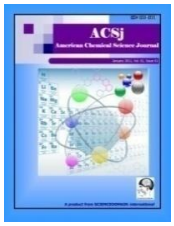

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Preparative Fractionation Analysis of *Acacia polyacantha* Gum Using Acetone as a Solvent

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Five authentic Acacia polyacantha whole gum samples were collected as natural exudates and five acetone fractions were obtained. Analysis of the whole gum samples and the fractions were carried out t for nitrogen (protein), specific rotation, intrinsic viscosity, pH, equivalent weight, uronic acid content, refractive index and average molecular weight. Results of refractive index for the whole samples and the fractions were found to share the same value (1.3337). Significant differences were obtained between the values of these parameters for the whole gum samples as well as the fractions and it is also among the fractions themselves.
In particular fractions 1,2,3,4and 5 showed nitrogen content of 0.32%, 0.35%, 0.40%, 0.39%and0.37% and protein content of (2.11%,2.31%,2.64%, 2.57% and2.44%) respectively. The mean values of specific optical rotation of the fractions was found-13.5 0, -14.50, -15.0 0,-16.00 and -14.50 respectively. The average molecular weight of the fractions gave 2.669x105,

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2.669X105, 2.714X105, 2.339X105 and 2.754X105 respectively. The statistical analysis showed significant differences (P ≤ 0.05) for some parameters such as, intrinsic viscosity, pH and the average molecular weight. These data confirms the heterogeneity of Acacia polyacantha gum, and their fractions.

Keywords: Gum Arabic; acacia; Fractional precipitates.

1. INTRODUCTION

The Sudanese major gums of economic importance are in the order of gum arabic gum Talha and *polyacantha* gum. Gum arabic, sometimes known as the dried gummy exudation of *Acacia. Senegal* closely related species of *Acacia* family *Leguminous* [1]. Defaye [2] defines gum arabic as a highly branched uronic acid type hetropolysaccharide produced as exudates from trees of the genous *Acacia* maintained under unhealthy conditions. It was noted that gum was very heterogeneous and it was described as 'hetropolymolecular' i.e. having either a variation in monomer composition and /or a variation in the mode of linking and branching of the monomer units, in addition to a distribution in molecular weight [3]. The mostly important property of a gum which make it unique amongst polysaccharides generally it is solubility and viscosity. The majority of gums dissolve in water at different concentration (e.g. Gum arabic can form solutions of up to 60% forming viscous solutions) .These properties of gums can be utilized in many applications, such as in food industry which is the major one, where emulsifying and stabilizing properties are utilized. The gum is also used in the pharmaceutical and medical fields, in addition to other industries (cosmetic, adhesive, paints and inks). Gum from *Acacia polyacantha* tree is a dried exudation obtained from stem and branches of *Acacia polyacantha* tree, family (*Leguminusae*) and genus(*Acacia*) , English name is Falcon's claw *acacia* and arabic name (Kakamut , Umsinina) [4 and 5]. Kakamut tree is widely distributed in Africa throughout tropical Africa. In Sudan there are several regional varieties, which usually occur along rivers and valleys where the water table is fairly high and soils are good [5]. In Sudan *Acacia polyacantha* tree is widely spread in Upper Nile province, Kordofan province *and* Blue Nile province. The wood is used mainly in fuel and charcoal of good quality, fence posts, farm implements and railway, sleeper, beams, and rafters. The gum is edible and used as adhesive in the treatment of textile fibers. The roots are used to act as a general health tonic as antidote for snake bite, and cure for venereal diseases. A preparation from the bark is used for general stomach disorders [5]. The aim of this work is to present and evaluate analytical data of authentic gum samples collected from three different locations, also to find out the effect of location on the gum properties.

Acacia polyacantha exudates is closely related to, an can hardly be distinguished from, the *acacia* exudates unless recognized by acknowledged gum expert or by studying the physicochemical characteristic, the two species *Acacia senegal* and *Acacia polyacantha* belong to the same group known as *Acacia senegal* complex, all gum from this group of *Acacia* species, have a laevorotatory(-ve) specific rotation [6]. Gummosis may be a protective mechanism against pathological conditions like bacterial or fungal infection, insect infestation, or mechanical injury.

