Physicochemical study of crude Gum and Foam Fractionated from

*AnogeissusLeiocarpus* (Sahab)

A Thesis Submitted in Partial Fulfillment of the Requirements of a

M.Sc. Degree in Chemistry

By

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Desember, 2017
الاستهلال

قال تعالى:

﴿ أَقْرِئْ بِنٍّاسِرَ ۖ رَبِّ ۛ إِنِّي خَلَقْتُكَۡ١ۚ خَلَقْنَا إِلَٰهَيْنَ ۚ مِنْ عَلَىٰۡۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢۢ
DEDICATION

All thanks from whole my heart to all members of my family.

To my dearest uncles & aunts

To my friends
Acknowledgements

Praise is due to Allah Almighty; who gave me the power and ability to complete this work.

I’m greatly indebted to my supervisor, Prof. Mohammed Elmubark Osman for proposing the idea, support, encouragement and response to my questions and queries. I would also like to thank all the members of staff teaching and technical staff of chemistry laboratories at Sudan University of Science and Technology for helping.

I would like to express my deep thank and sincere gratitude to staff of the institute of Research and industrial consultations and chemistry laboratories of Ministry of Minerals, especially Mr. Mohammed Alsawi and the Q.C staff of AmipharmaCo.Ltd, especially Mr. Ahmed Abdulhameed. I would also like to express my deep thanks to Mr. Naif, collage of pharmacy– Khartoum.

Finally, I thank everyone helped and supported me to complete this research.
Abstract

The gum from *Anogeissus Leiocarpus* was fractionated into foam fraction A (hydrophobic) and B fraction (hydrophilic) and the percent yields were 14.6% and 85.1% respectively.

Crude gum and fractions were analyzed using standard methods. The results have shown that moisture, ash, PH, nitrogen content, protein content and specific optical rotation, were 7.9%, 2.53%, 4.1, 0.392, 2.450 and −37.4 for crude gum then 8.7%, 2.51%, 5.17, 1.022, 6.39 and −38.7 for fraction A (foam/hydrophobic) and 11.3%, 2.54%, 4.8, 0.630, 3.94 and −35.2 for fraction B (hydrophilic) respectively.

Cationic composition of the fractions in ppm were Ca(522.9), K(262.5), Mg(38.4), Na(8.74), Zn(0.093) and Pb(0.124) for fraction A and for fraction B Ca(192.5), K(252.8), Mg(34.86), Na(11.71), Zn(0.093) and Pb(0.052).

The study has shown closely similar results for the percentage ofuronic acid percent between crude gum (14.7%) and fraction B (12.1%) but fraction A (3.23%) has shown significantly different value.

The intrinsic viscosity in g/cm³ for fraction B (0.65) was approximately three times greater fraction A (0.24) and double the value of the crude gum (0.43). In addition the value of intrinsic viscosity of the crude gum was found to be double that of fraction A.
This sugar composition of the crude gum sample was found to be:
Rhamnose (2.31%), Xylose (4.94%), Arabinose (34.95%) and
Galactose (47.67%). In case of fraction A it was found to be
Xylose (6.51%) Rhamnose (6.51%), Arabinose (36.3%) and
Galactose (36.3%) while fraction B consists of Rhamnose (10.5%),
Xylose (6.84%), Arabinose (27.35%) and Galactose (40.12%).
مستخلص الدراسة

جزأ صمغ الصهب إلى جزأين، شق الرغوة أو الكاره للملاء (أ) والمحب للماء (ب) ووجدت النسبة المنوية لكل شق على النحو التالي: 14.6% و 85.1% لكل من أ و ب بالترتيب.

أجريت دراسة تحليله لكل من صمغ الصهب ومشتقاته، حيث تم توصيف الخصائص الفيزيوكيميائية التي تتضمن: الرطوبة، الرماد، الأس الهيدروجيني، محتوى النيزوجين، محتوى البروتينات والدوران النوعي على التوالي لكل من الصمغ والشق A والشق B: (35.89, 85.39, 78.93, 61, 0.392, 24.50, 0.686, 4.288, 0.374, 0.630, 3.988, 0.688, 4.8, 2.54, 4.1, 3.87, 2.23, 6.47, 11.2723, 0.36, 35.2.

أجريت تحليل للكشف عن محتوى المعادن كجزء من المليون ووجدت كالآتي:
الكالسيوم (522.9) البوتاسيوم (262.6)، المغنيسيوم (38.4) الصوديوم (8.74) الزئبق أو الخارجية (0.093) الرصاص (0.124) الثقل (أ) والكالسيوم (25.2) البوتاسيوم (11.71) المغنيسيوم (0.93) الرصاص (52.0) الصوديوم (34.62).

أيضاً أوضحت الدراسة تقارب نتائج نسبة حمض الجلوكوزونيك بين الصمغ الخام والشق (ب) وأظهر الشق (أ) اختلاف كبير.

