بسم الله الرحمن الرحيم

**Sudan University of Science and Technology**

**College of Graduate Studies**

# Preparation and Characterization of Glucuronic Acid and Magnesium Glucuronate from *Acacia senegal var. senegal* (GUM)

**تحضير وتوصيف حمض الجلوكيرونيك وجلوكيورنات الماغنيسيوم من الصمغ الهشاب**

**By**

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# الآيـــة

قال تعالى:

(وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا)

سورة الإسراء الآية (85)

صدق الله العظيم

**Dedication**

My father, My mother,  Sisters and brothers

# Acknowledgement

I am greatly in debted to Allah Almighty, who gave me the fitness, strength to conduct this work.

My sincere gralitude to my Prof: Mohammed Elmobark Osman for his kind administration practical, guidance and fruitful criticism that helped me to continue this work.

Thanks are due to National Research Center and members of the Department of Chemistry Sudan University of Science and Technology, and Iam also thankful to my friends technical and moral support during this work.

## Abstract

Asample collected from *Acacia senegal var. senegal* natural exudate and prepare Glucurnic acid, compounds magnesium glucuronate, and anti acid and conducted an analytical study describes The physic-chemisal properties of *Acacia senegal* the average specific rotation was polarized light ($-30.4^{0}$) moisture (%12.1)ash(%3.2), content of nitrogen (%0.3) and PH (4.3), . The moisture (% 0.14), ash (% 0.2), of the mg. gluuronate.

The elements composition gum calcium (36300 ppm) and magnesium (920 ppm) for calcium, (13740 ppm) and magnesium (12620 ppm) mg. glusuronate .

**المستخلص**

تم جمع عينة من صمغ الهشاب الناتج بصورة طبيعية في السودان وتحضير حماض الجلوكرونيك ومركبات جلوكرونات الماغنيسيوم المضاد للحموضه واجريت دراسه تحليليه لتوضيح الخواص الفيزيوكيميائيه لصمغ الهشاب وكان متوسط الدوران النوعى للضوء المستقطب ( -30.4˚)، الرطوبه ( 12.1%)، الرماد(3.2%) ، محتوى النتروجين ( 0.3%)، والاس الهيدورجينى (3.2) ، اما بالنسبه جلوكرونات الماغنيسيوم فكانت الرطوبه (0.14%) الرماد (2.3%) .

اما بالنسبه للعناصر المعدنيه ظهر الكالسيوم (36300 ppm) ،الماغنيسيوم (920 ppm ) لصمغ الهشاب، وكما ظهر الكالسيوم (13740 ppm)، و الماغنيسيوم (12620 ppm) جلوكرونات الماغنيسيوم.

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# Introduction

## 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.(* Elgaili. A. Omer et). *Acacia polyacantha* (family *Leguninosae*) exudates are closely related, and can be distinguished from *A. senegal* exudates by differences in physicochemical characteristics. The two species, *Acacia senegal* and *Acacia polyacantha* belong to the same group known as *Acacia senegal* complex. All gum exudates, from this group of *Acacia* species, have a laevorotatory (-ve) specific rotation in contrast to the *Acacia seyal* complex which produce gum exudates, that have a dextrorotary (+ve) specific rotation, other structural, botanical characteristics are noticeable even within the same species.

Gum arabic is a dried exudate obtained from the stems and branches of *Acacia senegal* (L.) Willdenow or *Acacia seyal* (fam. Leguminosae)

Gum arabic consists mainly of high-molecular weight polysaccharides and their calcium, magnesium and potassium salts, which, on hydrolysis, yield arabinose, galactose, rhamnose and glucuronic acid. Items of commerce may contain extraneous materials such as sand and pieces of bark, which must be removed before use in food. It is a branched molecule with protein content of about 2.0–2.5.( Malik A. Abdelrahman Elsheikh et.al.2015).

In Sudan, the term gum arabic is used in a wider context to include two types of gum which are produced and marketed, but which are, nevertheless, clearly separated in both national statistics and trade: "hashab" (from *A. senegal*) and "talha" (from *A. seyal*). In a still wider sense, gum arabic is often taken to mean the gum from any *Acacia* species (and is sometimes referred to as "Acacia gum"). "Gum arabic" from Zimbabwe, for example, is derived from *A. karroo*.

In practice, therefore, and although most internationally traded gum arabic comes from *A. senegal*, the term "gum arabic" cannot be taken as implying a particular botanical source. In a few cases, so-called gum arabic may not even have been collected from *Acacia* species, but may originate from *Combretum*, *Albizia* or some other genus.

## 1-1 LITERATURE REVIW:

### 1-1-1 Acacia Senegal:

### 1-1-2 Definition:

**Botanical classification**

Class: Equisetopsida

Subclass: Magnoliidae

Superorder: Rosanae

Order: Fabales

Family: Leguminosae/Fabaceae - Mimosoideae

Genus: Acacia

### 1-1-3 Description of the gum:

*Acacia senegal* is a small to medium sized thorny tree, with a stem, which is irregular in form and often highly branched. In leaf, like many other *Acacias*, it has a dense, spreading crown. In common with other members of the *A. senegal* complex, it has characteristic sets of prickles on the branches, usually in threes with the middle one hooked downward and the lateral ones curved upward. The bark is not papery or peeling (Tieguhong, et.al,2004).The tree is deciduous, droops its leaves in November in the Sudan (FAO,2007).

Gum arabic *(A. senegal)* is a pale white to orange-brown solid, which breaks with a glassy fracture. The best grades are in the form of whole, spheroidal tears of varying size with a matt surface texture. When ground, the pieces are paler and have a glassy appearance. Gum from (A. seyal) is more brittle than the hard tears of gum arabic (A. senegal).

*A. senegal* Shrubs or small trees 2-12m high brak yellow to light brown or grey,rough ,fissuring or flaking ,young brancnlets with horizontal slit- like lenticels,stipules non. Spinescent, prickles at nodes in threes, 2 lateral pointing upward or forward and one central pointing down ward or back ward, falcate ,4-7mm long, pinnae 2-6 paris, 0.5-3cm long ,leaflets 8-18 paris, linear to elliptic oblong, 1-6x 0.5-2 cm .inflorescence spicate, 2-10 cm long on peduncles 0,7-2cm long ,flower white or cream, sessile ,sepals 2x0.7 mm, pubescent, petals 2.5 x0.3 mm glabrous ,stamens 4-7 mm long, glandular. Fruit flat straight oblong membraneous dehiscent pods 3-24 x1-3.3 cm pale brown to straw-coloured,seeds vertical in pog, orbicular ,compressed, 8-12 mm across, yellow or pale brown, areoles crescent – shaped. Centrals, 1.5- 6 x2.5 – 5 mm funicles 7.5 mm long . flowers nov – feb , fruits Jan - April On sandy and clay plains in short grass savanna forming a continuous bell from east to west in central sundan .more common one the western sand plains of kordofan and Darfur as pure stands assoiated with Amelliteca.


**Acaia Senegal (L.) willd , var, Senegal brenan a. flowering branch , b. seed , c. flower , d. fruit, e. prickles** (hamza Mohammad El Amin – 1990).

