جامعة السودان للعلوم والتكنولوجيا

Sudan University of Science and Technology

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Characterization of *Acacia gerrardii var. gerrardii* Gum

توصيــف صمــغ الســلجم صنف السلجم

A Thesis submitted in partial fulfillment of the requirements for a master degree in chemistry

By

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بسم الله الرحمن الرحيم

استهلال

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صدق الله العظيـم

DEDICATION

To my parents

To my daughters

To my brothers and sisters

Acknowledgment

First and foremost I would like to thank god for all blessings he has bestowed upon me and for giving me knowledge and wisdom which helped me to accomplish this study.

I am deeply indebted to my supervisor Dr. Mohammed Elmubark who provided me with advice, support and suggestions which helped me to complete this study.

I would like to extend my special thanks for Dr. Amira Abd Alaziz for her guidance providing a lot of help and advice during this work.

Also I am grateful to the technical staff of the laboratory, Sudan University of Science and Technology, Environment & Natural Resources Institute, Faculty of Agriculture, Khartoum University for their help and support throughout the project.

Finally, I would like to thank all those who helped me in this work.

ABSTRACT

Sample of authentic *Acacia gerarrdii var. gerarrdii* were collected from south kordufan state of Sudan during the season of 2010. The physiochemical properties of *Acacia gerarrdii var. gerarrdii* gum were studied. Parameters such as solubility moisture content, ash content, pH value, specific optical rotation, intrinsic viscosity, nitrogen and protein content, acid equivalent weight and total uornic acid were determined. The results show that : solubility is 20% , moisture content 7.62 %, ash content 1.8%, pH value 5.32 , specific optical rotation +60 intrinsic viscosity 15.1 cm^3/ g , nitrogen content 1.88%, protein 13.16%, acid equivalent weight 2000 and total uronic acid 9.7%.

Acid hydrolysis of *Acacia gerarrdii var gerarrdii* followed by HPLC measurements revealed that sugar content was : Arabinose 39.3%, Galactose 5.5% and Rhmnose less than 1%.

Mineral composition of sample were studied using flame photometer and atomic absorption spectroscopy (AAS) techniques. The results showed that calcium has the highest value (70 mg/L), followed by potassium (5.3 mg/L), sodium (4.9 mg/L), magnesium (2.9 mg/L) and less than 1 mg/L for Zn, Cd, Pb and Cu.

The average number molecular weight of the sample was estimated using osmotic pressure measurements and found to be 1.1×10^5 Daltons.

المستخلص

تم الحصول علي عينة من صمغ السجلم (اكيشيا قرارداي صنف قرارداي) من ولاية جنوب كردفان بالسودان خلال الموسم 2010 تم توصيف للعينة باستخدام الطرق الفزيوكيميائية حيث تم تحديد نسبة الاذابة ومحتوي الرطوبة ومحتوي الرماد و قيمة الاس الهيدروجينى و الدوران الضوئى النوعى و اللزوجة الضمنية و محتوى النيتروجين والبروتين وحمض اليورنيك الكلى.

النتائج المتحصل عليها كالآتي : نسبة الذوبانية 20 % ، محتوى الرطوبة 7.69 % ،محتوى الرماد 1.8 % ،قيمة الاس الهيدروجينى 5.32 ، الدوران الضوئى النوعى 60.2+ واللزوجة الضمنية 15.1سم^3/ جم، محتوى النيتروجين 1.88 % ومحتوى البروتين 13.16% وزن الحمض المكافئ 2000 وحمض اليورنيك الكلى 9.7%.

التحلل الحمضى لعينة الصمغ متبوعا بقياسات كروماتوغرافيا السائل ذات الضغط العالى اظهرت ان محتوى السكريات الاحادية كالآتي : ارابينوز 39.3% ،جلاكتوز 5.5%، ورامنوز 0.33%.

تمت دراسة محتوى المعادن للعينة باستخدام جهاز مطياف اللهب الضوئى وجهاز مطياف الامتصاص الذرى.أوضحت النتائج أن الكالسيوم كانت له أعلي قيمة (70mg/L) يليه البوتاسيوم (5.3mg/L) ثم الصوديوم (4.9mg/L)، الماغنيسيوم (2.9mg/L) وأقل من (1mg/L) لكل من الخارصين، الرصاص، الكادميوم و النحاس.

تم حساب الوزن الجزيئى عن طريق قياسات الضغط الأزموزى ووجد أنه يساوى 1.1× 10^5 دالتون.

CONTENTS

Chapter three

List of Tables

List of Figure

Chapter one

Chapter One

1-Introduction

1.1Gums

1.1.1 Origin of *Acacia* gums:

Acacia gums are dry exudates obtained from the trees belonging to the various species of the genus *Acacia*. There are about more than 1000 species of *Acacia* species distributed over tropical and subtropical areas of Africa, India, Australia Central America and southwest North America (Martin, 1969).

Gum is produced in many countries but only a few of the known *Acacia* species are commercially important. The most important gum yielding area is the republic of the Sudan in Africa followed by French West Africa comprising Chad, Muretania and Frenchsudan. Nigeria, Tanganyika, Morocco, Abyssinia and Somaliland also produce sizable quantities of gum and lesser amount of acacia gums are collected in South Africa, India and Australia (Martin, 1969).

1.1.2 Gums Production in Sudan

More than two dozen distinct species of *Acacia* are found in the Sudan, these species are found throughout of the state of Kordofan in a large uninterrupted belt stretching westword through the province of Darfor and eastword to Nile River the best grade of gum comes from *Acacia senegal var senegal* and is commonly known as *Hashab* or Kordofan gum in the Sudan. About 90% of Sudan's production of gum arabic comes from these trees and less than 10% of that comes from *Acacia seyal var*. *seyal* which is prevalent in the south western part of the

country and in the Nile region. These trees are not tapped and only natural exudates are collected and sold as *Talh*a gum.

Several other species of *Acacia* occur in the Sudan and known to exude gums. Few of these are collected and some find their way to the market mixed with the other gums. These *Acacia*s are: *abyssinica, albida, Arabica, campylacantha, drepanolobium, farnesiana, flava, kirkii, gerrardii, laeta, mellifera, orfota, sieberiana, spirocarpa, stenocarpa* and *thunbergiana*.

1.1.3The aims of this project

- To characterize *Acacia gerrardii var gerrardii* gum by studying the physiochemical properties.
- To compare and constrast the result of physiochemical properties of *Acacia gerrardii var gerrardii* gum with some *Acacia gummiferae gums*.

1.2 Literature review

1.2.1Review of the gum:

Many reviews have been published in *Acacia gerrardii var gerrardii* gum with special emphasis on characterization, composition and chemotaxonomic aspects. Analytical data for gum exudates from *Acacia gerrardii* Benth. var. *gerrardii* concluded that species in sub-series *Gummifera* all have positive optical rotation, low viscosity, arabinose/galactose ratios greater than unity and low rhamnose content (Anderson, and Mc Dougall, 1987).

1.2.2 Derivation of specific name:

Gerraradii comes after Willian .T. Gerrard an English traveler and naturalist, who collected this gum in Kwazulu-Natal.

1.2.3 Botanical classification:

Name: *Acacia gerrardii* benth. *var gerrardii.*

Genus: *Acacia*.

Family: *Fabaceae* (alternative *Leguminosae*).