كما وجد أن اللزوجة الضمنية (جم/سم³) للشق B (0.65) تقريباً ثلاثة أضعاف الشق أ (0.24) وكذلك ضعف قيمة الصمغ الخام.

أظهرت الدراسة أربع أنواع من السكريات في الصمغ الخام:
الإكسيلوز (94.4%) الرامينوز (31.2%) الأريبينوز (4.95%) والجلاكتوز (47.67%)。”}

"أيضاً في الشق أ وجد أن الرامينوز (6.1)%، الإكسيلوز (6.1)%، الأريبينوز (3.3)% والجلاكتوز (36.3)% بينما في الشق B وجدت نسبة الجلاكتوز (12.0%) الرامينوز (10.5%) الأريبينوز (27.35%) والإكسيلوز (6.84%)."
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Introduction

Gums are high molecular weight polymeric compound composed of carbon, hydrogen, oxygen and nitrogen (Somiya, 2010). Plant gums are organic substance obtained as an exudation from fruit, trunk or branches of the trees spontaneously or after mechanical injury of the plant by incision of the bark, or after the removal of the branch, or after invasion by bacteria. The exudates becomes hard nodules or ribbons on dehydration to form sheath against micro-organism. They form clear glassy masses substance with dark brown color to pale yellow. The majority of gums dissolve in water at different concentration (e.g., gum Arabic can form solution of up to 60% forming viscous solution), that makes solubility and viscosity are fundamental property of gum. Gum Arabic is enjoy in applications and this is mainly due to the reduced surface tension and to extremely high solubility in water and low viscosity (Osman, 1993). The major application of gum is in food industry, the gum is also used in pharmaceutical and medical field, in addition to other industries such as cosmetic, adhesive, paint and inks.

1.1. Formation of Plant Gums

Numerous theories for the process by which the tree exudes gums have been proposed. It has been suggested that gums exudates may be a product of normal plant metabolism (Hirstand Jones, 1958; Smith and Montgomery, 1959), that they may arise from a pathological condition of the tree (Smith and Montgomery, 1959), or they may arise from microbiological infection of fungal (Vassal, 1972), or bacterial (Blunt, 1926 Mantell, 1954) origin. Another theory proposed is that starch
may undergo transformation into gum (Jones and Smith, 1949; Hirst, 1951). This seems unlikely according to Anderson and Dea (1986). As the enzyme systems necessary to transform starch into a highly branched arabinogalactan with galactose. Arabinose, rhamnose, glucuronic acid and its 4–0 methyl ether are complex. Further Anderson and Dea (1968) found that starch was not represent in the tissues of excised branches and therefore proposed that the gums have a he micellulose–type highly branched arabinogalactan precursor to which is addedrhnmmoseglucuronic acid and 4–0 methyl glucuronic acid terminated side chains in the final stages of gum production. Miskiel (1990) suggests that gum exudates are more likely to be formed by enzymatic glycosylation reactions rather than direct conversion of other plant polysaccharides.

1.2 Applications of gums

The major application of gum is in food industry 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).

1.2.1 Applications in Food Industry

According to Glickman and Schacht (1959) the major use (55%) of gum (gum Arabic) in the USA is in the food industry, primarily in confectionery. In Western Europe food uses of Gum Arabic accounted for 76% of the market, in 1988 with 4,800 tons were used in confectionery in France, Germany and the UK (Gordon, 1992). Its main functions in confectionery are the prevention of sugar crystallization and as an emulsifier in fat–based sweets, e.g. toffees. The gum acts as an
emulsifier by keeping the fat evenly distributed throughout the toffee thereby preventing the fat from 'leaching out' and forming an oxidisable film on the surface. The gum is also used to incorporate flavors in confectionery such as pastilles and gum drops and in the preparation of lozenges. Gum Arabic has also been used to stabilize frozen dairy products such as ice cream and sherbets due to its high water-absorbing properties. In this context the gum imparts a smooth texture to the frozen product by inhibiting the formation of ice crystals. In baking industry gum Arabic is used in glazes and topping, and in the encapsulation of spray-dried flavors into foods. In another application when used as a flavor fixative, the gum forms a thin and impenetrable film around the flavor particle protecting it from oxidation, evaporation and absorption of moisture. In the soft drinks industry gum is used as a flavor emulsifier in oil-in water emulsions and as a foam-stabilizing agent (Glicksman, 1969).

1.2.2 Gum in Pharmaceuticals and Cosmetics

Gum (gum Arabic) is used as a suspending and emulsifying agent in the Pharmaceutical industry. In the area of cosmetics gum Arabic imparts spreading, Viscosity and protective characteristics to lotions and protective cream by stabilizing the emulsion.

1.2.3 Other Applications
For their adhesive properties gums have been used in the manufacture of adhesives, for postage, stamps and also used in the formulation of paints and inks and have been applied in lithography and textile industry as a sizing and finishing agent (Meer, 1980).

1.3 Anogeissus leiocarpus (Anogeissusschimperi)

1.3.1 Nomenclature


1.3.2 Botanical classification


(USDA, 2010).