**1-1-4 Distribution:**

Has a wide distribution and remarkable adaptability. It is essentially a semi-arid zone species, but it is both drought, frost resistant, and can grow with a rainfall of between 100 and 800 mm per year. It grows across Africa, from Senegal to Ethiopia, through Mali, Nigeria, Chad and Sudan, to mention only the major producing areas. It is also found in the Middle East, Yemen, India and Pakistan. In the Sudan, particularly in the *Kordofan* and *Darfour* provinces, the species is uniform and found in pure stands giving the Sudan an important advantage of being the most important producer of this type of Gum Arabic. In other producing countries, *Acacia senegal* often found mixed with other species. Another feature of the Sudan system of production is that this species occurs| Page 10 both as a wild and as a cultivated species; man in village plantations ofter eplants it.(AIPG).

**1-1-5 Plant Sources**:

Botanical classification

Family Leguminosae (Mimosoideae):

*Acacia* spp., especially:

*A. senegal* (L.) Willd.

*A. seyal* Del.

Numerous *Acacia* species yield gum, either by natural exudation or after tapping, but almost all gum arabic of commerce originates either from *A. senegal* or *A. seyal*. There is disagreement over some aspects of *Acacia* taxonomy but *A. senegal* is generally regarded as occurring as four varieties:

*A. senegal* (L.) Willd. var. *senegal*

(syn. *A. verek* Guill. & Perr.)

*A. senegal* (L.) Willd. var. *kerensis* Schweinf.

*A. senegal* (L.) Willd. var. *rostrata* Brenan
*A. senegal* (L.) Willd. var. *leiorhachis* Brenan
(syn. *A. circummarginata* Chiov.)

*A. seyal* occurs as two varieties:

*A. seyal* Del. var. *seyal*
*A. seyal* Del. var. *fistula* (Schweinf.) Oliv.

Other species of *Acacia* from which gum is, or has been, collected for local use or as minor components of poorer quality shipments for export include:

*A. karroo* Hayne
*A. paoli* Chiov.
*A. polyacantha* Willd.
*A. sieberana* DC.

**1-1-6 Quality of gum Arabic :**

The quality of gum arabic as received by the importer depends on the source. Gum arabic (hashab) from Sudan is the highest quality and sets the standard by which other "gum arabics" are judged. Not only does Sudanese gum come from a species (*A. Senegal Var. Senegal* ) which intrinsically produces a high quality exudate with superior technical performance, but the collection, cleaning, sorting and handling of it up to the point of export is well organized and highly efficient.

Within Sudan, gum arabic from the Kordofan region has the highest reputation, and traders and end-users in importing countries often refer to "Kordofan gum" when indicating their preferences.

Gum talha from Sudan (produced from *A. seyal*) is intrinsically a poor quality gum than hashab , it has inferior emulsifying properties and even light-coloured samples of whole gum sometimes form dark solutions in water due to the presence of tannins and other impurities. It is more friable than hashab.

**1-1-7 Supply sources "geographical distribution:**

The gum belt referred to earlier occurs as a broad band across Sub-Saharan Africa Fig.1 from Mauritania, Senegal and Mali in the west, through Burkina Faso, Niger, northern parts of Nigeria and Chad to Sudan Fag.2, Eritrea, Ethiopia and Somalia in the east, and northern parts of Uganda and Kenya. Most of these countries appear in the trade statistics as sources of gum arabic, although they differ greatly in terms of the quantities which are involved.

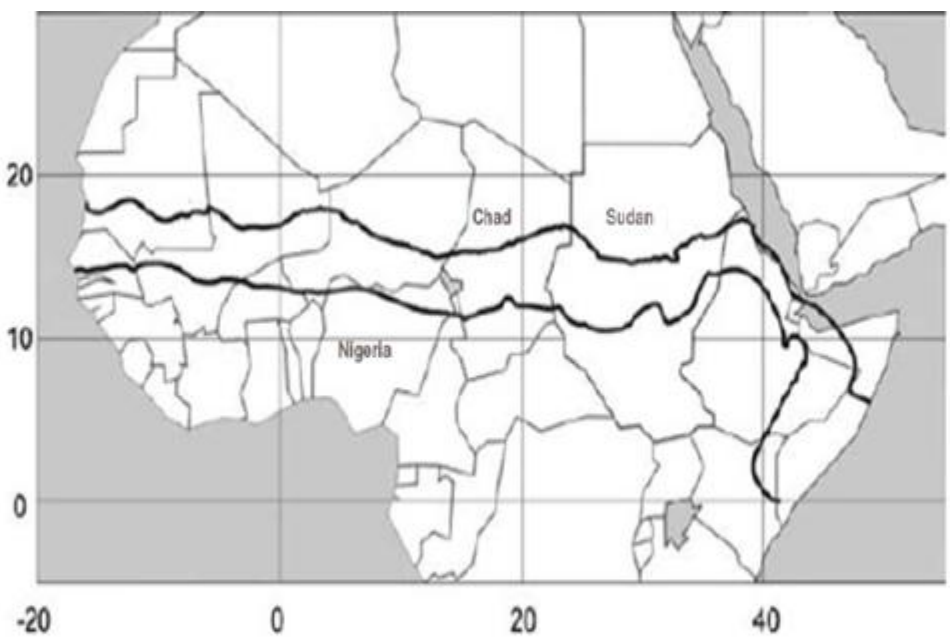



**Fig 1.1: The Gum Arabic Belt in Africa**

Sudan is the world's biggest producer of gum arabic, and since very little is consumed domestically it is also the main source of gum in international trade.