Fractionation is one of the most important of analysis, the preparative technique for fractionating polydispersed polymer is the simplest, and it involves the addition of precipitants to unaqueous isolation of fractions having different solubility's. Coprecipitations may occur [7]. Different fractions of different solubility's of the gum can be calculated, (fraction A), salt (fraction B), and the residual insoluble gel (fraction C) [8,9]. The gum solution has been fractionated previously using ethanol, saturated sodium sulphates solutions [7], ion exchange chromatography in DEAE- cellulose, phenyl sepharose Cl-4B 9, anti- arabinogalactan protein (AGP) antibodies [10]. Fractionation of *A. senegal* hydrophobic affinity chromatography reveals that it consists of at least three components: - Fraction 1 arabinogalactan (AG), fraction 2 (arabinogalactan protein) and fraction 3 glycoprotein (GP), [11,12]. But even those contain a range of different molecular weight components revealing the polydispersity of the gum [13] and electrophoresis [14]. Fraction 1 containing 88% of the total has only small amount of protein. Fraction 2 represents 10% of the total with a protein of 12%. Fraction 3 resembles 1.24% of the total, but contains almost 50% protein, which is 25% of the total protein present in the whole gum. Arabinogalactan protein (AGP) is responsible for the emulsifying

properties of gum arabic [11,12]. Preparative fractionation of polydispersed polymer using acetone is simple, acetone is less toxic and it can give best filaments of the fractions precipitants. The purpose of this study deals with some physicochemical properties of five successive fractions obtained from sample of *Acacia polyacantha* gum by fractional precipitation using acetone.

2. MATERIALS AND METHODS

Authentic samples of *A. polyacantha* gum were collected as natural exudates in nodule forms from *A. polyacantha* tree with the help of Gum Arabic Company Ltd, Khartoum, Sudan. The gum samples were collected in season 2001 and 2002. The gum samples were dried at room temperature about (30°C) then cleaned by hand, ground, sieved through the sieve No,16 and kept in container for analysis. The five samples were named as S1, S2,S3,S4 and S5respectively.

2.1 Methods

2.1.1 Fractionation

Solvent fractionation was carried out by a combination of the methods described by Tagar [15]. One liter of 10% clear homogenous of gum solution was placed into a three – necked round bottle flask connected with a mechanical stirrer and separating funnel. On addition of acetone 100 ml portion precipitation resulted in fine filament of gum. The precipitated phase was obtained by adding one liter of acetone. On standing over night, the precipitate was separated and dried in a desiccator (Fraction1). Different fractions (2 to 5) were separated successively by successive addition of 200ml of acetone portion.

2.1.2 Nitrogen content

Nitrogen was determined using semi micro khjeldahl method [16]. Accurately weighed 0.2 gm of gum samples in triplicates were taken in khjeldahl flasks and khjeldahl tablet [copper sulphate – potassium sulphate catalyst] was added to each tube. Three and half mls of concentrated nitrogen free sulphuric acid were added. The flask and contents were then heated over an electric heater until the solution attained a clear blue color and the walls of the flask were free from carbonized materials (85C). The contents of the flask were then transferred to a steam distillation unit, and 15 mls of 40 % sodium hydroxide solution were added, and distillation were carried out with steam. The distillation was collected in 10 mls of boric acid solution (2 %) to which three drops of methyl red were added, and titrated against 0.01N HCl. The same procedure was carried out for blank (distilled water)

$$N\% \quad \frac{(M1-M2) \times N \times 14.01 \times 100}{S \times 1000}$$

Where:

- M_1 : mls of HCL that neutralized the sample distillate
- M_2 : mls of HCl that neutralized the blank distillate
- N : normality of HCl titrant (0.01)
- S : sample weight (0.2 g).

The protein content was determined by multiplying nitrogen percent by the factor 6.6 [17].

2.1.3 Specific rotation

The specific rotation for the five whole gums and the five fractions was determined for 1.0 % aqueous solution at room temperature (30C) using an optical activity Bellingham and Stanley (Ltd.) polarimeter fitted with a sodium lamp with a cell path length 20 cm. The solution was passed through a No. 42 filter paper before carrying out measurements at room temperature. Triplicate readings were taken and averaged. The specific rotation for gum solution was calculated according to the relationship (Honig1964) [18]:-

$$\text{Specific rotation} = \alpha \times 100/C \times L$$

Where:

- α = observed optical rotation
- C = concentration of the solution
- L = length of the polarimeter tube

2.1.4 Intrinsic viscosity

An aqueous solution (1%) was prepared from each sample of the whole gum samples and the five fractions and the rate of flow recorded for successive dilutions using a capillary viscometer (shott Gerate type 50120/11) in a constant temperature bath at 30°C .The intrinsic viscosity was obtained by extrapolation of reduced viscosity against concentrations back to zero concentration [19].

