnsii de Wild gum as a substitute of Acacia Senegal and Acacia Seyal for use in some applications. A third example is a study of Cui & Mazza (1996) on durian seed gums in which sugar composition is very different compared to other commercial gums such as guar, and Gum Arabic [38].However, Gum Arabic structural characterization is incomplete and subject to many

corrections[39-43],it seems that sugars' content is not that property which gives Gum Arabic its distinguished unique quality.

We recommend future studies on vitamins and cellulose content of Gum Arabic.

CONCLUSION

Sugars' content of some most known Sudanese Gum Arabic varieties (Acacia Senegal, Acacia Seyalvar.seyal, and Acacia Seyalvar.fistula) was lower than that reported previously. particularly, similar sugars levels were observed in the two A. Seyal types. The levels of uronic acid, arabinose, and rhamnose in A.Senegal gum were apparently higher than in A.Seyal gum varieties. The variations in the results between this study and some previous studies may be partially due to the different methodologies of extraction and detection techniques. The approximate ratio of the three essential sugars {galactose: arabinose: rhamnose}in A.Senegal and A.seyalboth varieties were{9:5:2} and{13:2:1}, respectively. This sugars' ratio can be used as an indicator for the Acacia gums types.The Ascorbic acid content was very low in some Sudanese Acacia gums types.

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