Subfamily: *Memosoideae.*

Tribe*: Acacieae* or *Memecaceae.*

Specific epithet: *Gerrardii.*

Synonymy*:*

1- Acacia subtementosa DE WILD.

2- *Acacia hebecladoides Harms salgam*(Arabic).

Common names

Gray hair *Acacia* [Zimbabwae] English source

Red Thorn (English source)

Rooidring (African source)

1.2.4 *Acacia gerraradii* (Grey Haired *Acacia*) Fig.(1.1)

Foliage : deciduous

Mature height : 30' -35'

Mature width : 20' -40'

Growth rate : moderate

Hardiness : 18 degrees F

Exposure : full sun

Leaf color : green

Shade : dense

Flower color : white

Flower shape : ball

Flower season : spring

Thorns : present

Propagation method : seed

Figure (1.1) *Acacia gerrardii* tree

1.2.5 Description

Tree, usually 3 to 5m high, with a flat, umbrella –shaped crown and rough grey fissured bark. Young branch is densely pubescent, the epidermis splitting to reveal a rusty red inner layer. Spines straight , short , about 1 cm long leaves rather large ; pinnae five to ten pairs , 3 to 7,5 mm long , 1 to 2 mm wide ; flowers white scented , occur ring in globose heads.Pods large and sickle shaped, dehiscent, 7 to 16 cm long, 0.6 to 1.1 cm wide. Seed, olive brown compressed, 9 to 12 mm long and 7mm wide (Brenan, 1959) .

1.2.6 Distribution

Acacia gerrardii is distributed through out the African gum belt Fig (1.2) from Benin eastwards to Ethiopia and from there southwards to south Africa, other subspecies are distributed in Israel, Iraq, Jordan and the Arabian Peninsula (Brenan 1959).

1.2.7 Production of gums

Gums are unique that they are produced by trees only when they are in an unhealthy condition. Healthy trees have never been observed to yield gum. Most authorities believe that the formation of the gum is a pathological condition resulting from a bacterial or fungoidal infection of the injured tree, natural factors that tend to lessen the vitality of the tree, such as poorly nutritioned soil, lack of moisture, and hot weather improve gum yields. However, some observers suggest that the production of gum is a normal metabolic process in the plant and that the quantity and quality produced is a function of environmental conditions (Martin, 1969).

Figure(1.2) African gum belt

1.2.8 Tapping operation:

Gum producing infections start in breaks or wounds in the trees which are sometimes caused by nature but commonly by the natives. For the tapping operation a small axe is used to break the outer bark horizontally by making a shallow cut about 1.5 in. wide.The bark is then peeled one strip up and one strip down to from 2.3 ft. wounds. The gum then exude from the wound area in 3-8 weeks depending on weather condition.

As it exudes slowly from the exposed area, it forms tears or droplets which gradually dry and harden on exposure to the atmosphere Figure 1.3.

The average yield from young trees is about 900g and from old trees about 2000g. Collections are made every 10 days during the season since gum is produced continuously in the wound.

The gum is gathered in the dry season which usually lasts from October to May or June. During the rainy reason the trees are in full bloom and no gum is formed. As soon as the dry season is under way as indicated by the withering and falling of the leaves.

Figure (1.3) *Acacia gerrardii* gums

1.2.9 Physical properties of gums:

. Gum Arabic is a natural product complex mixture of hydrophilic carbohydrate and hydrophobic protein components (FAO, 1990). The physical properties of gum Arabic, established as quality parameters include moisture, total ash, volatile matter and internal energy Table (1.1), (1.2).

Analytica data for gum exudates from acacia gerrardii benth. Var gerrardii concluded that speices in sub-series Gummifera all have positive optical rotation, low viscosity, arabinose/galactose ratios greater than unity and low rhmnose content (Anderson, 1987).

Hydrophobic protein component functions as an emulsifier which adsorbs onto surface of oil droplets while hydrophilic carbohydrate component inhibits flocculation and coalescence of molecules through electrostatic and stearic repulsions. (Anderson *et al.*, 1990).

1.2.9.1 Shapes:

Natural gums are exuded in a variety of shapes and forms the best known being the tear-drop or globular shape and other shapes are flakes. Still other resemble stalactites and after collection and fracturing yield irregular fragments.

The surface of most gums is perfectly smooth when fresh but may become rough or covered with small cracks (Martin, 1969)

1.2.9.2 Color:

The color of gums in their natural exudates shape varies from almost water white (colorless) through shades of yellow, amber and orange to dark brown. The best grade is colorless with slight trace of yellow. Some gums possess pink, red or

green line, and some black or brownish gums are also found and that color is due mainly to the presence of various types of impurities (Martin, 1969).

1.2.9.3 Solubility:

Cum Arabic has a high solubility in water and can yield a solution of up to 50% concentrations at these high levels, it can actually form a highly viscous, gel.

Gum Arabic is insoluble in oils and in most organic solvents it is soluble in aqueous ethanol up to a limit of about 60% ethanol. Limited solubility can also be obtained with glycerol and ethylene glycol.

1.2.10 Processing:

The crude exudates of gum arabic is processed differently according to the quality finally required for it to be marketed.

Air drying is the easiest method to be applied which, together with mechanical milling (kibbling), are used in order to produce a granular material that is much more soluble than raw product. Other processing methods are spray drying. These methods involve dissolving exudates in water under controlled heating conditions and constant stirring. Heating must be mild to avoid distortion of the gum which could have a detrimental effect on its functional properties. After removing the insoluble material by decantation or filtration, the solution is pasteurized and subjected to spay or roller drying which involves spraying the solution into a stream of hot air. The water completely evaporates and the dry powder is separated from air by a cyclone, resulting in 50 to 100 mμ particles. During the roller-drying, the solution is passed to the hot rollers and the water is evaporated by the air flow. The thickness of the resulting gum Arabic film is controlled by adjusting the

distance between the rollers. The film is separated from the roll by scraping blades giving way to particle scales of several hundred μm in size (Maraina *et al.,* 2012).

Table (1.1) international specification of quality parameter of *gum Arabic* quality (FAO, 1990)

Property	**Value**
Moisture (%)	13-15
Ash content(%)	2-4
Optical rotation (degrees)	(-26) - (-35)
Nitrogen content (%)	0.26-0.39

Table (1.2) Cationic composition of total ash at 550 ˚C

Copper (ppm)	52 – 66
Iron(ppm)	730 – 2490
Manganese (ppm)	69 – 117
Zink (ppm)	45 – 111

1.2.11 Structure of gum:

The gums from *Acacia* are a complex polysaccharide, neural or slightly acidic salt containing calcium, magnesium and potassium cations. The gum is composed of six carbohydrate moieties, galactose, arabinoyranose, arabinofuranose, rahmnose, gulcuronic acid and 4-O-methylglucuronic acid the main structural feature is a centrol chain of β -galactopyranose units with 1,3 bonds and side chains of 1,6-

linked galactopyranose units terminating the glucuronic acid (Anderson *et al*., 1966).

Street and Anderson, (1983) proposed a structure, which is branches composed of 116 galactose unite linked by β (1,3) with six branches attached to the chain by (1,6) links. The average molecular mass of the structure is about 19.000. Then eventually a model for the whole molecule is obtained.

Fincher *et.al* (1983) suggested that the gum had a 'wattle blossom' structure with a number of polysaccharide units linked to a common polypeptide chain, Figure (1.4).