Kingdom: Plantae

Family: Combretaceae

Order: Myrtales

Genus: Anogeissus

Species: A. Leiocarpus

Latin name: Anogeissusleiocarpa (Dc.)

Arabic name: Sahab

English name: African birch

1.4 Gum from Anogeissus leiocarpus
These gums are classes of high molecular weight polymeric compounds composed mainly of C, H, O, and N are capable of possessing colloidal properties in an appropriate solvent, or swelling agent at low dry weight. They occur naturally as salts (specially of calcium and magnesium) and in some cases proportion of the hydroxyl group are esterified most frequency as acetates. In practical term gums are either hydrophobic or hydrophilic. Hydrophobic gums are insoluble in water and include resins, rubber, etc whereas hydrophilic gums are soluble in water and can be subdivided into natural, semi-synthetic and synthetic gums (Samia Eltayeb Ahmed, 2009).

Combertaceae is a family of twenty genera and six hundred species tropical and subtropical trees and shrubs. Generally Combertaceae was known as a rich source of tannin. Genera include Terminalia, Combertum, Quisqualis, Myrobalans and Anogeissus (Hans, 1990). Some gums from Combertaceae are being increasingutilized commercially for example *ghatt* gum and *Leiocarpus* gum.

### 1.4.1 Distribution

*Anogeissus leiocarpus* tree is widely distributed in Africa between isoheight of about 200 mm and the rain forest, from Senegal to Sudan and Ethiopia, in the south to Zaire (Hans, 1990). In the Sudan is widespread in Kassala province, Darfur province, Bahr El Gazal province, and Upper Nile province. In Equatoria province it is absent from the east bank of the Nile except for a small area within 20 km of Juba. In Yambio distinct it occurs in the gallery forests (Elamin, 1990).

### 1.4.2 Description
A medium size to a large tree particularly in Darfur province attaining a height upto 20m with a fluted bole. Bark: greyish whites, becoming very dark grey in oldtrees, fairly smooth flaking off, branches often drooping and slender, and branchessoftly pubescent. Leaves: alternate, elliptic to oval telanceolate, obtuseandmucronate, or acute at apex 2–8 cm long, 1.2–3.5 cm broad, at first densely silky, then laxly pubescent beneath leaves light green or ash coloured. Flowers: in globesheads, small, greenish yellow, with reddish disk and white hairs. Fruits: insubglobose cone– like heads, each fruit broadly winged coriaceous, 3 mm broad, braked by the persistent tubular portion of thereceptacle. Flowering of the tree during rains continues until October, fruiting in January andFebruary (Elamin, 1990).

1.4.3 Uses of *AnogeissusLeiocarpus*

The wood is used mainly for transmission and buildingpoles, fence posts, and forked poles and as a beam of localbuilding construction. It is also used for firewood andcharcoal. The leaves and barks contain tannin materials recognizedby Sudan tannin industry (Elamin, 1990). In Mali all partsof *AnogeissusLeiocarpus* (leaves, barks and roots) used as a medicine (antimicrobial anthelmintic activity) mixed withother plants in traditional system. Derivative of ellagic acids (anogelline) extracted from thebark have been shown to delay the degradation of collagenand the tree is grown commercially since 2000 for theproduction of cosmetics in the koro region of Burkina–Faso(Jansen, *et. al.*, 2005). Also *AnogeissusLeiocarpus*gum was used as food additives mixed with gum Arabic or assubstituted for it (Hans.1990).

1.5 Physiochemical properties and measurement
The physical appearance and properties of the natural gums are of utmost importance in determining their commercial value and their end use. These very considerably with gums of different botanical source, and there are even substantial difference in gums from the same species when collected from plant growing under different conditions or even collected from the same plant at different seasons of the year. The physical properties may also be affected by the age of the exudate, treatment of the gum after collection, such as washing, drying, sun bleaching, storage temperatures (Glicksman,M.,1969).

1.5.1 Solubility

Regarding to the solubility the true gums are divisible into three classes (Mantel.1947):

(i) Soluble gums that dissolve in water forming transparent solution, e.g. *Acacia Senegal* gum.

(ii) Insoluble gums which also absorb water but on addition of sufficient waterbreak down into very thick transparent solutions, e.g. *tragacanth* gum.

(iii) Half- soluble gums that partially dissolve but on addition of more water pass into solution; e.g. *ghhatti* gum.

1.5.2 Colour

The colour of the gums varies from almost colourless through various shades of Yellow, orange to dark brown, some of the best gum Arabic *“Acacia Senegal”* are almost colourless. On the other hand, dark or even black gums sometimes occur,
E.g. Mesquite gum (Howes. 1949). The colour of Anogeissus leiocarpus gum may be yellow or light brown (Smith and Montgomery. 1959).

1.5.3 Shape

Gums as collected in natural states are represented by a variety of shape and form. Usually the fragments are irregularly globular or tear shaped. The grading of gums is based on shape, size and color of gum nodules.

1.5.4 Moisture Content

It gives indication about the hardness of the gum and microbial counts and helps calculation of the dry gum samples (Malik, 2004).

