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Characterization and functional properties of *Acacia ehernbergiana* gum from Sudan

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By

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بسم الله الرحمن الرحيم

إِقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (2)

اقْرَأْ وَرَبُّكَ الْأَكْرَمُ (3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الْإِنْسَانَ مَا لَمْ

يَعْلَمْ(5)

صدق الله العظيم
سورة العلق

Dedication

I dedicate this work to my parents and my uncle, the late, Fath Elrahman.

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praise to Allah , Almightily most Gracious and most merciful for giving me the health to complete this work, I extend my gratitude to my supervisor Dr. E.A. Hassan for this supervision and guidance . I also acknowledge Dr. Layaly Ibrahim Ali Elsigar, Botany Department, Faculty of Science and Technology, University of Shendi, and Abd Elhameed Faroug and the staff of the chemistry department, Sudan University of Science and technology for technical support.

ABSTRACT

Two samples of ***Acacia*** *ehrenbergiana* gum from Elnaga and Elshagalwa of Sudanese origin characterized for a number of physicochemical properties. Results show that average values of moisture and ash of the samples were found to be 6.69% and 3.4% respectively, while the average specific optical rotation was (+2.52). Rrefractive index range has 1.3388. Iintrinsic viscosity was 6.41ml/g, nitrogen content 0.382%. The calculated average protein content values using a conversion factor 6.6, was 2.388%. The Tannin content was 0.015%. Acid equivalent weight and glucuronic acid were 1191 and 16.27% respectively. The number average molecular weight was 6.9×10^5Dalton. Cationic content shows Ca, Mg, K, Fe, and Cu, were the major cations of this gum. The monosaccharide contents of Elnagaa were Arabinose 31.9%, Galactose 27.6% and Rhamnose 9.7%. Elshagalwa sample that showed that Arabinose capacity was 36.6%, Galactose 22.7 and Rhamnose 11.8%. The emulsifying capabilities of *Acacia ehrenbergiana* compare well with that of *Acacia Senegal var senegal* gum.

المستخلص

في هذه الدراسة تم توصيف صمغ السلم من أصل سوداني (تم جمع العينات من منطقتي الشقالوه والنقعة) بعدد من الطرق الكيموفيزيائية، وكان متوسط القيم التي تم الحصول عليها لكل من الرطوبة والرماد 6.69% و3.4% بالترتيب. كما وجد أن قيمة الدوران الضوئي النوعي في المتوسط بلغ (+2.52). بينما أعطى معامل انكسار1.3388.وكان متوسط قيمة اللزوجة الضمنية 6.41 بينما نتيجة تحليل النيتروجين بطريقة كلدال 0.382 وكانت محتوى قيمة البروتين والتي حسبت باستخدام معامل تحويل النيتروجين 6.6 في حدود 2.39 في المتوسط وقيمة التانين المتوسطة 00.015% وكان متوسط الوزن الحمضي ألمكافئي ونسبة حمض اليورونيك 1191و16.3بالترتيب وأعطي متوسط وزن جزئي $10^5 \times$ 6.9 كما أشارت الدراسة الى أن الكاتيونات الغالبة في العينة كانت هي Ca وMn وMg وK وFe وCu. وأشارت الدراسة إلى أن قيم كل من الاربينوز والجلاكتوز والرهامينوز علي التوالي كانت 31.9 و27.6 و9.7 بالنسبة لعينة النقعة بينما كانت 36.6 و22.7 و11.8 بالنسبة لعينة الشقالوة. أظهرت دراسة الخصائص الاستحلابية لصمغ السلم بأنه يضاهي صمغ الهشاب في قدرته علي الاستحلاب .

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CHAPTER ONE

Introduction and Literature review

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1. Introduction and literature review

1.1 General introduction

Although there are more than 1100 species of *Acacias* botanically known distributed throughout tropical and subtropical areas of the world, most commercial gum Arabic is derived from *Acacia senegal* locally known as hashab gum (in the Sudan) and as Kordofan gum in the world. Gum Arabic has been known for, many, thousands of years and there are no artificial substitutes that match it for quality or cost of production (Gabb, 1997). In the Sudan more than thirty distinct *Acacia* species found, most of them produce gum, but *Acacia senegal var. senegal* is the more predominant, that made Sudan the world's largest producer of gum, followed by Chad and Nigeria (Verbeken *et al*., 2003). The gum belt in the Sudan covers an area of 520,000 km^2across central Sudan and accounts for one fifth of the country total area (IIED and IES, 1990). *Acacia* gums are, polysaccharides, obtained from the stems and branches of various plant species from the genus *Acacia* as dried exudates (FAO, 1990). They find, wide, applications in the food and beverages industries as a natural emulsifier, particularly, for citrus oils (Egadu *et al*, 2007) they are, also, used in the pharmaceutical industry as a suspending agent and stabilizer (Fennema, 1996). Ancient Egyptians used it, largely, in paintings as an adhesive form ineral pigments.(Caris, 1939). They are either hydrophobic or hydrophilic in nature. Hydrophobic gums are insoluble in water and include resins such as olibanum gum. Whereas hydrophilic gums are soluble in water and can be subdivided into natural, semi synthetic and synthetic gums (Glicksman, 1973). Chemically, gum Arabic consists mainly of high-molecular weight polysaccharides made up of rhamnose, arabinose, and galactose, glucuronic and 4-o-methoxylglucuronic acid, and the salts of calcium, magnesium, potassium, and sodium of the two acids (Gabb, 1997).

1.2 *Acacia ehernbergiana*:

1.2.1 Scientific classification:

Kingdom:	Plantae
Division:	*Magnoliophyta*
Class:	*Magnoliopsida*
Order:	*Fabales*
Family:	*Fabaceae*
Genus:	*Acacia*
Species:	*Ehrenbergiana*

Figure1.1*: Acacia ehrenbergiana* tree

1.2.2 Botanical description

Salam trees *(Acacia ehrenbergiana)* are multi-stemmed and spreading from the base, 2-7 meter height. The growing young trees form a lower canopy. Slash green outside and red inside. Leaves small with 1-2 pairs of pinnate, each with 8-12 pairs of leaflets. Thorns are 4-6 cm long, with, straight, set out in axillaries pairs, longer than the neighboring leaves. Flowers globosely gold-yellow in 1.0-1.5 cm diameter heads. Pods are narrow to linear 7-10 cm long, more or less curved, falcate and twisted, bright-red when young. Seed is small 4-6 mm long, 2-3 mm width, it has splay shape and dark brown.

1.2.3 Distribution

A. ehrenbergiana is a much branched tall shrub or small tree (2-7 m tall), which grows in dry semi-desert areas on sandy and clay soil and on stony screes. *A. ehrenbergiana* is one of the most drought-tolerant among the common African *Acacias* occurring in the rainfall belts 50-400 mm. Its distribution differs from that of *A. seyal*, as the latter is a typical Sahelian and Sudanian species occurring in rainfall belts between 400 and 800 mm, on fine-textured soils only.

The species is also known to occur in many ecoregions (WWF Ecoregions), such as Sahara desert, Sahelian Acacia savanna, southwestern Arabian foothills savanna, Red Sea Nubo-Sindian tropical desert and semi-desert, Arabian Desert and East Sahero-Arabian xeric shrub lands and South Iran Nubo-Sindian desert and semi-desert (Halwagy, 1961).

1.2.4 The uses of the plant

The wood is used mainly in fuel and charcoal of good quality, fence posts, farm implements, and railway, sleeper, beams and; rafters. The gum is edible and is used as adhesive in the treatment of textile fibers. The roots are 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). It used traditionally to alleviate the swelling and pain associated with rheumatoid arthritis.

The leaves Ethanolic extract of the plant showed standard antibacterial for gram

positive and gram negative bacteria. Also it showed antifungal activity (Al-Mamary *et al.*, 2002).