Qi *et .al*. (1991) separated the gum in two fractions using gel permeation chromatography (GPC). One high molecular mass fraction (corresponding to the arabinoglactan protein (AGP) fraction), which represented ~10% of the total, was found to contain ~10%protein, consistent with the findings of Randall *et al*., (1988). On deglycosylation, it yielded a hydroxyproline-rich polypeptide chain consisting of ~400amino acid residues with a possible repeating sequence of(HYP4 ser2 Thr Pro Gly Len His) Table 1.3. The fraction interacted with Yariv reagent, but differed from typical AGPs by its much lower alanine and acidic amino acid content. The carbohydrate component was subjected to alkaline hydrolysis and following subsequent analysis, they concluded that the carbohydrate was attached to the polypeptide chain in small units of ~30 sugar residues through galactosehydroxyproline linkages. Figure1.5.

This was supported by electron microscopy studies which revealed rod-like molecules ~150nm long. They suggested that the structure resembled a 'twisted hairy rope' in contradiction to the 'wattle blossom' model.

Mahendran *et al*., (2008) investigated the structural characteristics of the gum exudates from *Acacia senegal*. They found that the arabinogalactan protein complex has a molecular mass of 1-2×10^6 Da and consists of a polypeptide chain possibly containing ~250 amino acid with short arabinose side chains and much larger blocks of carbohydrate of molecular mass ~4.0×10^4 Da attached. The carbohydrate was highly branched. They suggested that these carbohydrate blocks may have a thin oblate ellipsoid carbohydrate structure, which has been reported for the arabinogalactan (AG) component Sanchez *et al*., (2008). They found that the molecule adopted a very compact conformation with Rg of 36nm , the structure is illustrated in Figure (1.6).

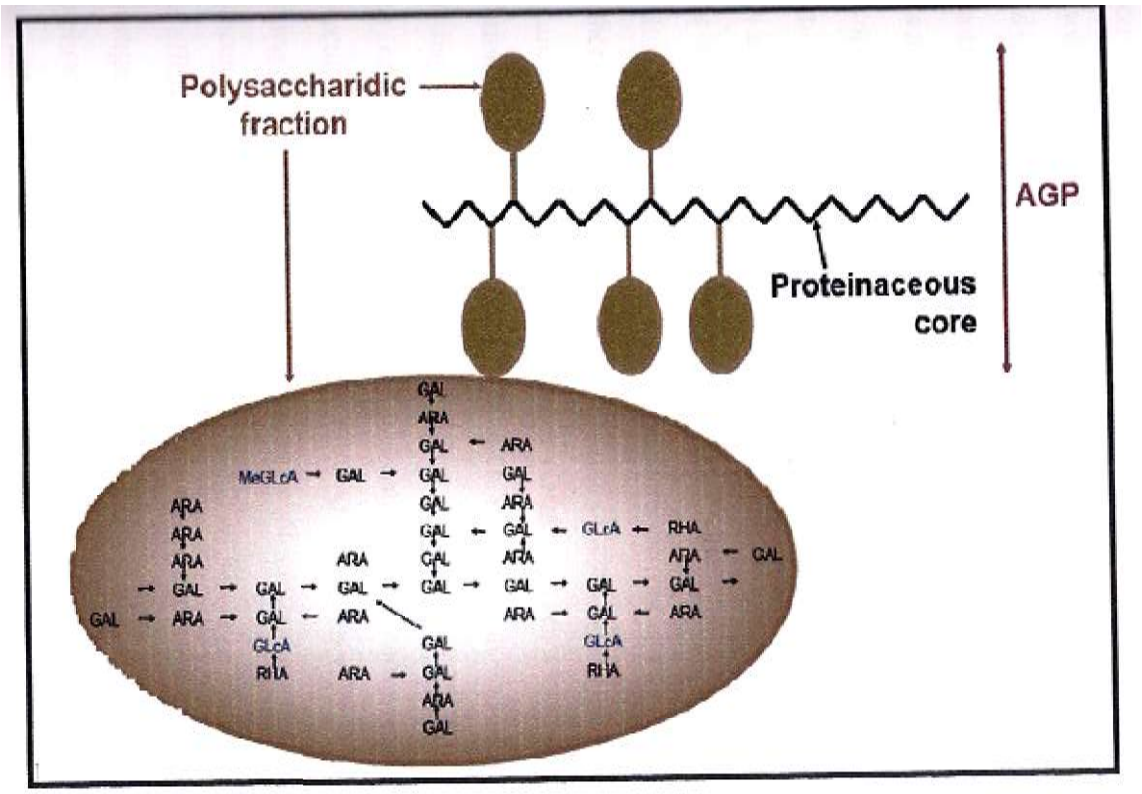


Figure1.4: Wattle blossom model of arapinogalactan-protein (Fincher *et.al*, 1983)

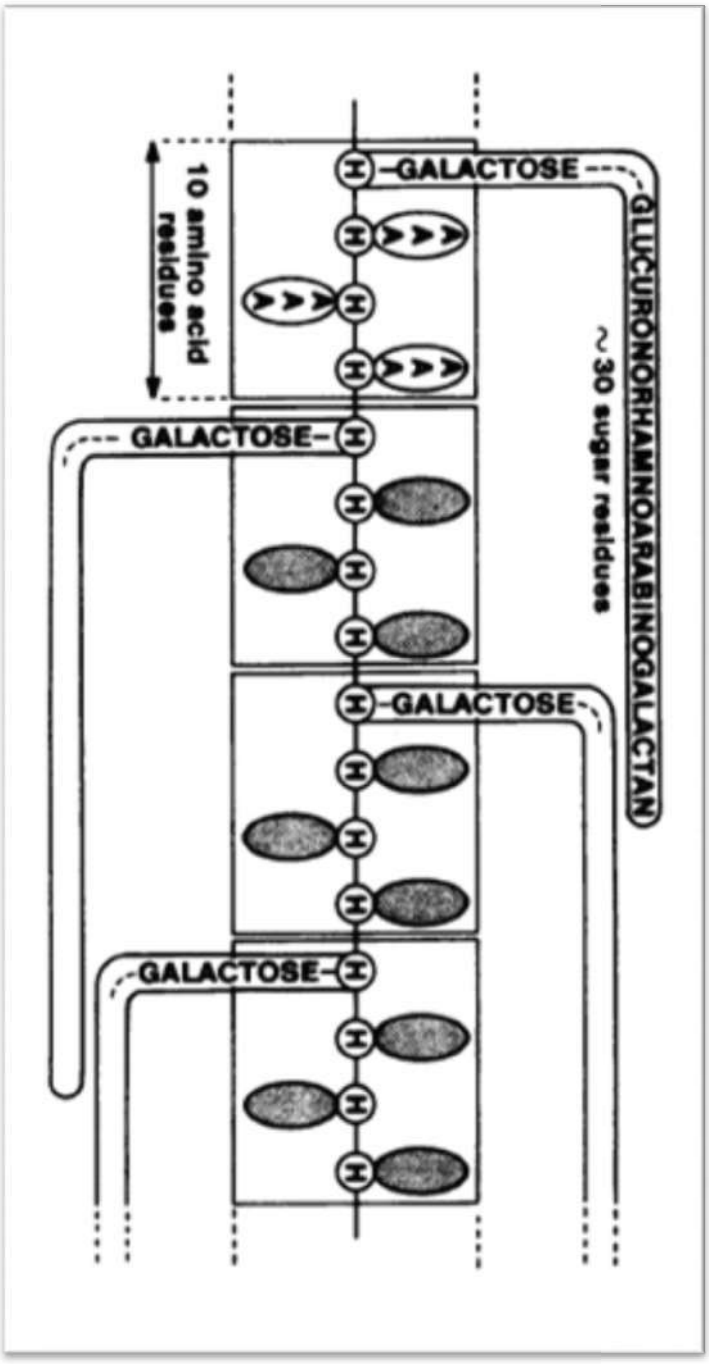

10 amino acid residues
GALACTOSE
GALACTOSE
GALACTOSE
GALACTOSE
~30 sugar residues
GLUCURONORHAMNOARABINOGALACTAN