1.5.5 Ash Content

Ash content is measure of inorganic residue remaining after organic matter has been burnt. The inorganic residue exist as compounds. The type of soil (clay or sand) affected the ash content significantly (Algaili, 2004).

1.5.5.1 Ashing and dissolution techniques for trace metal

Trace metals are likely to be in the mg K\(^{-1}\) concentration range. The concentration, however, will vary from species to species and throughout the growing season. In order to extract metals, the organic matter is decomposed by heat.

Dry ashing consist of heating the sample in muffle furnace, typically at 400–600°C for 12–15hrs. The resulting ash is then dissolved in dilute acid to give a solution of the metal ions. In accuracies can arise both from volatilization of metals in an insoluble form in crucible.
Wet ashing consists of heating the sample with oxidizing agent to break down the organic matter. A typical procedure would be heating first with concentrated nitric acid, followed by perchloric acid. Alternative combinations include sulfuric acid, hydrogen peroxide and nitric/sulfuric acids.

An advantage of wet digestion is lower losses from volatilization (due to lower temperature and liquid conditions) but it can give rise to higher metals blanks from impurities in the acids. (Reeve, R. N., 2002).

1.5.6 Cationic composition

Atomic spectroscopy is used for qualitative and quantitative determination of perhaps 70 elements. Sensitivities of atomic method lie typically in the part-per billion range. Additional virtues of these method are speed convenience, unusual high selectivity, and moderate instrument costs spectroscopic determination of atomic species can only be performed on a gaseous medium in which individual atoms (or sometimes, elementary ions, such as Fe$^{3+}$, Mg$^{2+}$, Al$^{3+}$) are well separated from one another. Consequently, the first step in all atomic spectroscopic procedure is atomization, a process in which the sample is volatilized and decomposed in such a way as to produce an atomic gas. The efficiency and reproducibility of the atomization step in large measure determinethe method’s sensitivity, precision, and accuracy, that is, atomization is by far the most critical step in atomic spectroscopy.

Several methods are available to atomize samples for atomic spectroscopic studies. The most widely used of these is flame atomization. The other atomization methods electro thermal, inductively coupled plasma, and direct current plasma. Three types of atomic spectroscopy are based upon flame atomization (1) atomic absorption
spectroscopy (AAS). (2) Atomic emission spectroscopy (AES) (3) atomic fluorescence (AFS).

In flame atomization a solution of the analyte (usually aqueous) is converted to mist, or nebulized, and carried into the flame by a flow of gaseous oxidant or fuel. Emission or absorption is generated in the resulting hot gaseous medium (Holler, S., 1995).

1.5.7 Acidity and PH measurement

The hydrogen ion concentration is very important in chemistry and industry of gums, therefore functional properties of gum may be affected by change in pH e.g. viscosity and emulsifying power. Arabic acid substance is the major content of commercial Gum Arabic and Arabic acid when decomposed gives arabinose, so that the Gum Arabic is called Arabic acid and hence the gum solution is moderately acidic (Algaili, 2004).

1.5.8 Viscosity

This is a property common to gases and liquids (i.e. fluids) is a measure of frictional resistance that a layer of a fluid in motion offers to another. Viscosity is produced by the shearing effect of one layer of the fluid moving past another. In case of liquid, there are strong attractive cohesive forces between the different molecules. Now when a layer is moving faster than the other, then due to strong attractive forces, there is showing down of the faster layer or viscous drag is there (Sharma, K. K., 1992).
Table (1.1) list of the different types of the solutions viscosities.

**Nomenclature of solution viscosity:**

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Recommended Name</th>
<th>Symbol and defining Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative viscosity</td>
<td>Viscosity ratio</td>
<td>$\eta_r = \eta/\eta_0 \approx \frac{t}{t_0}$</td>
</tr>
<tr>
<td>Specific viscosity</td>
<td>_</td>
<td>$\eta_{sp} = \eta - 1 = (\eta - \eta_0)/\eta_0$ $\approx (t-t_0)/t_0$</td>
</tr>
<tr>
<td>Reduced viscosity</td>
<td>Viscosity number</td>
<td>$\eta_{red} = \eta_{sp}/c$</td>
</tr>
<tr>
<td>Inherent viscosity</td>
<td>Logarithmic viscosity number</td>
<td>$\eta_{inh} = (\ln \eta_r)/c$</td>
</tr>
<tr>
<td>Intrinsic viscosity</td>
<td>Limiting viscosity number</td>
<td>$[\eta] = (\eta_{sp}/c)<em>{c \rightarrow 0} = \lim</em>{(\ln \eta_r)/c \rightarrow 0}$</td>
</tr>
</tbody>
</table>

The intrinsic viscosity $[\eta]$ is independent of concentration by virtue of extrapolation to $c=0$, but is a function of the solvent used.