1.3 Study objectives:

The objectives of this work are:

- To collect and authenticate sample of *Acacia ehrenbergiana* gum.
- To determine the physiochemical properties of *Acacia ehrenbergiana* gums (moisture content -water solubility, pH, relative density, intrensic viscosity, total ash, nitrogen content , protein content and total soluble fiber).
- To determine the cationic composition (Ca-Mg-Fe-Na-K-Mn-Cr-Zn-and pb) of *Acacia ehernbergiana* sample.
- To determine sugar composition of *Acacia ehernbergiana* samples.
- To determine the number average molecular weight(M_n) of *Acacia* sample.
- To compare the physico-chemical characteristic of *A. ehernbergiana* with other members of *gummifera* series.

1.4 Literature Review

1.4.1 Plant gums

Plant gums are organic substances obtained as an exudation from trunks, or branches of trees, spontaneously or after mechanical injury of the plant by incision of the bark, or by the removal of a branch, or after invasion by bacteria or fungi (Smith,1949).The term gum often describes materials which affect sense of touch, taste and sight in measure summed up as property of gummosis" which is difficult to define but visual and manual examination of the material may cause the observer, to call it gum (Mantell, 1947). Gum refers to any polysaccharide that is dispersible in water to give viscous solutions, gels or colloid dispersions. Generally gums are long chain high molecular weight polymers that dissolve or disperse in water to give thickening or gelling effect and exhibit related secondary functional properties, such as emulsification, stabilization, and encapsulation (Sharma, 1981). Gums or hydrocolloids are mainly long–chain, straight to branched polysaccharides that contain hydroxyl groups that can bond to water molecules. These chains consist of 2×10^3 to 1.084×10^3 monosaccharide units.

The sugar monomers can contain linked side unite, or substituent groups, such as sulphate, methyl, ether, ester and acetals (Kuntz, 1990). Gums composed mainly of C, H, O, and N elements; and acidic gums (eg. Gum Arabic) contain mainly Ca, Mg, Na and Fe as cations (Jones *et al.*, 1958).

1.4.2 Gum Arabic

Gum Arabic is the dried gummy exudates from the stems and branches of *Acacia Senegal* (L) Willd, or of other related species of *Acacia* (Family: *Leguminosae*) (Dondain and Phillips, 1999). It is defined by the FAO/WHO Joint Expert Committee for Food Additives (JECFA) as 'a dried exudation obtained from the stems of *A. senegal* or closely related species of *Acacia* (family *Leguminosae*)' (FAO/WHO. Compendium of food additives, 1999). Although, there are many species of *Acacia* trees botanically, only two species, namely *A. senegal* and *Acacia seyal* are acceptable to the Codex Alimentarius Commission (Al-Assaf *et al*., 2003; Dondain and Phillips, 1999; FAO/WHO. Compendium of

food additives, 1999). Gum Arabic has wide industrial uses as a stabilizer, thickening agent and emulsifier, mainly in the food industry (example, in soft drinks syrup, gummy candies and marshmallows), but also in the textile, pottery, lithography, cosmetics and pharmaceutical industries (Verbeken *et al*., 2003). It has been approved for use as food additives by the US Food and Drug Administration and is on the list of substances that is a generally recognized as safe (GRAS) with specific limitations (FDA Proposed affirmation of GRAS status for gum Arabic, 1974). In folk medicine, gum Arabic has been reported to be used for the treatment of inflammation of the intestinal mucosa, and externally to cover inflamed surfaces (Gamal el-din *et al*., 2003). It is an edible, dried, gummy exudates that is rich in non viscous soluble fiber (Williams and Phillips, 2000). Clinically, it has been tried in patients with chronic renal failure, and it was claimed that it helps reduce urea and creatinine plasma concentrations and reduces the need for dialysis from 3 to 2 times per week (Suliman *et al*., 2000). Despite the fact that gum Arabic is widely used as a vehicle for drugs in experimental physiological and pharmacological experiments, and is assumed to be an ‘‘inert” substance, some recent reports have claimed that it possesses anti-oxidant, nephroprotectant and other effects (Ali *et al*., 2008; Gamal el-din *et al*., 2003).

Pharmacologically, gum Arabic has been claimed to act as an anti-oxidant, and to protect against experimental hepatic, renal and cardiac toxicities in rats (Ali *et al*., 2009). Analysis of gum Arabic has indicated that it consists of three distinct components. Fraction 1, which represents 88.4% of the total, is an arabinogalactan with molecular mass 2.79 $\times 10^5$ and is deficient in protein. Fraction 2, which represents 10.4% of the total, is an arabinogalactan protein complex with a molecular mass of 1.45 $\times 10^6$, containing ~50% of the total protein. It is envisaged that on average each molecule of fraction 2 consists of five carbohydrate blocks of molecular mass ~2.8 $\times 10^5$ covalently linked through a chain of amino acid residues. Fraction 3 represents only 1.24% of the total gum but contains ~25% of the total protein and has been shown to consist of one or

more glycoproteins. Whereas the proteinaceous components of fractions 1 and 2 contain predominantly hydroxyproline and serine, this is not the case for fraction 3 (Randall *et al*., 1989).

Gum Arabic is a branched-chain, complex polysaccharide, slightly acidic, found as mixed calcium, magnesium and potassium salt of a polysaccharidic acid. The backbone is composed of 1,3-linked b-D-galactopyranosyl units. The side chains are composed of two to five 1,3-linked b D-galactopyranosyl units, joined to the main chain by 1,6-linkages. Only a few plant species are cultivated at present to obtain gums used in the food industry as additives; most of them belong to the *Leguminosae* family. Some examples are: *A. senegal*, source of *Acacia* or Arabic gum; *Astragalus* spp., source of tragacanth; *Cyamopsis tetragonolobus*, source of guar gum; *Ceratonia siliqua*, source of locust bean gum (Ibañez and Ferrero, 2003). The most commonly recognized is Arabic gum, but a wide range of other tree exudates is used for variety of uses in their countries of origin, such as mesquite gum (Anderson and Farquhar, 1982; Anderson, 1990; Vernon-Carter, *et al*., 2000; Williams and Phillips, 2000). *A. Senegal* trees grow widely across in Sahelian countries of Africa, especially in Sudan, and gum Arabic, as a food additive, has been an important item of commerce since ancient times (Glicksman, 1969). The gum belt in Sudan provides a natural buffer zone between the desert in the North and the more fertile agricultural lands in the South. Deforestation within the gum belt has lead to an increase in desert encroachment and threatens agricultural production (IEED and IES, 1990; Keddeman, 1994; Olsson and Ardö, 2002). Following the Sahel drought of the 1970s and 1980s a southward shifts in the tapping of gum has been reported (IEED and IES, 1990) as people moved from the more fragile environment in the northern parts of the gum belt to the less fragile and better environment of the south. Over the last three to four decades, the land use practices have moved from a rotation with long fallow periods (15 to 20 years) of gum cultivation interspersed with short period of cultivation (4 to 6 years) towards a more or less continuous cultivation (Barbier, 2000). Gum Arabic agriculture plays an important role as a cash crop produced in

the traditional rainfed areas of North Kordofan in western Sudan (El- Dukheri, 1997). Gum trees are managed in the Sudan in an agro forestry system known as the bush-fallow system (Obeid and Seif El Din, 1970). However, the recent disruption of this traditional agro forestry system due to the misuse of land, drought and desertification is considered to be among the main factors that have led to fluctuations in gum Arabic yield and the consequent instability of supply (Awouda, 2000; Seif El Din, 1995). It has been reported that rainfall and temperature have an effect on the time of tapping the tree and consequently on gum yield (Abdel, 1978; Awouda, 1973; Muthana, 1988). Apart from drought, desertification and mismanagement, gum arabic production also varies as a result of complex factors in the physical, biological and socio-economic environments. The impact of all or some of these factors on gum arabic production has been reported (Abdel Rahman, 2001). There is still an information gap regarding the factors that control gum Arabic yield. The International Institute for Environment and Development and the Institute of Environmental Studies (IIED and IES, 1989) reported that rainfall and its distribution pattern together with the minimum temperature during tapping and gum picking, and the relative humidity, are the main factors affecting gum Arabic yield.