Figure 1.5 The extesin-like model for gum arabic glycoprotein as proposed by Qi *et al.* (1991)

Table 1.3 Amino acid content in gum arabic taken from *Acacia senegal var senegal*

Amino acid	(n mol/mg) GA	% Amino acid
Hydroxyproline	54.200	0.711
Serine	28.700	0.302
Threonine	15.900	0.208
Proline	15.600	0.180
Leucine	15.100	0.198
Histidine	10.700	0.166
Aspartic acid	10.600	0.141
Gultamic acid	8.290	0.122
Valine	7.290	0.085
Phenylalanine	6.330	0.105
Lysine	5.130	0.075
Alanine	5.070	0.045
Isoleucine	2.380	0.031
Tyrosine	2.300	0.042
Arginine	2.120	0.037
Methionine	0.110	0.002
Cysteine	0.000	0.000
Tryptophan	0.000	0.000

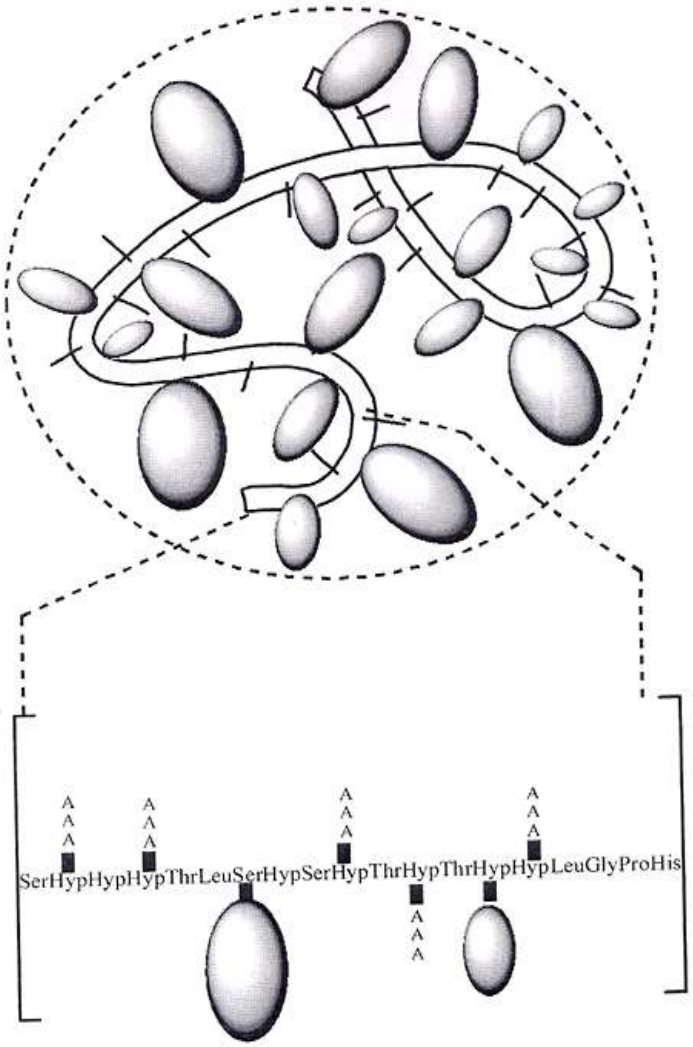


Figure (1.6) Schematic structure of the gum arabic arabinogalactan protein complex (Mahendran *et al*., 2008).

1.2.12. Applications of gums

1.2.12.1 Food applications:

Gum arabic is mainly used in the confectionery industry, where it is incorporated in a wide range of products. It has a long tradition of use in wine gums, where it produces a clarity that is higher than can be obtained with other hydrocolloids. Furthermore, it prevents sucrose crystallization, provides a controlled flavor release and slows down melting in the mouth, making the wine gum long lasting. It also provides the appropriate texture to these candies, which are easily deformed in the mouth but don't adhere to the teeth (Arja *et al.,* 2011).

In lower calorie candy, gum arabic is used to compensate for the loss of texture, mouthfeel and body, resulting from the replacement of sugars by artificial sweeteners. It is also used in chewing gum as a coating agent and as a pigment stabilizer. In aerated confectionery products, such as marshmallows, nougats and meringues, gum arabic acts as whipping and stabilizing agent. It is also used in toffees and caramels as an emulsifier, to maintain a uniform distribution of the fat across the product (Tadesse *et al.,* 2007).

Gum arabic is widely used as an emulsifier in the manufacture of soft drinks. Due to its stability in acid conditions and its high solubility, gum arabic is well suited for use in citrus and cola flavor oil emulsions. High levels of gum are used to ensure a complete coverage of the interface and to prevent flocculation and coalescence of oil droplets. Normally, a weighting agent is added to increase the oil-phase density, inhibiting destabilization due to creaming (Wyasu and Okereke, 2012).

1.2.12.2 Non food applications:

Gum arabic was once extensively used in the pharmaceutical industry, but is now replaced by celluloses and modified starches in many applications. It is still

used as a suspending agent, emulsifier, adhesive and binder in tabletting and in demulcent syrups. In cosmetics, gum arabic functions as a stabilizer in lotions and protective creams, where it increases viscosity, imparts spreading properties and provides a protective coating and smooth feel. It is used as an a adhesive agent in blusher and as a foam stabilizer in liquid soaps (Arja *et al*., 2011).

Gum Arabic is also used in the preparation of etching and plating solutions in the lithography industry. It is used as a dispersant in paints and insecticidal/acaricdal emulsions, respectively keeping the pigments and active components uniformly distributed throughout the product. In the textile industry, it is used as a thickening agent in printing pastes for the coloration of knitted cellulose fabrics. Other applications are ink and pigment manufacture, ceramics and polishes (Verbeken *et al*., 2003).

1.2.12.3 Emulsifying properties:

Gum is well recognized as an emulsifier used in essential oil and flavor industries. Randall *et al*., 1998, reported that the Arabinoglactan-Protein (AGP) complex is the main component responsible for gum arabic ability to stabilize emulsions by the association of the (AGP) amphiphilic protein component with the surface of oil droplets, while the hydrophilic carbohydrate fraction is oriented toward the aqueous phase, preventing aggregation of the droplets by electrostatic repulsion. However, only 1-2% of the gum is absorbed into the oil-water interface and participates in the emulsification; thus, over 12% of gum arabic content is required to stabilize emulsions with 20% orange oil If there is not enough gum arabic amount to cover all gum drops, unstable emulsion will be formed and flocculation and coalescence occurs (Williams *et al*., 1990).

Chapter two

Chapter two

2. Materials and methods

2.1 Materials and instrument

2.1.1 Materials

Sample of *gerrardii var gerrardii* gum were collected from Sudan in South Kordofan State during April, 2010.