2.1.5 pH value

The pH of the gum solutions was determined for the whole gum and the five fractions, using a Beckman Zeromatic IV pH meter at 30°C.

2.1.6 Refractive index

Refractive index of 1% *polyacantha* gum solution was determined at room temperature using Bellingham and Stanley – London No, 918095 England Refractometer.

2.1.7 Equivalent weight

Apparent equivalent weight was determined according to the method reported in encyclopedia of chemical technology vol. 11, Encyclopedia of chemical technology [20] with some modification. The aqueous gum solution (3%) was treated with acid washed Amberlite Resin 120 (H^+) [2 gms per 10 mls gum solution] for an hour and then titrated against 0.02 N sodium hydroxide solution using phenolphthalein as indicator and the equivalent weight was determined as follows:

$$\text{Equivalent weight} = \frac{\text{weight of the sample X1000}}{\text{volume of titer X molarity of alkali}}$$

2.1.8Uronic acid anhydride

Uronic acid percentage was determined according to the relationship (Elamin1972) [21]:

$$\text{Uronic acid} = \frac{\text{molar mass of uronic acid anyhydride X100}}{\text{acid equivalent weight}}$$

2.1.9 Molecular weight

The molecular weight was calculated from intrinsic viscosity using Mark-Houwink equation [22,23], based on Anderson and Rahman [24] for *Acacia senegal.*

2.1.10 Statistical analysis

Analysis of samples and fractions was carried in triplicate and then averaged. Data obtained were assessed by Mann- Whitney test. Basic statistics for all parameters were computed (mean, standard deviation and P-value). Finally the significance and insignificance differences were determined in the appropriate mean at a probability level of (0.05).

3. RESULTS AND DISCUSSION

The present study, fractionation of authentic sample of *A. polyacantha* gum by solvent (acetone) has been carried out and some physicochemical properties have been determined and results compared between whole gum and fractions and among gum fractions themselves.

Table 1 showed, the analytical data for five authentic samples of *A. polyacantha* gum. It illustrated that the mean value for nitrogen content for the five whole gum samples is 0.35% which is in agreement with that (0.35%) reported by Karamalla [25]. It is also in a close agreement with the mean value of nitrogen 0.34% reported by Ishag [26]. The values obtained it was in the range (0.33 to 0.36%), (2.18 to 2.38%) for nitrogen and protein content respectively reported by Siddig [27]. Such results obtained for nitrogen and protein content it was in the range (0.30 to 0.42%), (1.88 to 2.77%) respectively in the recent studies reported by Elnour [6]. Table 2 showed the nitrogen (protein) content for the five gum fractions. It was in the range (0.32 to 0.40%), (2.11to2.64%), and the mean value is (0.37%), (2.44%). Also Table 2, it has been shown that fractions F_3, F_4, and F_5 are richer in nitrogen than the whole gum. It is evident from the protein content in *polyacantha* gum that the high amount of protein in the molecules is associated with the high molecular weight fraction. This finding of protein content in gum fractions agrees with the observation in *A. senegal* and *A. seyal* [28]. Insignificant differences ($P \leq 0.05$) were observed on the analyzed data of each nitrogen and protein contents of the whole gum and its fractions.

Fractionation of gum arabic using gel filtration gave only two major fractions when monitored by UV absorbency at 220 nm and a specific uronic acid assay. The first fraction (10% of the total mass) contained 10% protein and 90% carbohydrate, the hydrogenous fraction (90% of the total mass) contained only a small 0.3 – 1%) protein [29]. This conclusions support the heterogeneity of *A. Polyacantha* gum with regard to nitrogen (protein) content. Karamalla [25], has been confirmed that protein rich high molecular weight component arabinogalactan protein (AGP) provides the functionality of gum arabic as an emulsion stabilizer. It can be seen from Table 1 aqueous solutions of all samples and its fractions (Table 2) were found to be optically active (laevorotatory). The values of specific rotation of the five authentic *A. polyacantha* gum