However, the relationship between gum Arabic yield and rainfall is complex and the available information is sparse and imprecise (IIED and IES, 1989). Large-scale planting programs with the help of local communities have been implemented since the early 1980s to restock the gum Arabic belt in order to curb desertification and to improve the gum Arabic yield and production in western Sudan (Afaf *et al*., 2007). Gummosis is widespread in plant kingdom and is known to be produced by stress conditions such as heat, drought and wounding. Gums form a barrier at lesions hindering the invasion of microorganisms.

Fungal and bacterial infections have been linked with the synthesis process, although, this has by no means been proved (Ghosh and Purkayastha, 1962; Greenwood and Morey, 1979; Luckner, 1990).

1.4.2.1 Grading of gum Arabic

Grading is done to improve quality of the gum coming into the market. The practice, in principle, involves sorting gum nodules by hand according to their size and color. The method of grading varies among the major producing countries (Adamson and Bell, 1974). In Sudan for example, the main grades are:

"Natural" grade-consists of gum Arabic as it is picked from the tree with all associated impurities "Cleaned" grade -one where impurities like bark, twigs and other varieties of gums together with smaller fragments of dust have been removed.

"Cleaned and sifted" –as for cleaned grade but where smaller pieces of gum have also been removed. Handpicked selected grade -a special grade that consists of only better pieces of gum, essentially the larger pieces of uniform pale color. “Siftings and dust” the waste from other grades, particularly the cleaned and sifted grades.

The Gum Arabic exporting of Sudan has adopted only the cleaned, cleaned and sifted and handpicked selected grades for export. These grades are regarded internationally as model grades for both quality and price.

In Nigeria, the main grade of gum Arabic is called 'Falli' or 'kolkol'. It is of good appearance and quality comparable to the kordofan gum though inferior as it tends to produce a slightly dark color in solution. French speaking countries in West Africa appear to export their gum under more or less same conditions. Principal producers are Senegal and Chad and smaller quantities also come from Mali, Niger and Mauritania. About six grades are recognized (Admson and Bell, 1974).

- Gomme blanche-colourless and comparable to kordofan handpicked selected.
- Gomme petit blanche-small pieces of the same size.
- Gomme blonde darker color.
- Gomme petit blonde-small pieces of the same size.
- Gomme vermicelle -a whitish to pale yellow gum
- Gomme fabrique –rejected pieces of gum (because of their dark color).

1.4.2.2 Application of gums.

Exudates gums are used in many applications, mainly situated in the food area. However, there are also considerable non-food applications. Gum readily dissolves in cold and hot water in concentrations up to 50%. Because of the compact, branched structure and small hydrodynamic volume of its molecule, gum solutions are characterized by a low viscosity, allowing the use of high gum concentrations in various applications. Solutions exhibit Newtonian behavior at concentrations up to 40% and become pseudo plastic at higher concentrations. The pH of the solutions is normally around 4.5–5.5, but maximal viscosity is found at pH 6.0 (Verbeken *et al*., 2003). Gum Arabic has excellent emulsifying properties, particularly the AGP fraction. The hydrophobic polypeptide backbone strongly adsorbs at the oil–water interface, while the attached carbohydrate units stabilize the emulsion by steric and electrostatic repulsion.

Fractionation studies show that, although emulsifying properties generally improve with increasing molecular weight and protein content, the best results are obtained with mixtures of different fractions (Ray *et al*. 1995). Also, the heterogeneous nature of the gum makes it an excellent emulsifier (Buffo *et al*. 2001) found that stability of beverage emulsions is influenced by a number of processing factors, such as pasteurization and demineralization, and by the pH of the emulsion.

The use of gums in foods has to be in accordance with the FDA Code of federal regulations in USA. Gums are 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, where it produces a clarity that is higher than can be obtained with other hydrocolloids (Williams and Phillips 2000).

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).

1.4.2.3. Physicochemical properties of gums:

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.

1.4.2.3.1 Solubility

Gums can be classified into three categories with regard to their

Solubilities:

- Entirely soluble gums: e.g. *A. Senegal, A. seyal.*
- Partially soluble gums: e.g. *Gatti* gum.
- Insoluble gums: e.g. *Tragacanth* gum (Omer, 2004).

1.4.2.3.2 Color

The colors of gums vary from water- white (colorless) through shades of yellow to black. The best grades of gum are almost colorless with slight traces of yellow; some possess pink likes (Siddig, 2003).

1.4.2.3.3 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).

1.4.2.3.4 Moisture

The hardness of gum would be determined by moisture content. The moisture content of good quality gum does not exceed 15 and 10%for granular and spray dried material respectively (FAO, 1999).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.

Anderson *et al*., (1963) reported the moisture content of *A. seyal var. seyal* gum in the range from 11% to 16.1%. Randall *et al*., (1988) found that the moisture content of Kordofan *A. senegal var*. *senegal* 15.5%. Osman (1993) reported the moisture content of *A. senegal var. senegal* gum ranged between12% to 15%.Osman (1993)reported the moisture content of *A. senegal var*. *senegal* in the average of 13.0%.Karamallah *et al*., (1998) reported a mean value of moisture content for 803*A.senegal var. senegal* gum samples collected in season 1994/1995 was 10.75% and the range was 8.1% –14.05%.Also they reported the moisture content for authenticated *A. senegal var. senegal* gum samples collected in season 1995/1996, the minimum value was 9.15% and the maximum value was 14.3%. Moisture content for100 commercial samples of *A. senegal var. senegal* gum collected between 1992 and1996 in the same study had a mean value of 14.16%.The moisture content of *A. senegal var*. *senegal* gum samples collected from trees of various ages and different locations by Idris *et al* (1998) was found to be in the range of12.5%-16%.Karamallah (1999) reported a moisture content for *A. senegal var. senegal* and *A. seyal var. seyal* gum collected between 1960 and 1999 in Sudan, as 10.75% and 9.4% respectively. Hassan (2000) in the study of *A. seyal var. seyal* gum from different locations of Sudan reported an average of 8.5% moisture content.

Hassan *et al*., (2005) reported the moisture content of *A. seyal var. seyal* gum in the range from 7.4% to 8.3%. Siddig *et al*., (2005) reported the value of 12.6% for the moisture content of *A. seyal var. seyal* gum. Omer (2006) found that the moisture content of *A. senegal var. senegal* and *A. seyal var. seyal* gum were in the range of 11.76% to 14.8% and 5.66% to 11.11% respectively. Moisture

content in *A. senegal var. senegal* and *A. seyal var.seyal* gum was determined by Abdelrahman (2008) and it was found to be 11.01% and 11.07% respectively. Younes (2009) reported the mean value of moisture content for *A. senegal var. senegal* gum as 11.01% and the range was 9.91% – 14.72%, and the mean value of moisture content for *A. seyal var. seyal* gum was 10.10% and the range was 9.90% – 10.35%. Satti (2012) reported the mean value of moisture content for *A. nilotica var. nilotica* the range was 10.33% -10.81%.

1.4.2.3.5 Ash

Ash content is a measure of inorganic residue remaining after organic matter has been burnt. The inorganic residues exist as elements (Table 1-2). Anderson *et al*., (1963) reported the ash content of *A. seyal var. seyal* gum in the range from 1.94% to 3.55%. Anderson (1977) reported the ash content of *A. senegal var. senegal* and *A. seyal var. seyal* gum in the value of 2.87% and 3.93% respectively. The same author in (1991) in the final report of the safety assessment of *Acacia* gums reported a mean value of ash content 3.61% for *A. senegal var. senegal* samples provided by importers in 1990/1991. Juraseket*al* (1993) reported an ash content of *A. senegal var. senegal* 3.0%. Osman (1993) reported an ash content of *A. senegal var. senegal* in the average of3.6%. The mean value of ash content had been determined for 803 *A. senegal var. senegal* gum samples collected in season 1994/1995 by Karamallah *et al*.,(1998) and was found 3.77%.The same author reported value of 3.7% ash content for authenticated sample and 3.62% for commercial sample of *A. senegal var. senegal* gum. Again Karamallah (1999) in table 1-1, reported values of 3.7 and 2.3 ash content for *A. senegal var. senegal* and *A. seyal var. seyal* gum respectively collected between 1960 and 1999 in Sudan.