Sulphuric acid, Hydrochloric acid, Sodium hydroxide, Amberlite resin reagent and some other chemicals for analysis.

2.1.2 Description of gum sample

Acacia *gerrardii* gum collected from the tree is irregular-shaped nodules, unlike other species of gum which exudates on the trunk of the tree with uniform spherical nodules. The majority is found in aform of spiral or tears firmly attached to the bark and branches.

Nodules are brown to reddish in colour where as spirals and strips are yellowish white to colourless.

2.1.3 Instruments

- Pestle
- Mortar
- Oven
- pH meter
- Furnace

- Atomic Absorption Spectrometer
- Flame Photometer
- HPLC system

2.1.4 Purification of gum sample

A dried sample of *Acacia gerrardii* var *gerrardii* gum is cleaned by hand to remove foreign particles and adhering bark. The sample was ground using a mortar and pestle and kept in a glass container for analysis

2.2 Analytical Methods

2.2.1 Solubility

The solubility test was done by adding gradually a known weight of sample to 20ml of water and dissolved by the continuous stirring until it become saturated solution (gel) at room temperature and determined the dissolved weight of gum.

$$\text{Solubility \%} = \frac{\text{w.t of gum /g} \times 100}{\text{Volume of water/ ml}}$$

2.2.2 Determination of moisture content

Moisture content of the gum samples was determined using to pre-weighted shallow weighing dish. The weighted dish and its contents were dried in an oven (Heraeus. Functionline T6-kendro) at 105˚C for five hours, then cooled in a desiccator and reweighted. The loss on drying was calculated as follows:

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

W_1: original weight of sample (g).

W_2: weight of sample after drying (g).

2.2.3 Determination of ash content:

Accurately three grams of the dried sample were weighted on dry porcelain crucible and ignited at 550˚C in a amuffle furnace (Herauns. Function line T6-kendro) until free from carbon, cooled in a desiccator and weighted. Then the total ash % was calculated as follows: (FAO, 1991)

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

Where:

W_1: weight of the empty crucible (g)

W_2: weight of the crucible +sample (g)

W_3: weight of the crucible + ash (g)

2.2.4 pH measurement

Corning –Pinnale -555 pH meter was calibrated by using three of different buffer solutions one adjusted at pH4,7 and other at pH11. Then after calibration it was used to determine the pH of crude gum sample of 1g/100ml aqueous solution(w/v) calculated on dry weight basis.

2.2.5 Determination of specific optical rotation $[\alpha]^{T}_{D}$

Specific optical rotation was determined for 1.0 % solution (on dry weight basis). The sample was dissolved in distilled water mixing on a roller mixer until the sample fully dissolved (approximately 5 hours), after filtration of the gum solution through whatman cellulose nitrate membrane filter paper (0.8µm), optical

rotation was measured at 25˚C using a polarimeter (AA -5 optical Activity Ltd)with a D –line of Na (589.3nm) fitted with a cell of path length of 20.0cm. The specific optical rotation was calculated according to the relationship:

$$\text{Specific optical rotation } [\alpha]^{T}_{D} = \frac{\alpha \times 100}{L \times C}$$

Where:

α: observed angle of rotation.

L: the length of sample holder in decimeters (dm).

C: concentration in g/100mL.

T: temperature.

D: wavelength of the light (Sodium line 589nm).

2.2.6 Determination of the number average molecular weight (osmometric)

Original gum solution (12%w/v) was prepared by dissolving the gum in sufficient amount of distilled water and leaving the solution overnight before filling with distilled water up to the mark .Each gum solution was filtered through whatman 41 filter paper. Different concentrations were made from the original solution and their corresponding osmomat^{R} 050 (Colloid – osmometer). The temperature of measurement was recorded. The plot of $(\pi/C)^{0.5}$ versus (C) gave straight line for each solution of gum sample and was extrapolated to zero concentration, from which the molecular weight of the gum sample was calculated according to Van Hoff's equation:

$$\pi/C = RT/M_n\ (1+0.5\ A_2M_nC)^2$$

Or,

$$(\pi/C)^{0.5} = (RT/M_n)^{0.5} (1+0.5A_2M_nC)$$

The intercept = $(RT/M_n)^{0.5}$

The slope = $(RT/M_n)^{0.5}A_2Mn/2$

Where:

π : the osmotic pressure.

T : the absolute temperature.

R : gas constant.

C : the concentration of the solution in g/ml.

A_2 : the virial coefficient, which was calculated from the slope of the straight line and its value depends on the gum-solvent interaction; its value equal zero for an ideal solvent, greater than zero for a good solvent and less than zero for a poor solvent.

2.2.7 Determination of cationic composition

The direct dry ashing method was used in sample preparation (one g) of gum sample was accurately weighted and put in glazed porcelain dish.

The sample was placed in a furnace and heated gradually to 550° C. the temperature was maintained for 6 hours. then the sample were cooled and 30ml of 3N HCl were added to the sample and then covered with watch glass and boiled gently for 10 minutes .

The content of sample was then cooled, filtered into a $100cm^3$ volumetric flask, and diluted to volume with distilled water.

Na, K and Ca were determined using model 410 classic flame photometer Cu, Zn, Mg, Pb and Cd were determined using Atomic Absorption Spectrophotometer (AAS). Standards of specific elements were used for the

preparation of the calibration curves. They were prepared by suitable dilution of stock standard solution for each element.

2.2.8 Determination of sugar content:

Method:

According to FAO specification (FAO, 1982) by boiling solution containing 0.5g of gum in 12.5 ml of 4% w/w H_2SO_4 for a period of 7hrs .The hydrolyzed gum solutions were neutralized by the addition of 2grams of $BaCO_3$ to each sample. The neutralized hydrolyzates were filtered to remove insoluble barium salts and the clear supernatant analyzed for neutral sugar residues using HPLC.

2.2.9 Determination of equivalent weight and total uronic acid:

Method:

2.2.9.1 Equivalent weight:

The equivalent weight was determined according to the method described in the Encyclopedia of Chemical Technology (1966) with some modification. Ten mls of 3% aqueous gum solution were treated with 2 grams of Amberlite Resin IR-120(H) for an hour, filtered and were then washed with distilled water.

The filtrate was titrated against 0.02 N NaOH solution, using phenolphthalein as an indicator and the equivalent weight (E.W) was calculated according to the following equation

$$\text{Acid Equivalent weight} = \frac{\text{weight of sample} \times 1000}{\text{volume of titer} \times \text{normality of NaOH}}$$

2.2.9.2 Total uronic acid content:

The uronic acid percentage (UA) was calculated for each sample by multiplying the molecular weight of uronic acid (194) by 100 and dividing by the acid equivalent weight as in the following equation:

UA% = 194 × 100/E.W

Where

UA%: uronic acid percentage.

E.W: acid equivalent weight.

2.2.10 Determination of nitrogen and protein content

Nitrogen was determined using a semimicro kejeldahl method 0.2g of gum sample were accurately weighted in triplcate and placed in kejeldahl digestion flask and one tablet of mercury-copper sulphate –potassium sulphate catalyst was added to each flask. Then 5mls of concentrated free nitrogen sulphuric acid were added to the flask. The contents were heated over an electric heater until the solution attained a clear blue color and the walls of the flasks were free from carbonized materials. The contents of the flasks were then transferred to a steam distillation assembly. The distillate was collected in 10ml of boric acid distillation was continued after neutralization of the contents with 20ml of 40% NaOH followed by 25mls distilled water. Three drops of methyl red were added and titrated against 0.01M HCl. The same procedure was carried out for a blank (distilled water).