samples were found to be in the range(-13.0° to -20.5°) with the mean value of specific rotation is(-17.40°) which is higher than that (-10.3°) reported by Biswas [30], and also greater than the range (-7° to –13.0°) showed by Siddig (2003). This value was less than the range (-24.5° to –36.7°) for *A. senegal* reported by Siddig (1996).These findings were within the range of recent results (-8.7° to-25.0°) reported by Elnour et al. [31]. Table 2 showed results of the specific optical rotation of the fractions, it ranged from (-13.5° to –16.0°) but the values are generally less than those for unfractionated whole gum (-13.0° to -20.5°). The results showed the increasing in the values of specific optical rotation for the first four fractionsF1, F2, F3 and F4, (–13.5°, -14.5° –15.0 and –16.0°) respectively. In addition it seems that the mean value for the specific optical rotation for the five fractions (calculated as –14.7°) is less than the mean value for the whole gum (-17.40°). Analysis of data showed in significant differences (P ≤ 0.05) between samples and fractions.

Aqueous solution of all samples of *Acacia polyacantha* gum is viscous. Table 1 representsthe intrinsic viscosity of all samples ranged from (10.2 to 10.6 ml/g) with the mean value of (10.34 ml/g). This mean value of intrinsic viscosities of the crude gum samples (10.3 ml/gm) were significantly less than those (10.3 ml/g) of the fractions (11.18 ml/g) (Table 2). However, the values of intrinsic viscosities of*A.polyacantha* gum was closer to the results obtained by Siddig [27] (12.7 ml/g), and less than (14 ml/g) for *A. seyal* reported by Elkhatim [32]. Present results were in a close agreement with value (10.2 ml/g) reported by Elnour et al. [31]. Statistics analysis showed significant differences (P ≤ 0.05) between the samples and the fractions. The variation in the intrinsic viscosity values of the fractions indicate the heterogeneity of *polyacantha* gum, such variation may be due to the variation in molecular size, branching and the degree of polymerization of monosaccharide in the gum polymer system.

The pH of *Acacia polyacantha* gum aqueous solution was found to be slightly acidic. It is clear from Table 1 that the pH value of whole gum ranges from (4.7 to 5.0).

The mean value of pH of the whole gum samples was found to be (4.84) which is greater than that of the fractions (4.70), (Table 2). These results werein agreement with that value of *senegal* gum (3.19-5.61)(Siddig1996), and in agreement to the range of (4.9) reported by Siddig [27]. It is closely related to the result (5.96) represented by Elnour [6]. The statistic analysis showed significant differences (P ≤ 0.05) between the samples and fractions.

Refractive index of all samples and fractions, (Tables 1 and 2) shared the same value (1.3337), it is in a close agreement with the result (1.3337) reported by Elkhatim [32] for *Acacia polyacantha* gum .

Concerning the equivalent weight, samples (Table 1) and the fractions, (Table 2) showed a minimum value (1170.07) for samples and maximum value (1619.87) (1515.15-1630.43 for fractions). However the above result is in agreement with the range (1136.0 to 1875.0) for *Acacia senegal* reported by Karamalla [33]. This result is also greater than that obtained in the recent studies (1280.80) reported by Elnour [6]. The equivalent weight showed insignificant differences (P ≤ 0.05) between the samples and fractions.

The presence of uronic acids in all samples of *Acacia polyacantha* gum indicated that all samples have acidic sugar (Glucuronic acids). (Table 1) illustrated that *polyacantha* whole gum samples and fractions, uronic acid content of the whole gum and fractions ranged from (12.54 to 16.56%) and (11.90to12.80%) respectively. It clear that the mean value of uronic acid content of the whole gum is (14.5%) which is higher than that for fractions (12.26%) (Table 2). The mean value (14.5%) for the whole gum samples which in the range of *senegal* gum (10.34 to 23.32%) reported by Siddig [34] and Eltayab [35] for *Anogesissus Leiocarpus.* Results indicated here were comparable to the values ranging from (12.02 to17.30%) reported for *Acacia polyacantha* gum Elnour et al. [31]. Statistical analysis indicated insignificant differences (P ≤ 0.05) between the samples and fractions.