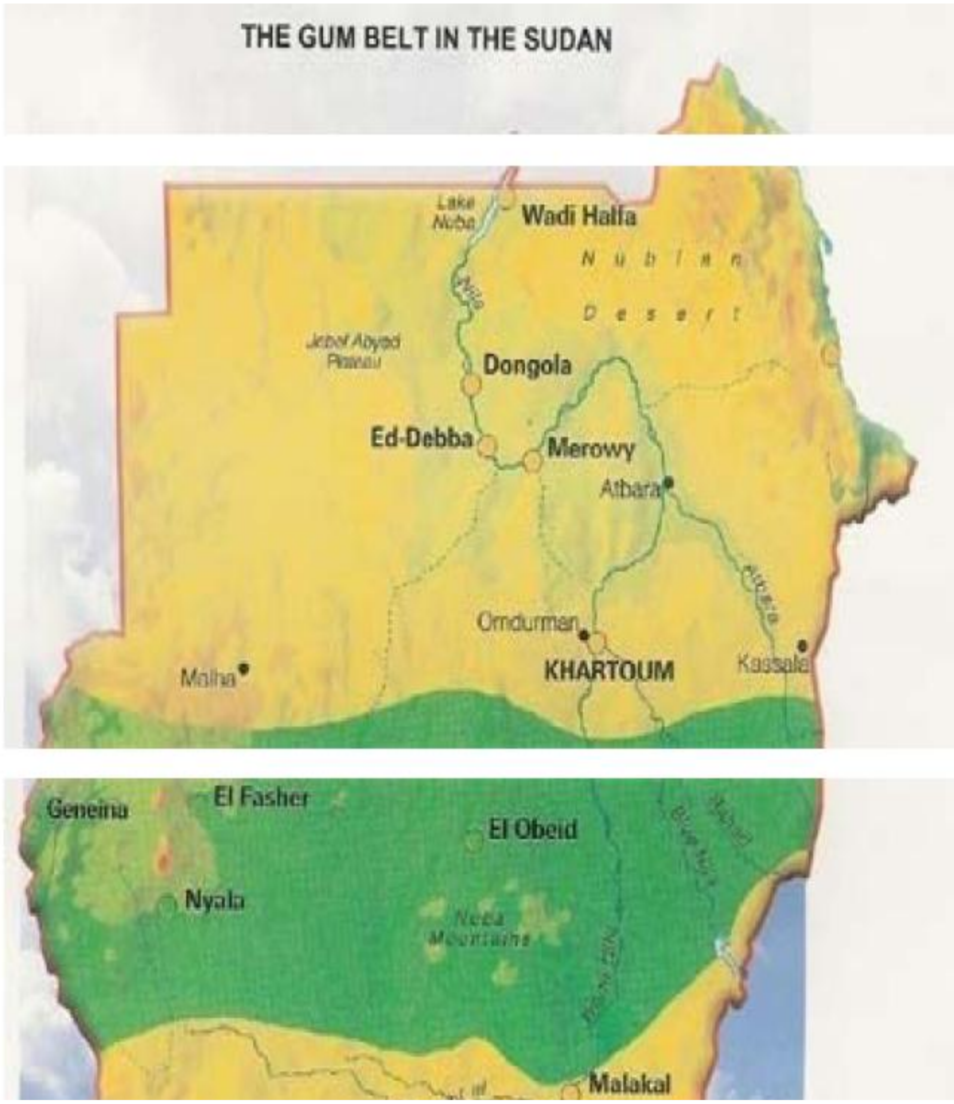

THE GUM BELT IN THE SUDAN
Wadi Halfa
Nubian Desert
Dongola
Ed-Debba
Merowy
Atbara
Omdurman
KHARTOUM
Kassala
Malha
Geneina
El Fasher
El Obeid
Nyala
Nuba Mountains
Malakal

**Fig 1.2: The Sudan Gum Belt**

A few countries which have gum-yielding Acacias produce gum for the local market, but not in sufficient quantities to enable exports to be made. Two such examples are Zimbabwe and South Africa, which produce gum arabic from *A. karroo*. Outside Africa, India produces small amounts of gum, similar in quality to gum talha, but a proportion of her exports of gum arabic consists either of re-exports of African gum or locally produced gum ghatt (From. *Anogeissus latifolia*) misclassified as gum Arabic.

(FAO,2007).

**1-1-8 Structure of plant gums:**

Gum nodules contain polysaccharide material of complex nature usually contaminated with impurities such as bark fragments, entrapped dust and insects. Inert pertinacious material and a few amounts of terpenoid resins can also be present. Gums are polyuronides; the uronic acid residues may carry acetyl or methyl groups and, generally, occur at least in part as methyl groups or as metallic salts. The hexose residues are present in the pyranose configuration, while the pentose residues occur in the furanose(Stephen et al., 1955 and 1957) beside the foregoing gums, *Sterculiatermentosa* gum contains rhminose, galactose and probably galacturonic acid, *Olibanum*gum was found to be of an arabino - galactan and polysaccharide containing galactose and galactouronic acid (Elkhatem et al., 1956). It was noted that the gum was very heterogeneous and it has been described as heteropolymolecular, i.e. having either a variation in monomer composition and/or a variation in the mode of linking and branching of the monomer unites, in addition to distribution in molecular weight(Lewis and Smith, 1957; Dermyn, 1962 and Stoddart, 1966). According to Philips(1988) and Williams (1989), fractionation by hydrophilic affinity chromatography revealed that *Acacia Senegal* gum consists of at least three distinct components. Fraction 1 (arabinogalactan) AG, fraction 2 (arabinogalactan-protein) AG and fraction 3 (galactoprotein) GP. But even those contain a range of different molecular weight components revealing the polydiverse nature of the gum (Osman, 1994).

Fraction 1 containing 88% of the total has only small amount of protein content. Fraction 2 represents 10% of the total and had 12% protein content. Fraction 3 resembles 1.24% of the total but contains almost 50% of protein AGP are responsible for the emulsifying properties of gum Arabic. No mention has been made to detailed comparison between the structures of gums from different species of trees, but is believed that D-galactose and uronic acid residues generally constitute the backbone of gum polysaccharide with 1- 3 and 1-6 linkages predominating side chain are characterized by the presence of D xylopyranose, L-arabinose, and L-arabino-furanoselinkage (Elnour, 2007).

**1-1-9 Theories of gums formation:**

There are many theories of gum formation theories and functions which have been formulated to explain the phenomena of gummosis, formation of gum exudates is pathological condition resulting from microbial(fungal and bacterial) infection of in juredtree, natural factor that tends to lessen the vitality of the trees, such as poor soil, lack of moisture, and the weather improve gum yields.Other considers the production of gum to metabolic process in the plant with quantity and quality produced being function of environmental condition, some believed that the gum formed as a defense mechanism to seal off the wound to prevent desiccation other proposed that the starch might undergo transition into gum.( the latter is refuted by Anderson and Dea 1968) , as the enzyme system necessary to transform starch into highly branched arabinoglactan with galactose , arabinose , rhamnose , glucuronic acid and its 4-o- methyl ether are complex, further(Anderson and Dea ,1968) found that the starch was not represent in tissues of excited branches and therefor proposed that gums have a hemicelluloses types, highly branched arabinoglactan precursor in which rhamnose glucronic acid and 4-o- methyl glucuronic acid are the peripheral groups(Omer, 2004)

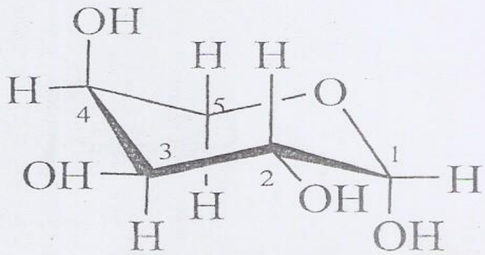
**1-1-10 Chemical structure of gums Arabic:**

Gum Arabic is branched, neutral or slightly acidic, complexpolysaccharide obtained as a mixed calcium, magnesium, and potassium salt. The backbone consists of 1,3- linkedb – d-galactopyranosyl units. The side chains arecomposed of two to five 1,3–linked b-d-galactopyranosylunits, joined to the main chain by 1, 6-linkages. Both themain and the side chains contain units of a-1-arabinofuranosly, a-1-rhamnopyranosyle,

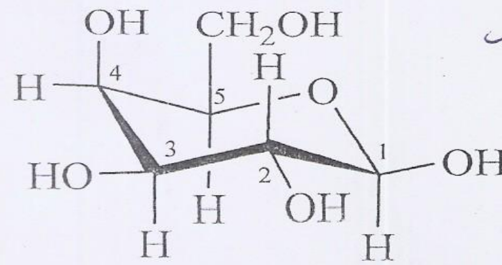
dglucuronopyranosyl, and 4-O-methyl -b-d-g lucuronopyranosyl, the latter two mostlyas end-units (Anderson and stoddart, 1966).