**1.5.8.1 Solution viscosity and molecule size**

Solutions viscosity is basically a measure of the size or extension in space of polymer molecules. It is empirically related to molecular weight for linear polymers; the simplicity of the measurement and the usefulness of the viscosity–molecular weight correlation are so great that viscosity measurement constitutes an extremely valuable tool for the molecular characterization of polymer.

Measurements of solution viscosity are usually made by comparing efflux time required for a specified volume of polymer solution to flow through a capillary tube.
with the corresponding efflux time $t_i$ for the solvent. From $t$, $t_0$, and the solute concentration are derived several quantities whose defining equations and names are given in the table (1.1) above. Two sets of nomenclature are in use for these quantities; one has had long and widespread application, the other (International Union 1952) was proposed for greater clarity and precision. In this system, the concentration $C$ is expressed in grams per deciliter (g/dl, g/100ml).

Dilute solution viscosity is usually measured in capillary viscometer of the Ostwald–Fenske or Ubbelohde type. The latter has the advantage that the measurement is independent of the amount of solution in the viscometer; measurement at a series of concentrations can easily be made by successive dilution. (Billmeyer, F. W., 1962).

The intrinsic viscosity has great practical value in molecular weight determinations of high polymers. This concept is based on the Mark–Houwink relation suggesting that the intrinsic viscosity of a dilute polymer solution is proportional to the average molecular weight of the solute raised to a power in the range of 0.5 to 0.9. Values of the proportionality constant and the exponent are well known for many polymer–solvent combinations. Solutions viscosities are useful in understanding the behavior of some biopolymers. (Steffe, J. F., 1996).

1.5.9 Specific optical rotation

The phenomenon of rotation of the plane of polarized light as a matter is known as “optical rotation”. This property is associated with the structure of organic molecules, such as proteins and biopolymers. Optical rotation is produced by chiral molecules i.e. when a carbon is bonded to four different groups and produces
mirror images (isomer) of a particular molecule. In principle the rotation produced by a molecule is equal in magnitude but opposite sign to that produced by its mirror image.

Substances that have the power of rotating the plane of polarized light through a certain angle are termed optically active. If the rotating is in clockwise direction the substance is called dextrorotatory (positive rotation) and if it in anticlockwise the substance is called laevorotatory (negative rotation). The extent of rotation of the plane of the polarized light can be measure by a polarimeter. In its simplest form this instrument comprises a source of monochromatic light, a polarizing prism (a Nicol prism of an Iceland spar), a sample holder tube, an analyzer prism (a second Nicol prism) which can be rotated against a scale and an eye piece. If the sample tube contains an optically inactive substance, the emerging light at the eye piece has maximum intensity when the two prisms are aligned. With optically active molecules in the light path, the analyzer has to be rotated for the emergent light to have maximum intensity.

The angle of rotation depends on the concentration and nature of the active molecules, the length of the light path, and the wavelength of the light used and varies only slight with temperature and solvent.

The physical parameter used for characterization is specific optical rotation, given by:

\[
[a]_w^t = \frac{100\alpha}{l.c}
\]

Whereas:
α: is the measured rotation in degrees

C: is the concentration of the solution in g/100cm³

L: is the light path length in dm

W: is the wavelength of light (sodium line, 589 nm)

T: is the temperature

(Kamal Mohamed Saeed, 2011).

Objective

This study aims to explain the information that may help for how to use this gum and its fractions in food, pharmaceuticals, cosmetics industry and industrial formula when mixed with other ingredients.
2.1 Materials

Sample of *anogeissus leiocarpus* was donated by NOPEC gum processing company (from south kordofan state).

2.2 Methods
Accurately weight 150g of gum were dissolved in 750 ml of distilled water (20% w/v).

2.2.1 Separation

The gum’s solution (20% w/v) is separated into two fractions (a) a hydrophobic (foam), and (b) a hydrophilic by air pressure apparatus. The last one was dried by freeze dryer apparatus.

2.2.2 Determination of Moisture content

Accurately weight 1.0096g of (a), 1.0091g of (b) and 1.0097 of sample in preheated and weighted dish. Then it was dried in an oven at 105°C for 12 hours to a constant weight. Moisture content was then calculated as a percentage of the initial weight from the following relation:

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).

2.2.3 Determination of Ash

Accurately weighted 3.3813g of (a), 3.2861g of (b) of dried samples were ignited in a muffle furnace at 550°C for 12 hours and ash% was calculated from the following relation:
Ash (\%) = \frac{W_3 - W_1}{W_2 - W_1} \times 100

Where:

\(W_1\) = Weight of the empty crucible.

\(W_2\) = Weight of the crucible + the sample.

\(W_3\) = Weight of the crucible + ash.

2.2.4 PH measurement

PH meter was calibrated using two different buffers one adjusted at pH 4 and other at pH 11. Then after calibration it was used for determination of the pH of the gum fractions, of 1g/100 ml aqueous sodium hydroxide solution (w/v).