Nitrogen calculated as follows:

$$N\% = \frac{14.04 \times N \times (V_1 - V_2) \times 100}{W}$$

V_1: volume of the titrant.

V_2: volume of the blank.

N : normality of HCl.

W: weight of sample in grams

Protein content was determined by multiplying N% by nitrogen conversion factor (NCF). Equals 7.0 for *Acacia gerrardii* (Osman, 1993)

Protein % = N%× NCF

2.2.11 Viscosity measurement:

The viscosity (flow time) was determined on a 1% gum solution using Ubbehode viscometer (B2 USA)immersed in a constant temperature water bath set at 25˚C. Gum solution was prepared by dissolving one gram of the gum in 100ml of 1M NaCl, filtered and transferred into the viscometer. The initial relative viscosity was determined. Further dilution of sample were made by adding 2ml of the solvent and the flow time for each concentration was measured. Finally the flow time for the pure solvent was determined.

The intrinsinc viscosity was calculated as follows:

Relative viscosity $[\eta_{rel}] = \eta/\eta^{\circ} = t/t_{\circ}$

Specific viscosity $[\eta_{sp}] = \eta_{rel} - 1$

Reduced viscosity $[\eta_{red}] = \eta_{sp}/c$

Intrinsic viscosity $[\eta] = lim_{c\to 0} [\eta_{sp}/c]$

The intrinsic viscosity $[\eta]$ is determined from the intercept in the plot of $[\eta_{red}]$ as a function of sample concentration at zero concentration (infinite dilution).

The inherent viscosity is determined from the intercept in the plot of η_{red}/c as a function of sample concentration at zero concentration (infinite dilution).

Chapter three

Chapter three

3.1 Results and discussion

Table 3-1 shows some physiochemical properties for some variety of *acacia gummiferae* gums: *nilotica, siebrana*, and the sample under study, *gerrardii var gerrardii* from the same location of South Kordofan.

Acacia gerrardii gum was characterized by a low solubility up to 20% and the result of moisture content is depended on season of collection, climate and storage Scondition. The moisture content and ash of *Acacia gerrardii var gerradii* gum show no significant variation from other *Acacia* gums.

The pH value of *Acacia gerrardii* gum is 5.3 this value show no difference with Elassm (2012) and less acidic than that reported for *Acacia siebrana* (Saed, 2011) and for *Acacia seyal var.fistula* (Karamalla 1965).

The value of specific optical rotation of *A. gerrardii* gum was +60.2 this result agrees with the result of Kramalla (1965) and higher than that reported by Elassam, (2012) and lower than that *of A. nilotica var nilotica* (Satti, 2011).

The intrinsic viscosity was obtained by extrapolation to zero concentration of the plot of the reduced viscosity against the different gum concentrations Table (3.5) and it was found to be 15.1cm^3/g (Fig 3.1). These result agree with Satti, (2011), Saeed, (2011), and higher than Elassam, (2012).

Nitrogen and protein content of *Acacia gerrardii* gum were found to be 1.88% and 13.13% respectively this result is higher than that reported by Satti, (2012), Saeed, (2011) and Karamalla(1965), but it agrees with Elassam, (2012). That means that

Acacia gerrardii gum is characterized by a higher value of nitrogen and protein and lower solubility.

Equivalent weight of the sample was 2000 and total uronic acid 9.7%. The table showed that highest equivalent weight has lowest uronic acid value.

The number average molecular weight (Mn) of *acacia gerrardii* gum was obtained by osmotic pressure measurement and calculated from the intercept of $\sqrt{\pi}/c$ against concentration Table (3.4), Fig(2.1) .The value of (Mn) was found to be $1.1*10^5$. This result of *A. gerrardii* var *gerrardii* is lower than the result obtained for a variety *Acacia* gums from the same subgenus Table (3.1).

The sugar content of *Acacia gerrardii var gerrardii* were measured using HPLC technique and were found to be 0.33% Rhaminose, 39.3% Arabinose and 5.5% Galactose as shown in Table 3.2. Arabinose has a higher value than Galactose and Rhmanose (Fig 2.3).This result show the large different sugar content with a variety of *Acacia* gums.

Cationic composition of *A. gerrardii var gerraedii* gum was determined using atomic absorption spectrophotometer(AAS) and a flame photometer, the results showed Ca (70ppm) has the highest value followed by K, Na, Mg, Cu, and trace of Pb and Cd as shown in Table 3.3.

Table 3.1 physiochemical properties *of some Acacia gummiferae* gums

Variety	Moisture %	Ash %	pH	Optical rotation	Intrinsic viscosity g/ml	Nitrogen %	Protein %	Equivalent weight	Total uronic acid %	Mn	Reference
Acacia nilotica	10.84	1.9	5.08	+100	10.13	0.025	0.163	1899.7	10.21	$3.51*10^6$	Satti.A.A (2011)
Acacia siebrana	8.56	1.61	4.34	+104	8.56	0.37	2.45	-	9.2	$1.5*10^6$	Saeed.K. M (2011)
Acacia gerrardii	6.5	1.89	5.35	+46.3	22.4-33	2.7	18.9	1273	15.6	$5.5*10^6$	Elassam. H.A (2012)
Acacia seyal var. fistula	6.46	2.9	4.7	+60-+61	-	0.07	0.44	1530-2083	9.3-12.7	10^6	Kramalla. (1964)
Acacia gerrardii	7.6	1.8	5.3	+60	15.1	1.88	13.16	2000	9.7	$1.1*10^5$	This study (2015)

Table 3.2 Sugar content of *Acacia gerrardii var gerraedii* gum (in mg/ml)

Reference	Rhamnose%	Arabinose%	Galactose%
Satti, (2012)	10.68	41.20	17.43
Elassam.H.A (2012) mg/ml	0.49	55.6	9.7
Saeed.K.M (2011)	4.0	57	9.8
This study(2014)	0.33	39.3	5.5

Table 3.3 Cationic composition of *Acacia gerrardii var gerraedii* gum sample in mg/L

Cation	K^+	Na^+	Ca^{++}	Mg^{++}	Zn^{++}	Cd^{++}	Pb^{++}	Cu^{++}
mg/l	5.3	4.9	70	2.92	0.16	<LTD	<LTD	0.93

LTD mean less than detection, LTD for Pb =0,003, Cd = 0.001

Table 3.4 Variation of osmotic pressure of *Acacia gerrardii var gerraedii* gum with concentration

Conc.(g/ml)	√π/c
0.02	0.269
0.04	0.405
0.06	0.461
0.08	0.60
0.1	0.96

Figure(2.1) Osmotic pressure concentrations profile of *A.gerrardii var gerraedii* gum