They further analyses the product by methylation and gelpermeation chromatography and found that theuronic acid and the rhamnoseresidues eliminated firstwhich proved that they are located at the periphery of themolecule and the core was consisted of a β1,3-galactopyranose chain with branches linked through1,6 position. Also found that the protein componentwas associated with the high molecular weight fractionand lower molecular mass fraction was virtuallyexclusively polysaccharides.

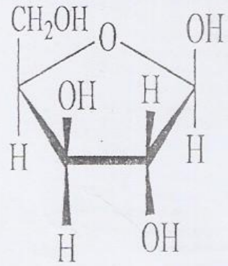
Figure (1.3) shows the polysaccharides in gum Arabic.(street et al., 1983) used computer modeling to analyze the previous data and proposed the structure illustrated in Figure (1.4), (churms et al.,1983) subjected the gum to smithdegradation leaving the reaction to reach completion aftereach stage of degradation procedure. They obtained different values for the composition and size of th emolecule of each degradation product than those previously obtained by (Anderson, 1966b), and proposed amore regular structure than the previous one proposingthat the galactan core consisted of 13β-, 3-D- galactopyranosyl residues Fig (1.4) having two branches which give single repeatingsubunits having molecularmass of $8\times 10^3$ within the molecule .As the whole gum was found to have molecular weight of 560,000 thus it was proposed that the molecule consists of 64 of these subunits and that they were symmetricallyarranged.(Defye and Wang, 1986) in their structural studiesof gum Arabic using A25.182MHz $^{13}$C-NMR (Alaa.M.M, 2015)



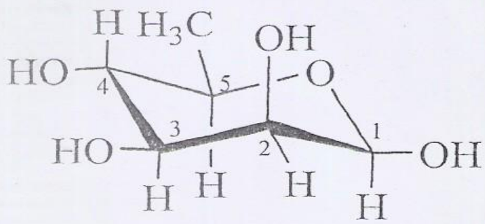
a- Arabinopyranose



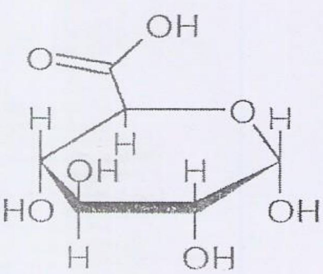
β- Galactopyranose



Arabinofuranosyl



β- Rhamnopyranose



The α-D form of glucuronic acid

**Figure(1.3) structural of carbohydrates units of gum molecule (Alaa.M.M, 2015).**

A Arabinofuranosyl, Ap Arabinopyranosyl.

R Rhamnopyranosyl.

Um 4-O-Methyl glucopyranosyluronic acid.

U Uronic acid.