2.2.5 Determination of Specific Optical Rotation

1 g/100cm aqueous solution of the gum was prepared, the solution was filtered. Optical rotation was measured using a 2 dm tube filled with the test solution, at room temperature. The specific rotation was calculated according to the relationship:

\[\alpha = \frac{\alpha \times 100}{L \times C}\]

Where:

\(\alpha\) = Observed angle of rotation.

\(L\) = the length of sample holder in dm.

\(C\) = the concentration of sample.
2.2.6 Total glucouronic acid

A glass column was packed with an Amberlite Resin IR (12H⁺). H₂SO₄ (2M) was passed through the column until the resin was thoroughly washed with the acid. Then this was followed by distilled water until the column was sulfate free. 50 ml of 20% gum solution was passed through the column, followed by the distilled water until the volume of 250 ml of the eluent and washing were collected. This was titrated against 0.1M NaOH. The apparent equivalent weight of the acid was calculated by:

\[
A.E.W. = \frac{\text{Weight of the sample}}{\text{Volume of titrate} \times \text{molarities of alkali}} \times 100
\]

% of uronic acid anhydride is calculated by:

\[
\text{U. A. A} = \frac{194 \times 100}{A.E.W}
\]

Where:

A. E. W = is the apparent equivalent weight.

194 = Molecular weight of uronic acid.

2.2.7 Intrinsic Viscosity

Measurement of dilute solution viscosity (resistance to flow) provide the simplest and most widely used technique for routinely determining molecular weight, it is not an absolute method, and each polymer system must be first
calibrated with absolute molecular weight determination (usually light scattering) run on fractionated polymer samples. Viscosities (on successive dilutions) are measured by determining the flow time of certain volume of solution through a capillary viscometer at constant temperature. The viscosity of solution may have a complicate variation with composition due to possibility of hydrogen bonding among the solute and solvent molecules hydroxyl groups make high viscosities because of hydrogen bonding to these O–H groups. Viscosity can be expressed in several terms.

\[ \eta_{rel} = \frac{\eta}{\eta_0} = \frac{t}{t^0} \] ..........................(1)

Where \( \eta \) and \( \eta^0 \) refer to solution and solvent viscosity respectively, in poise units (dynes cm\(^{-2}\) sec\(^{-1}\)) or Pascal seconds, \( t \) and \( t^0 \) the flow time of the solution and pure solvent through a capillary viscometer respectively.

The specific viscosity \( \eta_{sp} \) is the relative viscosity minus one:

\[ \eta_{sp} = \eta_{rel} - 1 \] ..........................(2)

The division of \( \eta_{sp} \) by concentration of the solution (C) gives reduced viscosity (viscosity number) \( \eta_{red} \).

\[ \eta_{red} = \frac{\eta_{sp}}{C} \] ..........................(3)

The inherent viscosity (logarithmic viscosity number) can be expressed in the equation.

\[ \eta_{inh} = \ln \eta_{rel}/C \] ..........................(4)
Intrinsic viscosity (limiting viscosity number) $[\eta]$ is determined by extrapolating a plot of either reduced or inherent viscosity versus concentration to zero.

$$[\eta] = \lim (\eta_{sp}/C)_c = 0 = (\eta_{inh})_c = 0 \hspace{1cm} (5)$$

Mark and Houwink arrived at an empirical relationship between molecular weight and the intrinsic viscosity.

$$[\eta] = KM^a \hspace{1cm} (6)$$

Where $K$ and $a$ are constants which depend on the nature of the polymer and solvent. The terms $K$ and $a$ represent the slope and intercept, respectively, of a plot of log $[\eta]$ versus log molecular weight of a series of fractionated polymer samples whose molecular weight have been determined by absolute methods (i.e. light scattering).

### 2.2.8 Nitrogen content

Nitrogen content was determined using a semi – micro Kjeldahl method according to (AOAC, 1990).

Kjeldahl nitrogen determinations are performed on a variety of substances such as meat, feed, grain, waste water, soil gum and many othersamples. Various scientific associations approve and have refined the Kjeldah method, including the AOAC international by which the nitrogen is determined (micro – kjeldahl) according to (AOAC, 1990).

The Kjeldahl method is performed under three main steps: digestion, distillation and titration.
**Digestion**

Digestion is accomplished by boiling a homogeneous sample in concentrated sulfuric acid. The end result is an ammonium sulphate solution. The general equation for the digestion of sample is shown below:

Sample (N) + H₂SO₄ → (NH₄)₂SO₄ + H₂O + CO₂ + Other products

**Distillation**

Excess base is added to the digestion product to convert NH₄ to NH₃ as indicated in the following equation. The NH₃ is recovered by distillation the reaction product.

(NH₄)₂SO₄ + 2 NaOH → 2 NH₃ + Na₂SO₄ + 2 H₂O

**Titration**

Titration quantifies the amount of ammonia in the receiving solution. The amount of nitrogen in a sample can be calculated from the quantified amount of ammonia ion in the receiving solution.