Hassan *et al*., (2005) in the study of *A. seyal var. seyal* gum from different locations of Sudan reported an average of 0.21% ash content. Omer (2006) reported the ash content of *A. senegal var. senegal* in the average of 3.27% and an average of 2.61% for *A. seyal var. seyal* gum. The mean value of ash content reported by Abdelrahman (2008) in *A. senegal var. senegal* and *A. seyal var.*

seyal gum in the average of 3.32% and 2.43% respectively. Younes (2009) reported the mean value of ash content for *A. senegal var. senegal* gum was 4.89% in the range of 4.0% – 5.23%, and the mean value of ash content for *A. seyal var. seyal* gum 4.47%. Satti (2012) reported the ash content in *A. nilotica var. nilotica* gum in mean value the range from 1.82% - 1.91%

Table 1.1: Analytical data of the gum exudates from *Gummifera Acacia* species of the Sudan (Karamalla, 1999)

Species	Moisture (%)	Ash (%)	Nitrogen (%)	Protein (%)	pH	Relative Viscosity	Sp.Rot (degree)
*A. sieberana*var.*sieberana*	5.30	1.90	0.35	02.19	3.95	1.36	+74.16
*A. sieberana*var.*vermesenii*	4.90	2.10	0.35	02.19	3.88	1.47	+77.16
A. nubica	4.60	0.03	0.35	02.19	3.50	0.50	+64.16
A. tortilissubsp.Raddiana	4.40	1.80	1.84	11.50	3.60	0.77	+71.33
A. tortilissubsp.Spirocarpa	6.40	2.03	1.40	07.50	3.85	0.76	+68.66
A. tortilissubsp.Tortilis	6.10	1.90	1.20	08.75	4.15	0.80	+69.00
A. drepandolobium	6.10	0.01	0.87	05.44	4.05	1.01	+75.83
A. grrardii	5.90	3.10	2.31	14.44	4.40	2.75	+48.50
A. ehrenbergiana	7.90	2.60	0.22	01.37	3.45	0.37	+5.66
A. niloticasubsp.Nilotica	6.10	0.03	0.06	00.37	4.10	0.69	+97.66
A. niloticasubsp.tomentosa	5.80	0.04	0.10	0.62	4.48	0.90	+80.16
A. niloticasubsp.Astringen	5.60	0.06	0.06	00.37	3.75	0.68	+75.16
*A. seyal*var.*seyal*	7.20	2.30	0.10	00.63	4.35	1.28	+50.50

Table 1. 2: chemical analysis of some African *Acacia species* gums

Species	Ash %	N%	$\propto_D^{25}$	[η]	$M_w \times 10^6$	A.E.W	Uronic acid%	References
A.ehrenbergiana	3,10	0.09	-0.7	07.00	1060	1060	17.00	Anderson et al.,(1984)
A.hokii	1,30	0.23	+91	13.00	521	521	34.00	Anderson et al.,(1984)
A.karoo	0.56	0.13	+54	Nd	Nd	Nd	12.00	Anderson et al.,(1984)
A.kirbii	1.40	0.09	+54	08.00	1817	1817	09.70	Anderson &Farquhan (1979)
A.nilotica	2.48	0.02	+108	09.50	1890	1890	09.00	Anderson (1976)
A.nubica	1.54	0.20	+98	09.80	3030	3030	07.00	Anderson (1976)
A.rubusta	Nd	2.80	+36	Ns	1660	1660	09.00	Crmuns &Stephen (1984)
A.sieberana	1.50	0.19	+103	12.00	1230	1230	04.00	Anderson et al.,(1974)
A.acatechii	0.28	Nd	-30	Nd	Nd	Nd	03.30	Aganwwal & Soni (1088)
A.erubescens	3.90	1.06	-13	08.00	874	874	20.10	Anderson&Farquhan(1979)
A.fleckii	4.00	0.58	-32	13.00	918	918	19,20	Anderson&Farquhan(1979)
A.laeta	Nd	0.56	-42	20.70	1250	1250	14.00	Anderson (1976_1977)
A.mellifera	2.90	1.45	-56	23.50	843	843	20.90	Anderson&Farquhan(1979)
A.polyacantha	Nd	0.37	-12	15.80	2020	2020	09.00	Anderson (1986)

Table 1.3: cationic composition of some samples of *A. Senegal* and *A. Seyal*

Species	Mg	Ca	K	Na	Zn	Cu	Fe	Mn	Pb	References
Senegal	24000	20600	1600	8400	9.0	32	54	3	0	Anderson et al., 1984[a]
Senegal	39000	316000	221000	10200	40	66	110	57	11	Anderson et al., 1989 [b]
Senegal	38000	25600o	237000	940	24	52	128	106	6.0	Anderson et al.,1990 [f]
Senegal	1345-1937	5387-6314	6664-7735	3.9-12	0.2-0.4	1.1-1.5	2.5-6.9	2.4-8.8	0.84	Buffo et al 2001 [c]
Senegal	1009	6797	8057.9		23.96					Omer 2006 [f]
Senegal	2159.704	70922	9459.459	67. 1296	20.5125		37.0370		7.5757	Abdelrahman 2008 [f]
Senegal	267	6490	261	266						Younes 2009 [f]
Seyal	11.7	11200	7900	5,49	620	130		750		Siddig 2003 [d]
Seyal	27	7000	101100	9.67	13	51	190	200		Siddig 2003 [e]
Seyal	761	9824	2683	505.3		18.82	4339			Omer2006 [f]
Seyal	1229.0424	9417.20	2802.803	111.054	7.8632		43.9815			Abdelrahman 2008 [f]
Seyal	419	7370	380	195						Younes2009 [f]

A,b,d,e cited in YOUNES (2009), c ceied in Abd Elrahman (2008) and f cited in Amira (2011

1.4.2.3.6. Nitrogen and protein content

The role of nitrogen and nitrogenous component in the structure, physicochemical properties and functionality of gum Arabic was recently subjected to intensive investigation (Anderson. 1985; Gammon *et al*., 1968). Dickinson (1988) studied the emulsifying behavior of gum arabic and concluded that there is strong correlation between the proportion of protein in the gum and it is emulsifying stability. Idris (1989) showed that the protein contents of fresh samples were fairly constant (2%) irrespective of the age of the tree.

Anderson *et al*., (1963) reported that nitrogen content of *A. seyal var. seyal* gum ranged from 0.09 – 0.19% w/w. Nitrogen content of *A. senegal var. senegal* gum had been determined by Anderson (1977) and was found to be 0.29% and for *A. seyal var. seyal* 0.14%. Jurasek *et al*., (1993) reported 0.28 to 0.35% nitrogen content for *A. senegal var. senegal* samples and 0.14% for *A. seyal.* Osman (1993) reported that nitrogen content for the *A. Senegal var. senegal* gum to be 0.31% and protein content 2.4%. Idris *et al*., (1998) studied the nitrogen content of *A. senegal var. senegal* from trees of different ages and different locations and they found the range between 0.22- 0.39%, hence protein content ranged between1.5-2.6%. Karamallah *et al*., (1998) reported the mean value of nitrogen content for 642 *A. senegal var. senegal* gum samples collected in season 1994/1995 as 0.33% .Also they reported the mean value of nitrogen content for authentic *A. senegal var. senegal* gum samples collected in season 1995/1996 as 0.3%. Nitrogen content for 100 commercial samples of *A. senegal var. senegal* gum collected between 1992 and 1996 in the same study had a mean value of 0.32%.Karamallah (1999) reported nitrogen content in comparative analytical data for *A. senegal var. senegal* and *A. seyal var. seyal* gums collected between 1960–1999 in Sudan to be 0.33% for *A. senegal var. senegal* gum, and 0.11% for *A. seyal var. seyal* gum. Hassan *et al*., (2005) reported protein content of *A. seyal var. seyal* had a mean value of 0.96%. The nitrogen content of *A. seyal var. seyal* gum had been determined by Siddig *et al*., (2005), it was found to be 0.15% and

hence protein content found to be 1.0%. Omer (2006) determined the nitrogen content for samples of *A. senegal var. senegal* and *A. seyal var. seyal* from different locations, the values were 0.35% and 0.14% for *A. senegal var. senegal* and *A. seyal var. seyal* respectively, whereas protein content had a value of 2.3% and0.93 respectively. Abdelrahman (2008) reported the average value of nitrogen content of *A. senegal var. senegal* gum 0.37% whereas equal to 0.14% for *A. seyal var. seyal* gum, he found that protein content of *A. senegal var. senegal* gum 2.4%and equal to 0.95% for *A. seyal var. seyal* gum. Recent study by Younes (2009)determined nitrogen content for *A. senegal var. senegal* 0.35% and protein content2.3%, for *A. seyal var. seyal* the author reported nitrogen content 0.22% and protein content 1.4% . Satti (2012) reported the mean value of nitrogen content and protein content for *A. nilotica var. nilotica* were found 0.02% and 0.16% respectively.