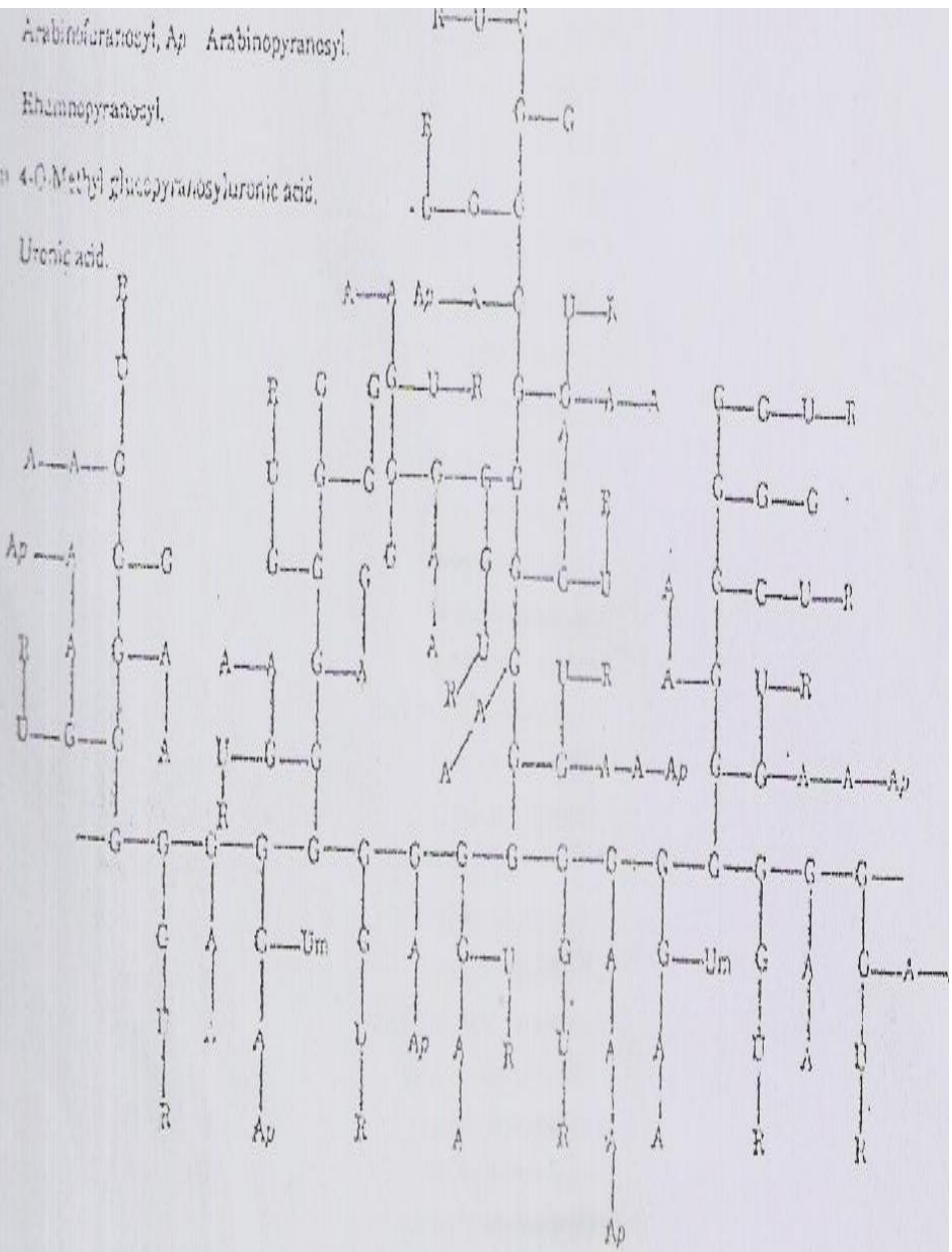


**Fig (1.4) The structural of *Acacia senegal* gum as proposed (street et al., 1983)**

1-1-11 **Structure of plant gums:**

Gum nodules contain polysaccharide material of complex nature, usually, contaminated with impurities such as bark fragments, entrapped dust and insects. Inert pertinacious material and a few amounts of terpenoid resins can also be present. Gums are polyuronides; the uronic acid residues may carry acetyl or methyl groups and, generally, occur at least in part as methyl groups and generally occur, at least in part, as metallic salts. The hexose residues are present in the pyranose configuration, while the pentose residues occur in the furanose (Stephen *et al.*, 1955 and 1957) Beside the foregoing gums, others have been studied; *khaya senegalese* gum contains galactose, rhaminose and probably 4-O-methyl, D-glucuronic acid and galactouronic acid (Aspinal *et al.*, 1956). *Sterculia termentosa* gum contains rhminose, galactose and probably galacturonic acid, Olibanum gum (*Boswellia carterii*) was found to be of an arabino-galactan and a polysaccharide containing galactose and galactouronic acid (Elkhatem *et al.*, 1956). It was noted that the gum was very heterogeneous and it has been described as heteropolymolecular, i.e. having either a variation in monomer composition and/or a variation in the mode of linking and branching of the monomer unites, in addition to distribution in molecular weight (Lewis and Smith, 1957; Dermyn, 1962 and Stoddart, 1966). According to Philips (1988) and Williams (1989), fractionation by hydrophilic affinity chromatography revealed that *Acacia Senegal* gum consists of at least three distinct components. Fraction 1 (arabino galactan) AG, fraction 2 (arabino galactan-protein) AGP, and fraction 3 (galactoprotein) GP. But even those contain a range of different molecular weight components revealing the polydiverse nature of the gum (Osman, 1994). Fraction 1 containing 88% of the total has only small amount of protein content. Fraction 2 represents 10% of the total and had 12% protein content. Fraction 3 resembles 1.24% of the total but contains almost 50% of protein AGP is responsible for the emulsifying properties of gum Arabic (Williams, 1989, and Phillips, 1988). No mention has been made to detailed comparison between the structures of gums from different species of trees, but is believed that D-galactose and uronic acid residues generally constitute the backbone of gum polysaccharide with 1-3 and 1-6 linkages predominating side chain are characterized by the presence of D-xylopyranose, L-arabinose, and L-arabino-furanose linkage.

**Applications of plant gums:**

The solubility and viscosity of gum are the most fundamental properties, which make it unique among polysaccharides, the majority of gums dissolve in water at different concentrations, and such properties are exploited in many applications.

**1-1-12 Applications in the food industry:**

Gums for their high viscosity in solutions and inability to crystallize, are particularly suited to serve in foodstuff such as: thickeners for beverages, stabilizers for oil and water emulsions and as wider application where function is to prevent agglomeration and setting of minute particles. They are also used to incorporate flavors in confectionery such as pastilles and gum drops, and the preparation of lozenges. The role of gum Arabic in confectionary products is usually either to prevent crystallization of sugar or to act as an emulsifier (Glicksman *et al.,* 1973).

**1-1-13 Pharmaceutical and cosmetic applications:**

Gums are used as a suspending and emulsifying or binding agents in pharmaceutical industries, it has been used in tablet manufacturing, where it functions as a binding agent or as a coating prior to sugar coating, some times in combination with other gums .*A. polyacantha* gum used to act as general health tonic as antidote for snake bite, and cure for venereal diseases. A preparation from the bark is used for general stomach disorders (Voget, 1995).

**1-1-14 Paints and coating composition application:**

The hydrophilic colloids and modified cellulose find application in paint industry because of their stabilizing effect on paint emulsions, waxes and numerous others products. (Gamble and Grady 1938) treated pigments with water soluble hydrocolloids such as gum Arabic to add controllable chemotropic properties to paints. The gum also finds application in coating composition Horne *et al*. (1953) developed non glare coating based on a water soluble dye dissolved in gum Arabic solutions.

**1-1-15 Other industrial uses**:

Due to its adhesive properties gum have been used in the manufacturing of adhesives for postage stamps and also in the formulations of paints and inks. Gum may serve as a source of monosaccharide, as e.g. mesquite gum (family prosopis) serve as a source of L-arabinose (51%) because of its easier hydrolysis, and availability of the gum in large quantities. The mesquite gum can be dialyzed by addition of ethanol (White, 1947 and Hudson, 1951), or alternatively, isolated by crystallization from methanol after removal of acidic oligosaccharides on ion exchange resin or precipitated by barium salts. Gums are widely used in textile industries to impart luster to certain materials (silk), as thickeners for colors and mordant in calico printing (Omer, 2004).

### 1-1-16 Physicochemical properties of *Acacia senegal* gum:

The physical properties of the natural gum are most important in determining their commercial value and their use. These properties vary with gums different botanical source, and even substantial differences in gum from the same species when collected from plants growing under different climatic conditions or even when collected from the plant at different season of the year (Hirst *et al.,* 1958). The physical properties may also be affected by the age of the tree and treatment of the gum after collection such as washing, drying, sun bleaching and storage temperature.

#### 1-1-16-1 Solubility:

Gums can be classified into three categories with regard to their solubilities:

1. Entirely soluble gums: e.g. *A. senegal, A. seyal.*
2. Partially soluble gums: e.g. *Gatti* gum.
3. Insoluble gums: e.g. *Tragacanth* gum (Omer, 2004).

#### 1-1-16-2 Colour:

The colours of gums vary from water- white (colourless) through shades of yellow to black. The best grades of gum are almost colourless with slight traces of yellow; some possess pink likes (Siddig, 2003). On the other hand dark or even black gums some times occur e.g. mesquite gum. There are also the pale rose pinks, darker pink and yellowish gums. The pink colour is probably due to the presence of different quantities of tannin materials (Omer, 2004).

**1-1-16-3 Shape:**

Natural gums are exuded in a variety of shapes and forms: usually the fragments are irregularly globular or tear globular or tear shaped. The best known being the tear or drop shape of various grades of gum Arabic. Other shapes are flakes or threat like ribbons with gum *tragacanth.* The surface is perfectly smooth when fresh but may become rough or crusty, covered with small cracks (Omer, 2004).

**1-1-16- 4 Moisture:**

The hardness of gum would be determined by moisture content. The moisture content of good quality gum dose not exceed 15 and 10% for granular and spray dried material respectively (FAO, 1999). The moisture content is weight lost due to the evaporation of water (Person, 1970). It shows the hardness of the gum and hence variability of densities, the amount of densities, and the amount of the air entrapped during formation. (Omer 2004) recently, reported that the moisture content of *A.*Senegal gum to be around 12.1 %.

**1-1-16 -5 Ash:**

In a study of 800 authentic formulations of gum from *Acacia Senegal* var *senegal* collected from 32 different localities of the gum producing belt of the Sudan, showed that the type of the soil had no significant effect on the ash content of the gum (Karamalla *et al.*, 1998). (Anderson *et al.*, 1983) found that the value of ash content of commercial gum arabic to be 4.4%. Later, (Anderson et al 1991) reported 3.6% Ash content for Sudanese formulations. FAO (1999) reported that the ash content of gum Arabic did not exceed more than 4%.

**1-1-16-6 Nitrogen and protein content:**

Gum arabic is a polymer with about 3% protein (Anderson et al., 1991). The protein fraction is responsible for the emulsification properties of the gum. The role of nitrogen and nitrogenous component in the structure, physicochemical properties andfunctionality of gum arabic was recently subjected to intensive investigation. Structurally the "Wattle blossom" model (Fincher et al., 1983) depends on the nitrogenous component (Fig.1).