There are two types of titration—back titration and direct titration. Both methods indicate the ammonia present in the distillate with a color change. Indirect titration, if boric acid is used as the receiving solution instead of standardized mineral acid, the chemical reaction is

\[
\text{NH}_3 + \text{H}_3\text{PO}_4 \rightarrow \text{NH}_4^+ \cdot \text{H}_2\text{BO}_3^- + \text{H}_5\text{PO}_3
\]

(Color change occurs)

The boric acid capture the ammonia gas, forming an ammonium–borate complex. As the ammonia collects, the color of the receiving solutions changes.

\[
2\text{NH}_4^+ \cdot \text{H}_2\text{BO}_3^- + 2 \text{HCl} \rightarrow 2 \text{NH}_4\text{Cl} + 2 \text{H}_3\text{BO}_3
\]
(Color change occurs in reverse)

2.2.8.1 Method

0.2 g dry weight was digested in small digestion flask using 1 tap of catalyst (Cu) and 2 ml of concentrated sulfuric acid were added. The digested was diluted and transferred to the steam apparatus using minimum volume of water, made alkaline with concentrated alkali (at least 15 ml NaOH 40%) and the ammonia was distilled into 10 ml boric acid solution (2%) plus indicator (methyl red 1% W/V) for 5–10 min. After lowering the receiving flask clear of condenser the apparatus was heated for a further 5 min, the distillate was then titrated with 0.01N of hydrochloric acid.

\[
\text{Nitrogen}\% = \frac{14.01 \times N \times (V_{\text{titrate}} - V_{\text{blank}})}{\text{weight of sample (g)}}
\]

N: is the normality of HCl.

2.2.9 Protein content

Protein was determined by multiplying nitrogen content by the 6.25 as factor.

\[
\text{Protein}\% = N\% \times F
\]

Where: F is the nitrogen conversion factor.

2.2.10 Determination of cationic composition

Elements were determined using AAS which used dry ashing method in sample preparation, 3.2870g of (a) and 3.3807g of (b) were placed in porcelain dish. Start in a cold furnace, and then heated to 550°C, maintain temperature for 4 hours. The samples were cooled and 10 ml of 10% HCl were added then were
covered with watch glass and filtered into a 100 cm³ volumetric flask, and diluted to volume with deionized water.

2.2.11 Determination of sugar composition

Sugar composition was determined using hydrolysis method of polysaccharides with mineral acids (generally 72% of sulfuric acid is used) using HPLC technique (column: inert sustain NH₂ 5μm 250×4.6 id mm, mobile phase: Acetonitrile: Distilled water(75:25), detector: RI detector(temp:27.1°–27.8°C), flow rate: 1.0 ml/min, inject volume: 20μl, column temp: ambient).

Results and Discussions

3.1– Physiochemical properties of *Anogeissus leiocarpus*

Table (3.1) shows physicochemical properties of *Anogeissus leiocarpus* gum which reported by this study and
(Samia El Tayeb, 1995). The results expressed as the mean value of these properties: moisture content, Ash content, protein percentage, nitrogen percentage, pH value, optical rotation, intrinsic viscosity and uronic acid.

Percentage of fraction A and fraction B were: 16.902% and 68.487%. Moisture content determines the hardness of the gum.

The moisture content of these samples is the highest value, but it is nearly to the moisture content for sample collected from Abojebiha then El-Rosa and fraction A (hydrophobic) it’s closed to El-Fula sample (Samia El Tayeb, 1995).

The ash content indicates the presence of inorganic elements existing in salt form (Anderson and Dea, 1986). Ash content of this sample is nearly from the value of sample collected from Elfula and insignificantly lower than the mean values reported from that two locations (Samia El Tayeb, 1995).

The nitrogen and protein percentage of fractions of sample is so close to the value of Elrosaressample (Samia El Tayeb, 1995).

The pH of aqueous solution of samples indicated the acidity of Anogeissus Leiocarpus Gum which might be due to the presence of acidic sugars (glucouronic acid). Table (3.1) showed the pH value of these sample, fraction A is insignificantly higher than the mean value reported for three locations.
The presence of uronic acid in samples of *Anogeissus Leiodcarpus* and its fractions indicate that the samples have acidic sugar (glucouronic acid) and this confirmed qualitatively and quantitatively by using chromatographic method, table (3.1) showed the percentage of uronic acid of samples, fraction(B) is significantly higher than fraction(A) and near to crude gum value.

*Anogeissus leiodcarpus* gum is optically active, it has a negative value (laevorotatory). This result is so close to Elfula and Abojebihasamples (Samia Eltayeb, 1995).

Figure (3.1), (3.2) and (3.3) illustrated intrinsic viscosity which obtained from intercept of the plotting reduced viscosity with concentration it were found to be 0.65, 0.43 and 0.24 respectively.