1.4.2.3.7 Cationic composition

The most four abundant cationic elements present in gum are calcium, potassium, magnesium, and sodium. It had been cited in the final report of the safety assessment of different *Acacia* gum. United States Pharmacopoeia reported the specifications grade of *Acacia* has arsenic (3ppm), lead (0.001%) and heavy metals (0.004%). The specifications for food grade *Acacia* include arsenic (3mg/kg maximum), heavy metals (0.002%maximum) and lead (5mg/kg maximum) had been cited in the same report.

1.4.2.3.8 Tannin content

One of the most important tests that can be used to identify *A. senegal* and distinguish it from other *Acacia* gum is absence of tannins. A study had been done by Zahir (1998, cited by Karamallah, 1999) on raw gums from different *Acacia* species of Sudan-for their taxonomic classification, showed that these *Acacia* species could be divided into two main groups. Out of the thirteen gums tested, all but one fell into one group. The species falling in the large group showed presence of tannins in their gums. The tannin content ranged between 0.03 to 1.63%. The only gum that did not show presence of tannin was the gum from *A.*

senegal, thus distinguishing itself distinctly and distantly from other *Acacia* gums. This finding was of significant importance when considering gums as food additives. It was established that tannins are anti-nutritional (Karamallah, 1999).

1.4.2.3.9. Specific optical 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).

Anderson *et al*., (1963) reported the specific optical rotation of *A. seyal var. seyal* gum in the range from +44.0 to +56.0.Anderson (1977) reported a value of -30.0 specific optical rotation for *A .senegal var. senegal* and +51.0 for *A. seyal var. seyal* gum. Vavdevelde and Fenyo (1985) reported specific optical rotation of *A. senegal var. senegal* to be ranging between -29.0 to -34.40. Anderson (1991) reported the mean value of specific optical rotation -30.50 for *A.senegal var. senegal* samples provided by importers in 1990/1991. Jurasek *et al*, (1993) reported a range of -20.0 to -32.0 for *A. senegal var. senegal*, and a value of +51 for *A.seyal var. seyal* gum. Osman (1993) reported specific optical rotation of *A. senegal var. senegal* to be ranging between -29.0 to -31.0. Karamallah *et al*.,(1998) reported the specific optical rotation for the 789 authentic *A. senegal var. senegal* gum samples, between-26.0to-34.0.Specific optical rotation of *A. senegal var. senegal* gum samples collected from trees of various ages and different locations by Idris *et al*., (1998) was found to be in the range of -27.0 to –36.0. Karamallah (1999) reported -30.30 specific rotation for *A. senegal var. Senegal* and +50.60 for *A. seyal var. seyal* gum. Hassan (2000) reported that *A. seyal var. seyal* gum exhibit dextrorotatory optical rotation ranging from +40.0 to +62.0.Hassan (2005) reported +53.0 mean value of specific optical rotation of *A. seyal var. seyal* gum. Optical rotation of *A. seyal var. seyal* gum had been determined by Siddig *et al*., (2005) and found to be+45.0. Omer (2006) reported that an average values of specific optical rotation equal to -32.0, and +49.40 for *A.*

senegal var. senegal and *A. seyal var.seyal* respectively. Abdelrahman (2008) reported an average optical rotation of *A. senegal var. senegal* gum as -31.50 and +61.0 for *A. seyal var. seyal* gum. Younes (2009) reported a value of -30.0 specific rotation for *A. senegal var. senegal* and +52.0 for *A. seyal var. seyal* gum. Satti (2012) reported a mean value of specific optical rotation for *A. nilotica var. nilotica* the range was found +90.92 - +99.17.

1.4.2.3.10Viscosity

The viscosity of liquid is its resistance to shearing, to stirring or to 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). Viscosities increases due to formation of a network of hydrogen bonds are form between molecules. This net work extends throughout the liquid, thus making flow difficult. Viscosity of gum solutions is inversely proportional to temperature. They also found that the viscosity of gum Arabic solutions changes with pH, with a maxima 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. Anderson (1977) reported a value of 13.4 $cm^3 g^{-1}$ intrinsic viscosity for *A. senegal var*. s*enegal* and 12.4$cm^3 g^{-1}$for *A. seyal var. seyal* gum. Duvallet *et al*., (1989) reported that the intrinsic viscosity of *A. senegal var. senegal* had a value of 21.8$cm^3 g^{-1}$.Jurasek *et al*., (1993) found that the intrinsic viscosity ranged between 13.4-23.1$cm^3 g^{-1}$for *A. sengal var. senegal* and equal to 12.4$cm^3 g^{-1}$for *A. seyal var. seyal*. Idris *et al*., (1998) studied the intrinsic viscosity of *A .senegal var. senegal* from trees of different ages and different locations and concluded that intrinsic viscosity varies with age of the trees but no affect was seen from trees in different locations. They found that intrinsic viscosity of *A. Senegal var. senegal* was in the range from10.4 to19.8$cm^3 g^{-1}$.Karamallh *et al*., (1998) reported that the mean value of intrinsic

viscosity of 1500 samples of *A .senegal var. senegal* was 16.44$cm^3 g^{-1}$.Also Karamallh, (1999) reported an intrinsic viscosity of 16.6$cm^3 g^{-1}$for *A. senegal var. senegal* and 11.0$cm^3 g^{-1}$for *A. seyal var. seyal*. Hassan *et al*., (2005) reported that the intrinsic viscosity of *A. seyal var. seyal* in the ranges between 11.9–17.6$cm^3 g^{-1}$. Flindt *et al*., (2005), reported an intrinsic viscosity of *A. seyal var. seyal* in the range from 11.6 to 17.7$cm^3 g^{-1}$.Al-Assaf *et al*., (2005) reported the intrinsic viscosity of sixty seven samples of *A. senegal var. senegal* in the range 9.7-26.5$cm^3 g^{-1}$.The intrinsic viscosity of *A. seyal var. seyal* gum had been determined by Siddig *et al*., (2005), it was found to be 14$cm^3 g^{-1\cdot}$ Omer (2006) found that an average values of intrinsic viscosityequal to 14.6$cm^3 g^{-1}$,and 11.4$cm^3 g^{-1}$for *A. Senegal var. senegal* and *A. seyal var. seyal* respectively. Abdelrahman (2008) reported the average value of intrinsic viscosity of *A. senegal var. senegal* gum 15.4$cm^3 g^{-1}$ and 11.6$cm^3 g^{-1}$for *A. seyal var. seyal* gum. The intrinsic viscosity had been determined by Elmanan *et al*., (2008), they reported that the intrinsic viscosity ranged between 14.7 to 17.3$cm^3 g^{-1}$ for *A.senegal var. Senegal* and between 14.6to 14.9$cm^3 g^{-1}$ for *A. seyal var. seyal*. Younes (2009) reported a value of18.9$cm^3 g^{-1}$intrinsic viscosity for *A. senegal var. senegal* and 15.5$cm^3 g^{-1}$ for *A. seyal var. seyal* gum. Satti (2012) reported the mean value of intrinsic viscosity for *A. nilotica var. nilotica* the range of 10.19 to 10.56$cm^3 g^{-1}$.