An adsorbed layer of protein at oil /water interface provides the primary stabilizing structure in many food colloids (Dickinson, 1994). According to (Dickinson et al., 1988), the variability in theemulsifying properties of gums from different Acacia species is dependent not only on the total protein (on polypeptide content) but also on the distribution of the protein-peptide between the low and highmolecular weight fractions and on the molecular accessibility of the protein peptide for absorption according to (Randall *et al.,* 1989).The United States pharmacopoeia and European Union specification defined the minimum standards of the protein content for good quality gum arabic as 3%.(Anderson 1986) found that the average nitrogen content for commercial *Acacia senegal* gum formulations to be 0.37%.

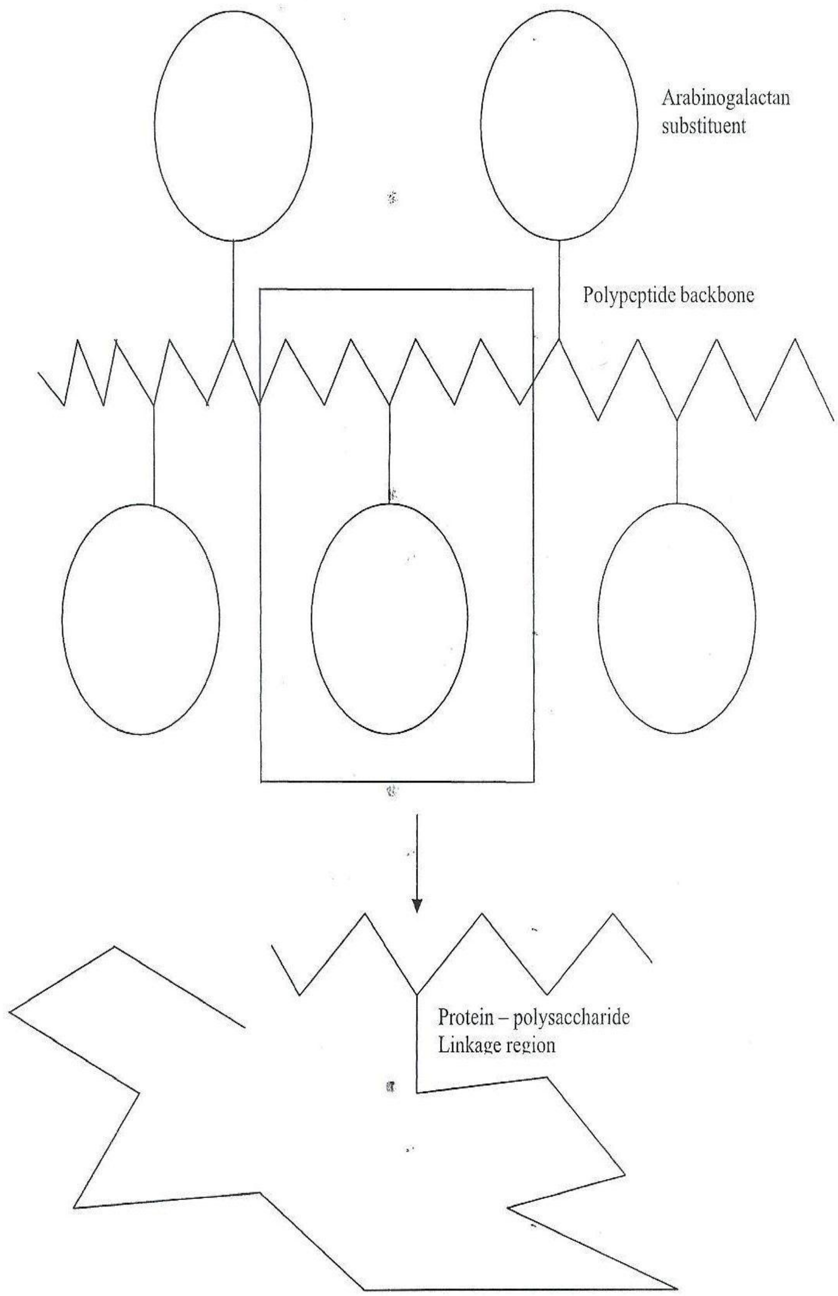

Arabinogalactan
substituent
Polypeptide backbone
Protein – polysaccharide
Linkage region

**Figure (1) The Wattle – Blossom model for *Acacia senegal* gum as proposed by (Fincher *et al.*, 1983 ).**

Investigations of protein in *Acacia senegal* gum have been carried out by (Akiyama *et al.,* 1984), they reported that gum Arabic contained 2.0% protein and they established that amino acid of gum arabic is rich in hydroxyproline and serine while alanine content is low. (Anderson *et al.*, 1985) described gum arabic as a proteinaceous polysaccharide with a protein content ranging from 1.5 to 3.0%. And concluded that the variation was mainly due to different localities. They reported the value of 0.23-0.58% nitrogen for commercial formulations. Osman (1998) reported 0.33-0.36% nitrogen (2.14-2.16% protein for *Acacia senegal* gum) and (Jurasak *et al.*, 1993) in achemo metric study for different Acacia species reported 0.27-038% nitrogen for commercial samples of Gum arabic from Sudan. Awad Elkarium (1994) reported that the average nitrogen contents of different commercial grades are around 0.28%. (Karamalla *et al.*, 1998)reported that the average nitrogen content of differentcommercial grades is around 0.33%.

**1-1-16-7 Specific rotation:**

The optical activity of organic molecules (saccharides and carbohydrates) is related to their structure and a characteristic property of the substance (Stevens *et al.,* 1987). The gum of natural origin, e.g. *A. senegal* gum, has the property of rotating the plane of the polarized light. The direction of the rotation, as well as the magnitude is considered as a diagnostic parameter (Biswas *et al.,* 2000). *Acacia Senegal* gum gives a negative optical rotation ranging between -20°to -34°. The Specific optical rotation is used to differentiate between *A. senegal* and other botanically related *Acacia* gums.( Anderson and Stoddart, 1966b) reported that the specific rotation for electrodialysed *Acacia senegal* gum was -31, 5°. Pure gum from *A. senegal* has specific optical rotation of -27° to 30° (Tioback, 1922). Certain variation in the degree of the optical rotation (-27°to-32°) has been noticed by (Anderson, 1968).( Karamalla *et al.* 1998) found that the mean of the specific optical rotation of commercial *A. senegal* gum was -30.54°. The optical rotation is not affected by both auto hydrolysis and variation, while mild acidic hydrolysis has a significant effect on optical rotation (Barron, 1991).

**1-1-16-8 Acidity and pH measurements:**

Comparative studies Show that gum from *Acacia senegal* has higher content of rhamnose (12-14%) and lower arabinose content (24-29%) compared to rhamnose and arabinose of other *Acacias* (Karamalla *et al.*, 1998). The main content of gum arabic is arabian (acid substance), and when it was decomposed it gave arabinose (Mantell, 1965). Thus gum arabic is called arabic acid therefore, the gum solution is slightly acidic with pH 4.66, as reported by( Karamalla *et al.*, 1998).