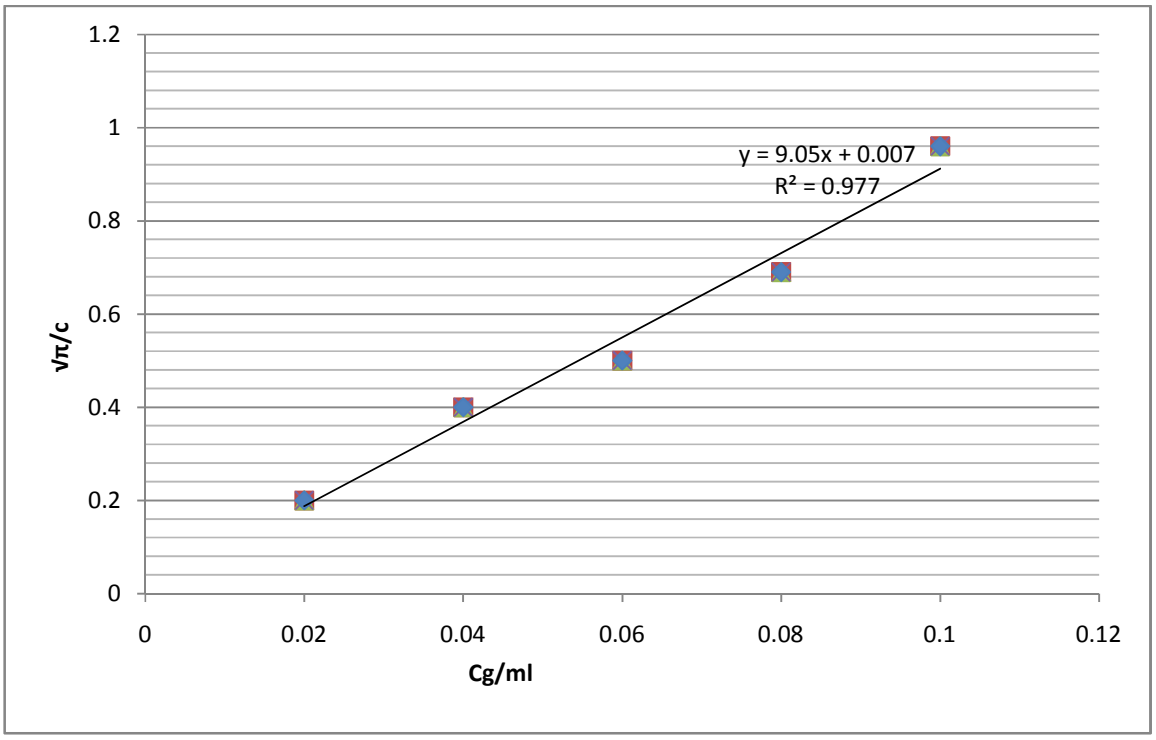
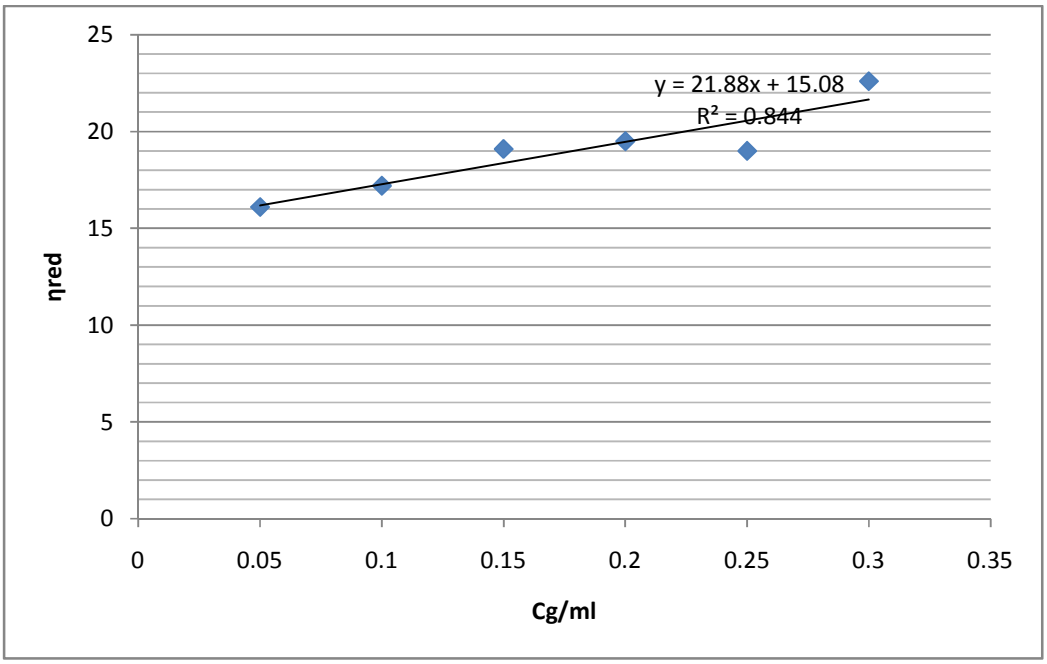


Table 3.5 Relative, specific and reduced viscosity of *A. gerrardii* gum *var gerraedii*

Conc.(g / ml)	Mean time of flow (t)	η_{rel}	η_{Sp}	η_{red}
0.005	94	1.08	0.08	16.1
0.010	102.3	1.17	0.17	17.2
0.015	112	1.29	0.29	19.1
0.020	121	1.39	0.39	19.5
0.025	128	1.46	0.46	19
0.030	146	1.68	0.69	22.6

Acacia gerrardii , solvent NaCl(1M) .Temp. 25°C average flow time in seconds t_0 =88.66 second