1.4.2.3.11Acidity and pH measurements

The hydrogen ion concentration is very important in chemistry and industry of gums, therefore functional properties of gum are affected by changes in pH e.g. viscosity, emulsifying power. Arabic acid substance is the major component of commercial gum Arabic and when decomposed, it gives arabinose, so that the gum Arabic is called Arabic acid and hence the gum solution is moderately acidic (pH= 4.5). Karamallah *et al*., (1998) reported the pH mean value of 4.66 for the 755 authentic *A. senegal var. senegal* gum samples, collected in season 1994/1995. The same author in the same study reported the mean value of 4.54

for commercial samples collected between 1992 and1996, also they reported an average value of 4.4 for *A. senegal var. senegal* gum samples, collected between1960 and 1995. Karamallah (1999) reported 4.66 pH values for *A. Senegal var. senegal* and 4.2 for *A. seyal var. seyal* gum. The pH value had been determined by Younes (2009), he reported a value of 4.78 for *A. Senegal var. senegal* and 5.16 for *A. seyal var. seyal* gum. Satti (2012) reported the mean value of pH for *A. nilotica var. nilotica* the range 5.15 to 5.24.

1.4.2.3.12 Acid equivalent weight and uronic acid anhydride

Titrable acidity, which is the number of ml of 0.02N sodium hydroxide that neutralize 10 ml of 3% gum solution, represents the acid equivalent weight of gum, from which the uronic acid content can be determined (Karamalla, 1965; Jurasick, 1993). Uronic acids constitute major component of many natural polysaccharides. They are widely, distributed in animal and plant tissues. A number of methods have beenb developed for determination of uronic acids. They include colorimetric, decarboxylation and acid base titimetric methods. Gums differ widely in their equivalent weight and uronic acid content (Karamalla, 1965).Anderson *et al*., (1963) reported that the uronic anhydride of *A. seyal var. seyal* sample after electro dialysis was found to be in the range between 12.1 – 16.8%,while the crude gum in the range between 9.0–16.4%. Anderson (1977) reported value of 16% uronic acid for *A. senegal var. senegal* and 12% for *A. seyal* gum. Jurasek *et al*., (1993) reported uronic acid for *A. senegal var. senegal* was found to be in the range between 12-28.3% and for *A. seyal var. seyal* 6.5%. Hence acid equivalent weight found to be in the range between 1430-1125 and for *A. seyal var. seyal* 1470.Osman *et al*., (1993) reported a value of 21% uronic acid for *A. senegal var. senegal*. Karamallah *et al*., (1998) reported the mean value of uronic acid for 115 *A. senegal var. senegal* gum samples collected in season1994/1995 as 13.7% and a mean value of 1436 acid equivalent weight. Idris *etal*., (1998) found the uronic acid of *A. senegal var. senegal* from trees of different ages and different locations in the range of 15-16%, hence acid equivalent weight ranged between1118-1238. Karamalla(1999) calculated that the

glucuronic acid percentage for *A. senegal var. senegal* gum in the range 16-17%. While for *A. seyal var. seyal* gum was in the range of 11-12%. Hassan *et al*., (2005) study seventy four authenticated different samples of *A. seyal var. seyal* from different location by using acid–base titrimetric method; he reported the mean value of equivalent weight 1489 and the uronic acid 11.9%. Siddig *et al*., (2005) reported uronic acid for *A. seyal var. seyal* 16%. Omer (2006) reported that the acid equivalent weight was to be 1161 in average, and glucouronic acid was to be15.2% in average for *A. senegal var. senegal*, whereas acid equivalent weight was to be 1107.9 in average and glucouronic acid was 15.9% in average for *A. seyal var. seyal*. Abdelrahman (2008) reported a value of 16.8% uronic acid of *A. senegal var. senegal* gum and 1153.8 acid equivalent weight. He found the value of uronic acid of *A. seyal var. seyal* 16.4% and 1185.8 acid equivalent weight value. Acid equivalent weight and uronic acid had been determined by Younes (2009), he reported the mean value of acid equivalent weight of 1620 and uronic acid of 11.89% for *A. senegal var. senegal* gum, and also he reported a value of 1180 acid equivalent weight and 16.34% uronic acid form *A.seyal var. seyal.* Satti (2012) reported the mean value of acid equivalent weight and uronic acid for *A. nilotica var. nilotica* the range were found 1904.48% -1910.61% and 10.17% - 10.20% respectively.

1.4.2.3.13 Molecular weight

The molecular weight of 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 approximate 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). Saverborn (1944) using ultra centrifugation method reported values of molecular weight in the range of 2.56 x 10^5- 3.26 x 10^5 g/mol for *A. senegal var.*

senegal. Mukherjee *et al*., (1962) reported molecular weight values of 2 x10^5 to 11.6 x10^5 for *A. senegal var. senegal* gum. Anderson *et al*., (1966) reported a value of 5 x10^5 and 8.5 x10^5 for the number average molecular weight (M_n) using molecular sieve chromatography and for weight average molecular weight (Mw)using light scattering technique for *A. senegal var. senegal* gum.

Using the same method Anderson *et al*., (1969) reported the value of 5.8 x10^5 For *A.senegal var. senegal* and this value were very close to the value reported by Churms *et al*., (1983). They reported a value of 5.6 x10^5 using steering exclusion Chromatography. Anderson *et al*., (1969) estimated the weight average molecular weight for *A. seyal var. seyal* and the value was 8.5 x10^5. Vandeveldeand Fenyo (1985) reported that *A. senegal var. senegal* has weight average molecular weight in the range 2.5 x10^5 to 1 x 10^6 g/mol. Connolly *et al*., (1988)calculated the molecular mass of the blocks of *A. senegal var. senegal* gum and found it to be of the order 2x10^5.Duvallet *et al*., (1989) reported value of 7.2x10^5 for molecular weight of *A. senegal* gum using low angle laser light scattering technique in IM NaCl at25.00C, they also obtained the value of 1.9 x10^5 for the number average molecular weight (Mn) using osmometry in 0.01M NaCl at37.00C. Randall *et al*., (1989) reported the value of 9.0 x10^5 for *A. Senegal var. senegal* gum using hydrophobic affinity chromatography (HAC). Using GPC-MALLS, Idris *et al*., (1998) obtained values between 2 x10^5 and 7.9 x$10^5$of the weight average molecular weight and values between 1.6 x10^5 and4.5 x10^5 of the number average molecular weight (Mn) for *A. Senegal var. senegal* gum.

Hassan *et al*., (2005) obtained the molecular weight of *A. seyal var. seyal* from the light scattering measurement using multi angle laser light scattering system. The value of Mw, Mn and Mz were found to be 1.94 x10^6, 1.08x10^5 and 1.11x10^6 respectively.

Al-Assaf *et al*., (2005) reported a value of 5.99 x10^5 for the weight average molecular weight using GPC-MALLS of *A. senegal var. senegal* and a value of10.4 x10^5 for *A. seyal var. seyal* (Abdelrahman 2008) estimated the molecular weight using GPC-MALLS technique. The values of Mw and Mn of *A. seyal var.*

seyal were found to be 15.5 x10^5 and 5.16 x10^5. For *A. senegal var. Senegal* were found to be 8.64 x10^5 and 2.86 x10^5. He also determined the Mn using osmotic pressure technique and it was found to be 4.7x10^5 and 2.4x10^5 for *A. seyal var. seyal, A. senegal var. senegal* respectively. Younes (2009) obtained the weight average molecular weights of *A. senegal var. senegal* and *A. seyal var. seyal* samples; it was ranged from 8.08 x10^5 to 1.34 x10^6 for *A. senegal var. senegal* and ranged from 6.40 x10^5 to 1.90 x10^6 for *A. seyal var. seyal*. For *A .nilotica var. nilotica* gum Anderson *et al*., (1969) reported a value of2.27 x10^6 g/mol, and a value of 6.74 x10^5 was reported by Al-Assaf *et al*.,(2005).