**1-1-16-9 Viscosity:**

The viscosity of liquid is its resistance, to shearing, to stirring or flow through a capillary tube (Bancraft, 1932).

Studies of flow of gum solutions play an important role in identification and characterization of their molecular structure. Since viscosity involves the size and the shape of the macromolecule, it was considered as one of the most important analytical and commercial parameter (Anderson *et al.*, 1969). The viscosity of a solution may have a complicated variation with composition, due to the possibility of hydrogen bonding among the solute and solvent molecules (Pimentel e*t al.*, 1960). More hydroxyl groups makes high viscosities, because a network of hydrogen bonds is formed between the molecules, this net work extends throughout the liquid, thus making flow difficult. The viscosity of gum solutions is inversely proportional to temperature. They also found that the viscosity of gum Arabic solutions changes with pH, but they found a maximum viscosity at pH 6-7. Viscosity can be explained in different terms such as relative viscosity, specific viscosity, reduced viscosity, inherent viscosity and intrinsic; it is also represented as kinematics or dynamic viscosity.

**1-1-16-10 Intrinsic viscosity:**

It can be used to determine the molecular weight of *Acacia Senegal* gum (Anderson and Dea, 1971). Karamalla, (1999) showed that wide variations in values for intrinsic viscosity and viscosity average were obtained indicating that such parameters cannot be used as qualifying Indices.

**1-1-16-11 Equivalent weight and Uronic Acid:**

Titrable acidity represents the acid equivalent weight of the gum, from which the uronic acid content could be determined (Karamalla, 1965, Anderson *et al.,* 1983). (Karamalla *et al.,* 1998), assessed the potentials of new parameters such as equivalent weight and total uronic acid content as additional qualifying indices. They found that the mean values for gum of *Acacia senegal* for the equivalent weight was 1436 and for uronic acid was 13.71%.

**1-1-16-12 Molecular weight:**

The molecular weight of the polymers can be determined from physical measurement or by application of chemical methods. The applications of chemical methods require that the structure of the polymer should contain well known number of functional groups per molecule and they invariably occur as end groups. The end group analysis method gives an approximately number of molecules in a given weight of sample; they yield the average number of molecules for polymeric materials. This method becomes insensitive at high molecular weight, as the fraction of end groups becomes too small to be measured with precision (Meyer, 1971). This is due to the fact that fraudulent 14 sources of the end groups not considered in the assumed reaction mechanism steadily become consequential as the molecular weight increases and the number of end groups diminishes to such an extent their quantities determination is not feasible. Those reactions confine frequent application of chemical methods to condensation polymers with average molecular weight seldom exceeding $2.5 \times 10^3$ (Flory, 1953). Physical methods frequently used for establishing polymer molecular weight are osmometry, polymer viscosity, measurement of coefficient of diffusion, ultra centrifugation and light scattering. One of the most recent advanced methods is light scattering (LS), which provides an absolute method for polymer molecular weight and size measurement. LS are rapid, accurate and requires small amount of sample. The molecular weight of gums varies greatly in values due to gum heterogeneity as well as variation in techniques used to separate, purify and determine the molecular weight. A $3.0 \times 10^3$ was reported by( Saverbon 1953) using centrifugal method. Using the light scattering technique gave higher values (Veil and Eggenberger 1954) reported a Mw =1.0×106; (Mukherjee and Deb 1962) reported Mw up to 5.8 × 105 and( Fenyo ,1988) reported a range of 4.0 × 106 to 2.2 × 106. Recently GPC coupled

on line to multi angle laser light scattering (MALLS) has been demonstrated to be a very powerful method for characterizing highly polydisperse polymer systems and the molecular weight of *A. senegal* gum was found to be equivalent to 5.4 × 105 (Picton, 2000).

**1-16-13 The objectives of this study:**

1:preparation of glucuronic acid from *aciace sengnel* (gum Arabic).

2:preparation of magnesium glucuronate from *aciace sengnel* (gum Arabic).

3:preparation of anti acid  tablets from magnesium glucuronate.

# CHAPTER TWO
# MAERIALS AND METHODS

## 2- MAERIALS AND METHODS:

### 2-1 Chemicals and Materials :

Authenticated samples of *A sengnel var sengnel* were collected from *aciace sengnel var sengnel* trees and identified by experts from the Forestry Department of Ministry of Agriculture and Forestry, and Khartoum Gum Arabic Processing Company.

-sulphuric (2M).

-Barium chloride.

-Magnesium bicarbonate.

-Amberlite cation exchange resin.

-Distilled water.

- magnesium glucuronate.

- micro crystalline cellulose .

-,starch binder .

-sugar.

### 2-2Apparatus and Instruments:

-Beakers.

-Measuring cylinder.

-Weighing bottle.

-Sensitive balance.

-Hot air oven.

-Thermostaic water bath.

-PH meter.

- Polarimeter.

-Atomic Spectro photometer.

-Mortar and pestle.

## 2-3 Preparation of samples:

Gum nodules were dried at room temperature, and then hand cleaned by hand to insure freedom from sand, dust and bark impurities,

## 2-4 Analytical Methods:

The following analytical methods were adopted in this study:

### 2-4-1 Preparation of uronic acid in gum arabic:

Action exchange colnmn packed with Amberlite (IR-120H+)resin was thoroughly washed with 2.0 mol $dm^2$ $H_2SO_4$ by distilled water until the column was sulphate free .

Gum Arabic samples (85g in 500 ml ) were slowly passed down the column .

### 2-4-2 Moisture content:

Crucibles were dried in Hearus oven at 105C for 30 minutes, cooled in a desiccator and then weighed ($M_1$). About two grams of sample were placed in the crucible and weighed accurately ($M_2$). Contents were heated in an oven for 5 hours at 105C cooled in desiccator and re weighed ($M_3$). Loss percentage on drying was calculated as follows:

$$\frac{M2 - M3}{M2 - \mathrm{M}1} \mathrm{X}\ 100$$

Where: The determination was conducted according to AOAC (1990).

$M_1$: Weight of the empty crucible

$M_2$: Weight of crucible +sample

$M_3$: Weight of crucible +sample after drying

### 2-4-3 Total ash:

Total ash was determined according to AOAC (1990). Crucibles were heated in an oven for 30 minutes cooled in a desiccator and then weighed ($W_1$). About two grams of sample were placed in the crucible and accurately weighed ($W_2$), then ignited at 55C in a Heracus electronic muffle furnace for 6 hours, cooled in a desiccater and weighed ($W_3$).