<table>
<thead>
<tr>
<th>Property</th>
<th>EL–Rosales</th>
<th>EL–Fula</th>
<th>Abojebiha</th>
<th>Present study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture %</td>
<td>9.6</td>
<td>8.7</td>
<td>9.2</td>
<td>7.8934</td>
</tr>
</tbody>
</table>

Table (3.1) show the physicochemical properties of *Anogeissus leiodcarpus*:–
<table>
<thead>
<tr>
<th>Ash %</th>
<th>3.4</th>
<th>2.1</th>
<th>4.8</th>
<th>2.53</th>
<th>2.51</th>
<th>2.54</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen %</td>
<td>0.61</td>
<td>0.83</td>
<td>0.72</td>
<td>0.392</td>
<td>0.686</td>
<td>0.630</td>
</tr>
<tr>
<td>Protein %</td>
<td>4.02</td>
<td>5.47</td>
<td>4.75</td>
<td>2.450</td>
<td>4.288</td>
<td>3.938</td>
</tr>
<tr>
<td>PH value</td>
<td>4.4</td>
<td>4.1</td>
<td>4.1</td>
<td>4.1</td>
<td>5.17</td>
<td>4.8</td>
</tr>
<tr>
<td>Specific Rotation</td>
<td>-40.6</td>
<td>-37.4</td>
<td>-38.3</td>
<td>-37.4</td>
<td>-38.7</td>
<td>-35.2</td>
</tr>
<tr>
<td>Uronic acid %</td>
<td></td>
<td></td>
<td></td>
<td>14.7</td>
<td>3.23</td>
<td>12.1</td>
</tr>
</tbody>
</table>

Cationic composition of the sample was determined (using atomic absorption spectrophotometer in Ministry of Minerals) and the average values were shown in table (3.2).

Table (3.2): shows average values of element composition of the sample

<table>
<thead>
<tr>
<th>sample</th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>Na</th>
<th>Zn</th>
<th>Cr</th>
<th>Pb</th>
</tr>
</thead>
</table>

26
\[ y = 0.98x + 0.6533 \]
\[ R^2 = 0.9733 \]

- Figure (3.1) shows intrinsic viscosity of hydrophilic fraction of *AnogeissusLeiocarpusGum*. 
Figure (3.2) shows the reduced viscosity of crude gum of *Anogeissus Leiacarpus*.

Figure (3.3) shows the intrinsic viscosity of hydrophobic fraction of *Anogeissus Leiacarpus* Gum.
Fig(3.4) shows HPLC analysis of sugar in crude gum
Fig(3.5) shows HPLC analysis of sugar in hydrophilic fraction
Fig(3.6) shows HPLC analysis of STD mixture
National Center for Research
Environment & Natural Resources Research Institute
Central Lab
HPLC Analysis Report

Chromatogram Info:
File Name: C:\Clarity Life\sugar\Data\Sample 2_Hydrophic.prm
Origin: Acquired, Acquisition started 1/1/2007 5:20:20 AM
Project: C:\Clarity Life\Projects\sugar.pro
File Created: 1/1/2007 5:22:33 AM
Acquired Date: 1/1/2007 5:21:26 AM
By:

Sample 2 Hydrophic - Detector 1

Voltage
[mV]

Time
[min]

Result Table (Uncal - Sample 2_Hydrophic - Detector 1)

<table>
<thead>
<tr>
<th>Reten. Time</th>
<th>Area [mV.s]</th>
<th>Height [mV]</th>
<th>Area [%]</th>
<th>Height [%]</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3670.664</td>
<td>246.701</td>
<td>79.9</td>
<td>91.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>314.416</td>
<td>2.977</td>
<td>2.3</td>
<td>1.1</td>
<td>1.930</td>
</tr>
<tr>
<td>3</td>
<td>96.983</td>
<td>3.436</td>
<td>2.0</td>
<td>1.3</td>
<td>0.378</td>
</tr>
<tr>
<td>4</td>
<td>74.574</td>
<td>4.969</td>
<td>1.5</td>
<td>1.8</td>
<td>0.619</td>
</tr>
<tr>
<td>5</td>
<td>287.250</td>
<td>8.357</td>
<td>5.8</td>
<td>5.4</td>
<td>0.691</td>
</tr>
<tr>
<td>6</td>
<td>472.952</td>
<td>4.546</td>
<td>8.5</td>
<td>5.7</td>
<td>0.769</td>
</tr>
<tr>
<td>Total</td>
<td>4966.837</td>
<td>270.108</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

1. This study involves fractionation of gum into two fractions; A and B and it were found 14.6% and 85.1% respectively and it showed four type of sugars: Rhaminose, Xylose, Arabinose and Galactose.

2. The results of physicochemical properties show that some properties near to each other (in crude, A, B) such as ash content (2.5%), pH (5.2, 4.8) in A & B also moisture between crude and A is approximating (7.9%, 8.7%), but it increase in B (11.3%) due to the efficiency of the freeze drying process which depend upon the surface area and the thickness of the sample, also fraction B is more viscous (0.65) than crude gum (0.43) and fraction A (0.24) that showed as intrinsic viscosity (g/cm³).

3. The cationic composition study show that: Ca is the highest followed by K, Mg, Na, Zn and Pb.
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• **Pakistan Journal of nutrition 8(6):782–786, 2009 ISSN 1680–5199/**

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  Freeman press. USA.