It is clear from (Table 1) that the mean value of the molecular weight of the five *Acacia polyacantha* whole gum samples is ($2.352X10^5$) which is in a close agreement to the mean value ($4.165X10^5$) for *Acacia polyacantha* gum mentioned by Siddig [27]. This result it is also within the range ($2.200X10^5$ to $4.00\ X10^6$) reported for *Acacia Senegal* [36], but it was high if compared to the range ($3.02X10^3$ to $3.90X10^3$) of *Acacia polyacantha* gum reported by Elnour [6]. This result is in a close agreement to the range value ($2X10^3$ to $2\ X10^5$) reported by Motlagh [14]. Also (Table 2) showed that the

Table 1. The analytical data of five authentic *Acacia polyacantha* gum samples(S)

Sample	Nitrogen %	Protein %	Specific rotation [α]	Intrinsic viscosity (η)	pH	Refractive index	Equivalent weight	Uronic acid %	Molecular weight (Mw)
S1	0.35	2.31	-16.0^0	10.60	4.8	1.3337	1273.34	12.54	$2.462x10^5$
S2	0.36	2.38	-20.5^0	10.20	5.0	1.3337	1173.71	16.53	$2.293x10^5$
S3	0.34	2.24	-20.5^0	10.20	4.9	1.3337	1619.87	11.98	$2.293x10^5$
S4	0.34	2.24	-13.0^0	10.40	4.8	1.3337	1304.34	14.87	$2.377x10^5$
S5	0.35	2.31	-17.0^0	10.30	4.7	1.3337	1170.07	16.56	$2.335x10^5$
Mean	0.35	2.30	-17.40^0	10.34	4.84	1.3337	1308.27	14.5	$2.352x10^5$
S.D	0.01	0.06	3.19	0.17	0.11	0.0000	184.08	2.16	7018

S.D= Standard deviation. Each value in table is a mean of three replicates

Table 2. The analytical data of *Acacia polyacantha* gum fractions (F)

Sample	Nitrogen%	Protein%	Specific rotation[α]	Intrinsic viscosity (η)	pH	Refractive index	Equivalent weight	Uronic acid %	Molecular weight (Mw)
F1	0.32	2.11	-13.5^0	11.07	4.7	1.3337	1515.15	12.80	$2.462x10^5$
F2	0.35	2.31	-14.5^0	11.07	4.7	1.3337	1595.74	12.16	$2.293x10^5$
F3	0.40	2.64	-15.0^0	11.17	4.7	1.3337	1578.95	12.30	$2.293x10^5$
F4	0.39	2.57	-16.0^0	10.31	4.6	1.3337	1595.74	12.16	$2.377x10^5$
F5	0.37	2.44	-14.5^0	11.26	4.6	1.3337	1630.43	11.90	$2.335x10^5$
Mean	0.37	2.40	-14.70^0	11.18	4.66	1.3337	1583.20	12.26	$2.352x10^5$
S.D	0.03	0.21	0.908	0.11	0.05	0.0000	42.41	0.33	7018

S.D= Standard deviation. Each value in table is a mean of three replicates

mean value of molecular weight of the fractions ($2.629X10^5$) which is greater than that for the whole gum samples ($2.352X10^5$). Fraction F1and F2 shared equal molecular weight ($2.669x10^5$) and F5 showed higher molecular weight ($2.754X10^5$). Statistic analysis revealed that significant differences ($P \leq 0.05$) between the samples and fraction. Successively we have obtained different compounds by fractional precipitation of *Acacia polyacantha* gum using acetone; such results obtained, it was already reported by Anderson [3] for *Acacia senegal* using sodium sulphate salt. According to Anderson [3] the *Acacia senegal* gum could be separated in to a number of different molecular mass fractions containing varying proportions of proteinaceous matter and monosaccharide constituents. Thus it has been concluded that gum arabic is a hetropolymolecular polymer consisting of molecules that differ in their sugar composition and their mode of linkage as well as in molecular mass. For all data presented here from physicochemical analysis indicated that the fractionated gums differed in several respects from crude gum samples.

4. CONCLUSION

We have been succeeded to obtain different compounds of different physicochemical properties by fractional precipitations of *Acacia polyacantha* gum using acetone. This fractionation of gum confirmed the hetropolymolecular nature of the material, such results it agrees with Anderson [3] and Osman [13]. Such data obtained from the physicochemical analysis indicated that the fractionated gum were insignificantly difference ($P \leq 0.05$) in several aspects from the authentic samples, but they differ significantly ($P \leq 0.05$) in their viscosity, pH and in molecular masses.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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