Total ash% was calculated as follows:

$$\frac{W3 - W1}{W2 - W1} \text{ X } 100$$

Where:

$W_1$: Weight of the empty crucible

$W_2$: Weight of crucible +sample

$W_3$: Weight of crucible +sample after drying

### 2-4-4 Specific optical rotation:

1 g/100cm$^{-3}$ aqueous solution of the gum was prepared, the solution was filtered to be highly pure. Optical rotation was measured using a(1dm=10cm) tube filled with the test solution, at room temperature specific optical rotation was calculated(Omer et al., 2015).

$$\text{Specific rotation} = \frac{\alpha \times 100}{C \times L} \quad = \cdots \ldots \text{dm} - 1 \text{ mL g} - 1$$

**Where:**

α = observed optical rotation

C = concentration of the solution (g/ml)

L = length of the Polari meter tube(dm)

### 2-4-5 Total nitrogen and protein content(Kjeldal method):

0.5 gram of sample (in duplicate) was weighed and transferred to Kjeldahl digestion flasks and Kjeldahltablet(copper sulphate potassium sulphate catalyst) was added. 10 cm$^3$ concentratedsulphuric acid was added. The tube was then mounted in the digestion heating system which was previously set to $240^{O}C$ and capped with an aerated manifold. The solution was then heated at the above temperature until a clear pale yellowish-green color was observed which indicates the completion of the digestion. The tubes were then allowed to attain room temperature. Their contents were quantitatively transferred to kjeldahl distillation apparatus followed by addition of distilled water and 30% (w/v) sodium hydroxide. Steam was then started and the released ammonia was absorbed in 25 $cm^3$ of 2% boric acid. Back titration of the generated borate was then carried out versus, 0.02M, hydrochloric acid using methyl red as an indicator. Blank titration was carried in the same way.(Omer et.al. 2015)

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

**Where:**

$M_1$: mills of HCl that neutralized the sample distillate

$M_2$: mls of HCl that neutralized the blank distillate

M: molarity ofHCl titrate (0.02M).

S : Sample weight(0.5g).

Protein content was calculated using nitrogen conversion factor resulting from amino.

acid analysis as follows:

% protein = % N x 6.6

Degradation: Sample+ $H_2SO_4 \rightarrow (NH_4)_2SO_{4(aq)} + CO_{2(g)} + SO_{2(g)} + H_2O_{(g)}$

Liberation of ammonia: $(NH_4)_2SO_{4(aq)} + 2NaOH \rightarrow Na_2SO_{4(aq)} + 2H_2O + 2NH_{3(g)}$

Capture of ammonia:$B(OH)_3 + H_2O + NH_3 \rightarrow NH_4+ + B(OH)_4^-$

Back-titration: $B(OH)_3$ + $H_2O$ + $Na_2CO_3$ → $NaHCO_{3(aq)}$ + $NaB(OH)_{4(aq)}$ + $CO_2(g)$ + $H_2O$

**2-4-6 pH value:**

pH was determined in 10% aqueous solution using Ion Meter 3205 JENWAY. Two standard buffer solutions of pH 4.00 and 7.00 were used for the calibration of the pH meter. The temperature was kept at 25°C and the pH was let to stabilize for one minute and then the pH of gum formulations were read directly.

The readings were repeated three time for each formulation.

**2-2 preparation of magnesium glucuronate:**

160g of magnesium bi-carbonate were weighed and then was added to 500 ml of glucuronic acid in 500 ml in beaker and then transferred in a poetry dish and was dried for three days at room temperature and the was grinded .

$$4Co_2 + 2mgHCo_3 \rightarrow 2 \begin{matrix} Coo \\ Coo \end{matrix} > mg + H2o + Co2\downarrow$$

**2-2-1 Elemental Analysis:**

Accurately weighed 2 grams of dry sample were ignited to ash in muffle furnace at 550$^o$C for 4 hours. Ten ml of 50% HCl and 5 ml of 33% $HNO_3$ were added to each crucible then allowed to warm for one hour to dissolve the minerals and cooled, then 10 ml of HCl and 10ml distilled water were added and allowed to stand for 15-20 minutes. The mixture was filtered with ashless filter paper No.41mm and distilled water was added to complete the volume to 100 ml in a volumetric flask.

**2-2-2 Preparation of formulation of anti acid tablet by direct method:**

Active substance magnesium glucuronate and excipients micro crystalline cellulose, starch binder, suger were weighed

(0.55g,2.4g,1.6g,0.6), then sifiting through No.15 mesh to reject all large particles. Excipient were added to active substance expect lubricant (Mg striate) and mixed by using amixer or binder for 15 minates. Mg striate ( lubricant) was added weight(0.2g) and mixed for 5 minutes mixed powder were compressed into tablets by using tableting machine (ERWEKA) Germany.

# CHAPTER THREE:

# Results and discussion

**3- Results and discussion:**

**3-1 Results:**

**3-1-1 Physicochemical properties of gum sample:**

**Table (3 -1)**

| Moisture % | Ash % | pH | specific rotation | Nitrogen % |
|---|---|---|---|---|
| %12.1 | %3.2 | %4.3 | $-30.4^0$ | % 0.3 |

**3-1-2 Physicochemical properties of magnesium glucuronate:**

**Table (3 -2)**

| **Moisture %** | **Ash %** |
|---|---|
| % 0.14 | %0.2 |

**3-1-3 quantitative mineral analysis of *Acacia senegal* samples:**

**Table (3 -3)**

| | Mg | Ca |
|---|---|---|
| Sample $A_1$ | 920 ppm | 36300 ppm |
| Sample $A_2$ | 12620 ppm | 13740 ppm |

**3-2 Discussion:**

**Table (3.1)** :The moisture of The gum is (12.1%). The moisture content of good quality gum should not exceed 15 of and 10% for granular and spray dried material respectively (FAO, 1999). the value of ash content of commercial gum (3.2%) (Anderson *et al.*, 1983) found that the value of ash content of commercial gum arabic to be 4.4%. Later, (Anderson et al 1991) reported 3.6% Ash content for Sudanese formulations. FAO (1999) reported that the ash content of gum Arabic did not exceed more than 4%.

**pH** Value of *Acacia senegal* gum (4.3%) the gum solution is slightly acidic with pH 4.66, as reported by ( Karamalla *et al.,* 1998).

The value of nitrogen content of commercial samples of Acacia senegal gum (0.3%) (Siddig 1996) reported that the average value of nitrogen content of commercial samples of *Acacia senegal* gum and 80% of authenticated samples analyzed were in the range (0.27-0.39%). reported that the mean of nitrogen content of *A.* senegal gum samples was 0.35%.

The *Acacia senegal var senegal* has specific optical rotation of (-30.4$^{o}$). Certain variation in the degree of the optical rotation (-27°to-32°) has been noticed by (Anderson, 1968).( Karamalla *et al.* 1998) found that the mean of the specific optical rotation of commercial *A. senegal* gum was -30.54°. The optical rotation is not affected by both auto hydrolysis and variation, while mild acidic hydrolysis has a significant effect on optical rotation (Barron, 1991).

**Table (3 -3):** elements composition of the mineral calcium (36300 ppm) and magnesium (920 ppm) Sample $A_1$ and calcium (13740 ppm) and magnesium (12620 ppm) for Sample $A_2$ ( Anderson et al 1985) found that calcium (31600 – 5387), (Abdelranman 2008) magnesium and (2159 – 704) in *Acacia senegal*.

**Conclusion:**

- The physic-chemical proportion of the gum shoued that the gum was atypical *acacia senegal var senegal*.
- magnesium glucuronate can be prepared from arabic Acid and mg $(HCO_3)_2$.

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