1.4.2.3.14 Number average molecular weight

An important group of absolute methods allowing the determination of the molecular weight of macromolecules is based on the measurement of colligative properties. Here, the activity of the solvent is measured in a polymer solution via determination of the osmotic pressure πos. The value of πos required to determine the number-average molecular weight can be obtained using a membrane Osmometer. Here, in a measuring cell having two chambers separated by a semi permeable membrane, one chamber contains the pure solvent and the second one the polymer solution in the same solvent (a membrane is called semi permeable if only the solvent can pass through but not the polymer molecules). Due to the lower activity (lower chemical potential) of the solvent in the polymer solution as compared to the pure solvent, solvent molecules migrate through the membrane from the solvent chamber into that of the polymer solution and dilute it. Therefore, the volume of the polymer solution increases until an equilibration is reached between the osmotic pressure πos and the hydrostatic pressure generated by the diluted polymer solution

$\bar{\Lambda}_{os} = \sigma g \Delta h$

Where σ is the density of the solvent and *g* is the acceleration of gravity.

Following Van't Hoff, it is

$\bar{\Lambda}_{os} V = n R t$

For diluted solutions, with *V* being the volume of the polymer solution and *n* the

number of moles of the dissolved polymer. Since $n = m/Mn$ (*m* is the mass (in g) of dissolved polymer) and $c = m/V$ it follows that:

$$\bar{\Lambda}os = \frac{mRT}{VM_n} = \frac{c\,RT}{M_n}$$

Since van's Hoff's law is valid only for infinitely diluted solutions, one develop sπos/c in power law series (break after the linear term in c)

$$\frac{\bar{\Lambda}os}{c} = \frac{RT}{M_n} + A_2.c$$

Thus, the osmotic pressure is first measured at different polymer concentrations, πos/c is then plotted vs. c, the values are linearly extrapolated to c $\rightarrow$ 0, and the value of *Mn* is determined from the *y* axis intercept. A_2 is the second virial coefficient of the osmotic pressure. Solvents where $A_2 = 0$ are called "ideal" solvents. For membrane osmometry (as well as for all other techniques of molecular weight determination via colligative properties) it is very important that the samples to be analyzed are very pure. In particular low-molecular-weight impurities have to be removed reliably. Otherwise, they will migrate through the semi permeable membrane and lower the chemical potential of the solvent in the reference chamber. An overestimation of the molecular weight will follow. The same effect applies when there are very small oligomers in the test sample. Therefore, the lower limit of M for application of membrane osmometry is approx. 10.000 ~ depending on the available membrane pore size. On the other hand, M should be below approx.50.000 because of the limited sensitivity of this method. Moreover, complete dissolution and absence of aggregates is required for reliable measurements.

Table1.4: Analytical data of the gum exudates from *A. seyal var. seyal* and *A.nilotica var. nilotica*

	Physic-chemical characteristic										
Species	Moisture	Ash	Nitrogen	protein	pecific rotation	Viscosity	PH	E.W.a	Uronic	M_n	Reference
A. Seyal var. seyal	11-16.1	1.94-3.55	0.09-1.9	-	44-56	-	-	-	12.-17		Anderson *et al*., (1963)
A. Seyal var. seyal	9.4	2.3	o.11	-	50.6	11	4.2	1744.6	11.12		Karamallah(1999)
A. Seyal var. seyal	8.5	0.21	-	0.96	40-62	11.9-17	-	1489	11.9		Hassan(2000)
A. Seyal var. seyal	7.4-8.3	0.21		0.96	53	11.9-17	-	1489	11.9		Hassan *et al.*, (2005)
A. Seyal var. seyal	12.6	-	0.15	1	45	14	-	-	16		Siddig *et al.*, (2005)
A. Seyal var. seyal	5.66-11.11	2.61	0.14	0.93	49.4	11.4	-	1107.9	15.9		Omer(2006)
A. Seyal var. seyal	11.07	2.93	0.14	0.95	61	11.6	-	1185.8	16.4	4.7×10^5	Abdelrahman (2008)
A. Seyal var. seyal	10.10	4.47	0.22	1.4	52	15.5	5.16	1180	16.34		Younes(2009)
A. nilotica var. niolotica	10.33-10.81	1.82-1.92	0.2	0.16	90.92-99.17	10.19-10.56	5.15-5.24	1904.48-1910.61	10.17-10.02	$(1.06\text{-}3.35) \times 10^7$	Satti (2012)

1.4.2.3.15 Sugar composition

Monosaccharide composition of gum is determined by acid hydrolysis of the gum where complete hydrolysis yields four basic sugar constituents, D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid. Anderson (1977) reported that sugar content of *A. senegal var. senegal* was 41%galactose, 27% arabinose, 14% rhamnose and 14.5% glucuronic acid. Jurasek *et al*., (1993) reported sugar composition as 34-46% galactose, 23-35% arabinose and 9-16% rhamnose for *A. senega var. senegal*, and 38% galactose, 46%arabinose and 4% rhamnose for *A. seyal var. seyal*. The sugar content of *A. Senegal var.senegal* studied by Osman *et al*., (1993), they reported the value of 35%galactose, 27% arabinose, 14% rhamnose and 21% glucuronic acid.

Idris *et al*., (1998) studied the sugar composition of *A. senegal var. Senegal* samples collected from trees of various ages and different locations. They found that the sugar cotent values were in the rang 39-42% galactose, 24-27% arabinose and 12-16%rhamnose. Karamallah (1999) reported comparative analytical data for *A. Senegal var. senegal* and *A. seyal var. seyal* gums collected between 1960 and 1999 in Sudan, he reported sugar content had a value of 36-42% galactose, 24-29% arabinose,12-14%rhamnose and16-17% glucuronic acid for *A. senegal var. senegal*, whereas he reported value of 37-38% galactose, 41-45% arabinose, 3-4% rhamnose and 11-12% glucuronic acid for *A. seyal var. seyal*. Islam *et al*., (1997) and Williams *et al*., (2000) reported the sugar content of *A. senegal var. senegal* 44% galactose, 27% arabinose, 12% rhamnose and 14.5% glucuronic acid. Also they reported the sugar content of *A. seyal var. seyal* as 38%galactose, 46% arabinose, 4% rhamnose and 6.5% glucuronic acid. Flindt *et al*.,(2005) reported the sugar content of *A. seyal var. seyal* (table1.5) 34.9% galactose, 26.5%arabinose,11.5% rhamnose and 11.6% glucuronic acid. Siddig *et al*., (2005)reported the sugar content of *A. seyal var. seyal* 36% galactose, 44% arabinose,3% rhamnose and 16% glucuronic acid. The average values of sugar content determined by Abdelrahman (2008) of *A. senegal var. senegal* 29.7%

galactose, 21% % rhamnose and 10.1% rhamnose. He also found the sugar content of *A. seyal var. seyal* as 28.8% galactose, 34% arabinose and 1.6% rhamnose. Also Satti (2002) reported suger content value of 10.6% rhamnose, 41.2% arabinose and17.4% galactose.

Table1. 5 : Constituent sugar analysis of *A. seyal var. seyal* gum exudates and *A. nilotica var. nilotica*

Species	**% rhamnose**	**arabinose %**	**galactose %**	**References**
A.Seyal var. seyal	4	46	38	Jurasek *et al*., (1993)
A.Seyal var. seyal	3-4	41-45	37-38	Karamallah (1999)
A.Seyal var. seyal	4	46	38	Williams *et al.*, (2000)
A.Seyal var. seyal	11.5	26.5	34.9	Flind *et al*., (2005)
A.Seyal var. seyal	3	44	36	Siddig *et al*., (2005)
A.Seyal var. seyal	1.6	34	28.8	Abdelrahman (2008)
A. nilotica var. niolotica	10.6	41.2	17.43	Satti (2012)

1.4.2.3.16 Amino acid composition

The most abundant amino acids of gum Arabic are hydroxyl proline and serine (Qi *et al*.,1991, Osman *et al*.,1995). Randall *et al*.,(1989) fractionated gum using hydro phobic affinity chromatography, they found that while hydroxyl proline and serine were the major amino acids in fractions 1and 2, the amino acid composition of fraction3 was significantly different with aspartic acid being the most abundant (Randall *et al*.,(1989) and (Qi *et al*.,1991) on deglycosylation of gum Arabic found that it yielded a hydroxy proline-rich polypeptide chain consisting of~400 amino acid residues.