Figure(2.2) intrinsic viscosity of *A. gerrardii gum var gerraedii*



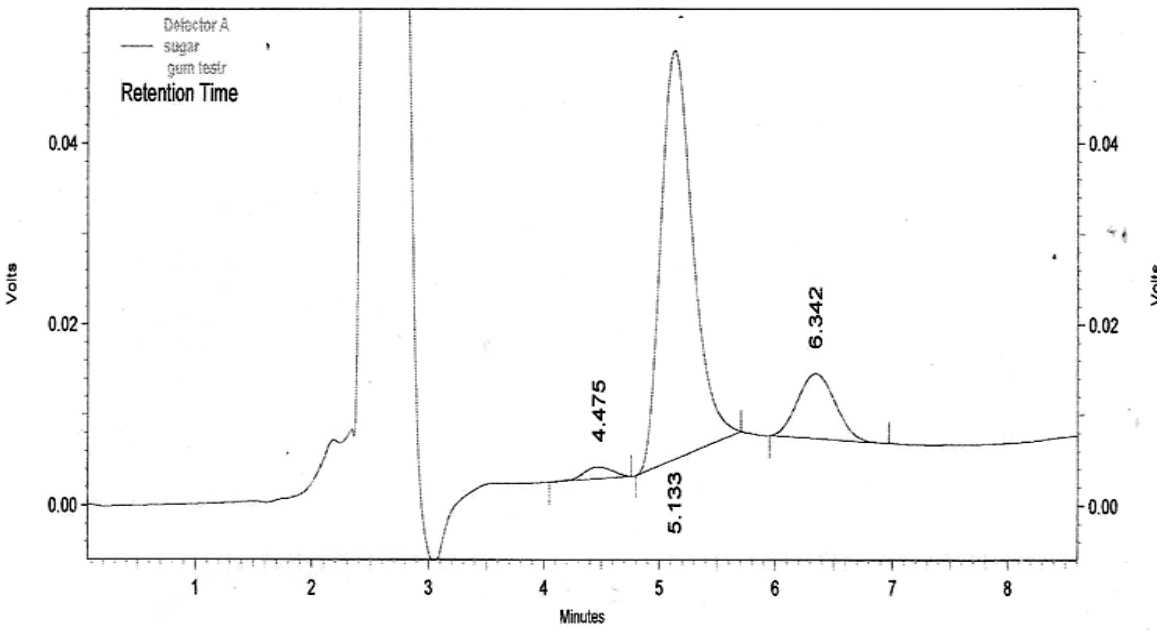
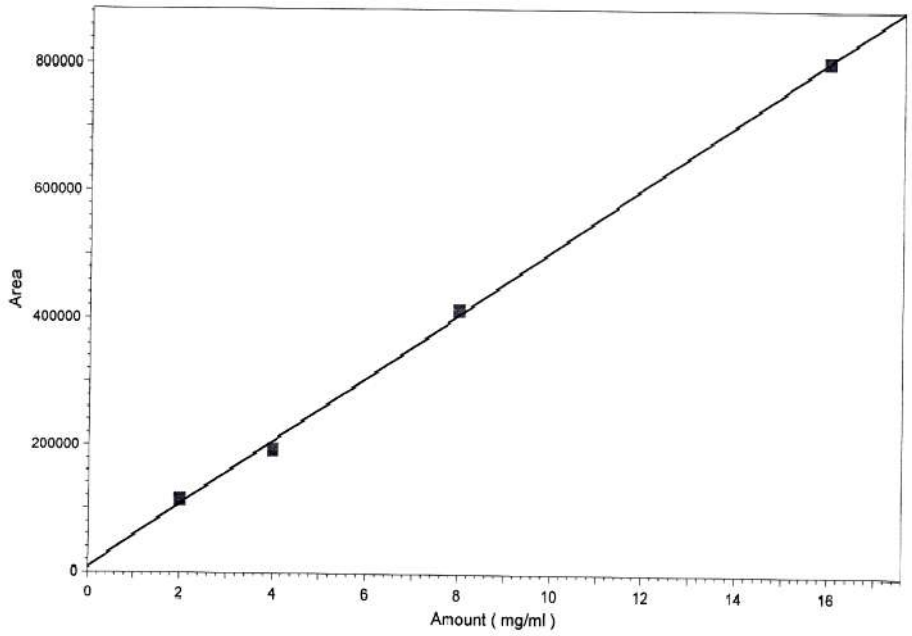
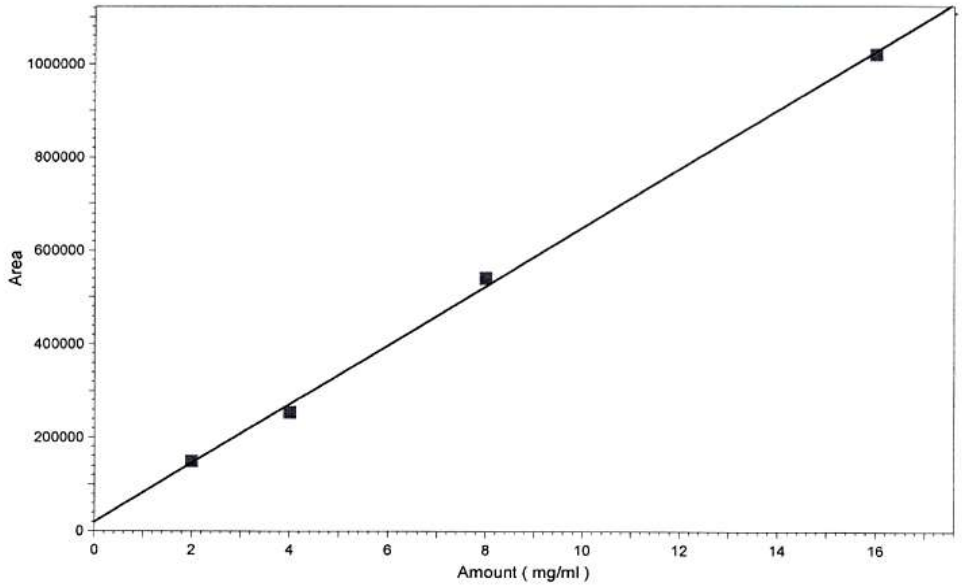


Figure (2.3) HPLC Profile of *Acacia gerrardii var gerrardii* gum sugarcomposition

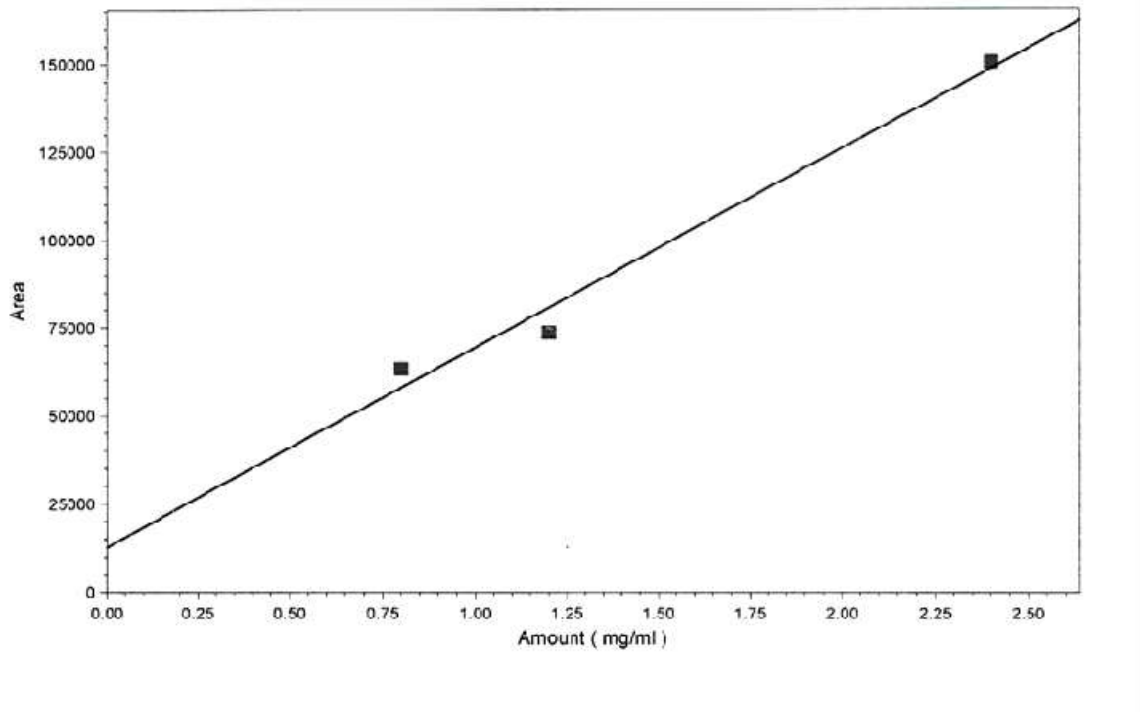
Figure (2.4) HPLC profile of Arabinose, Galactose, and Rhamnose standards



Arabinose



Galactose


Area
150000
125000
100000
75000
50000
25000
0
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25
2.50
Amount (mg/ml)

Rhmanose

3.2 Conclusion:

The physiochemical properties of the sample tested agree with previously reported studies on A. *gerrardii* gum which characterized by low ash content, high protein content, low viscosity, low solubility and high value of calcium content which is important for some physiological activities.

3.3 Suggestion for further work:

- The emulsifying stabilizing and thickening properties of *Acacia gerrardii* var *gerrardii* gum has to be investigated.

- Safty of *Acacia gerrardii var gerrardii* gum should be studied.

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