1.5 Spectroscopic analysis of some *Acacia* gums

The gum samples from *Acacia* species namely *Acacia Senegal var. senegal, Acacia mellifera, Acacia seyal var. seyal, and Acacia tortilis var. raddiana*. The

^{13}C and ^{1}H NMR spectra of gum samples showed similarity in individual sugar components, but characteristic patterns of each gum, were observed. FTIR spectra of the studied gums show the presence of the same functional groups in the four gums

1.5.1. Infra red spectroscopy (IR)

Infrared spectrophotometery is one of the most powerful tools available to the chemist for identifying pure organic and inorganic compounds because each molecular species has a unique infrared absorption spectrum. Thus, an exact match between the spectrum of a compound of known structure and that of an analyze unambiguously identifies the latter (Daly et al., 1990). The FTIR spectra for *A. Senegal* gum sample collected from gum Arabic Company and *A. seyal* gum sample collected from South Kordofan (Fig 1.2 and 1.3) showed clear peaks with abroad one at about 3401.60 cm-1 and 3398.29 cm-1 most likely for hydroxyl groups (OH), respectively. In addition to two another peaks at 1700-1600cm-1 probably for carboxyl, aldehyde or ketone groups, the remaining peaks (at 600-700cm^{-1}) were specific for the gum type (Mohammed, 2006).

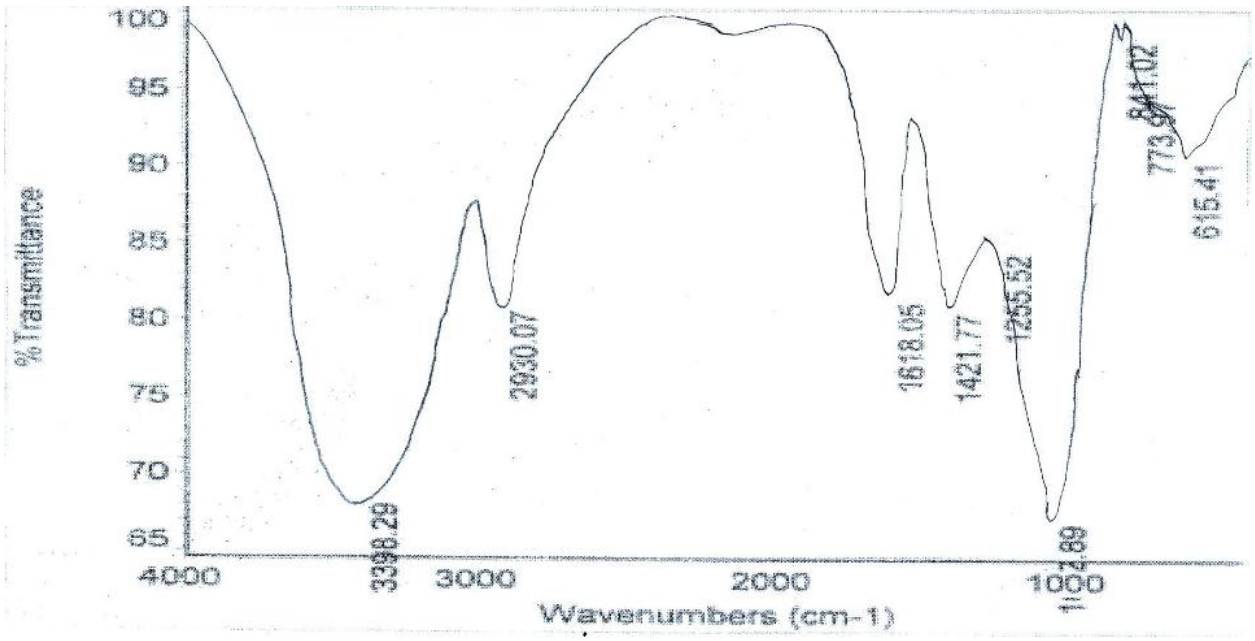

Figure1.2: FT.IR spectrum of *Acacia seyal* sample collected from Southern Kordofan (Mohammed 2006).

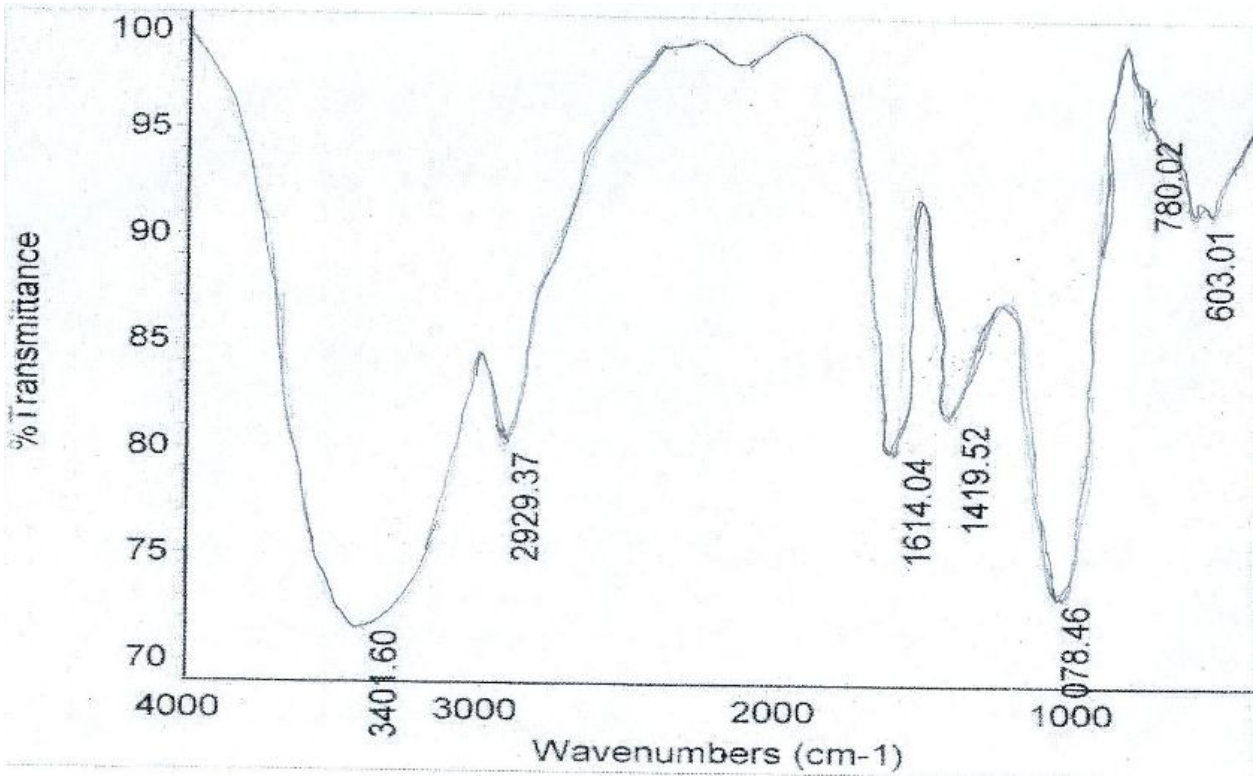

Figure1.3: FT.IR spectrum of *Acacia senegal* provided by Gum Arabic Company Ltd Elobied branch (Mohammed, 2006)

CHAPTER TWO

Materials and Methods

CHAPTER TWO

2. Material and Methods

2.1 Materials

2.1.1 Sampling

Natural exudates samples of the crude gum were collected from Elnagaa and Elshagalwa near Shendi (River Nile State),by the author with assistance of Natural Foresters Authority officers.

2.1.2 Purification of crude gum

The gum samples, used in this work, were relatively pure; however, impurities such as wood pieces and sand particles were carefully removed by hand. Then the samples were reduced to a fine powder using a mortar and pestle and kept in labeled sealed polyethylene bags.

2.1.3 Physical properties of *Acacia eherbergiana* gum

2.1.3.1 Color

The color of the collected gum nodules was pale yellow to brown.

2.1.3.2 Shape

The shapes of the gum nodules, as exuded naturally, were irregular or tear Shaped.

2.1.3.3 Solubility

Acacia ehernbergiana gum was highly soluble in water forming transparent solution, when dissolved cold water.

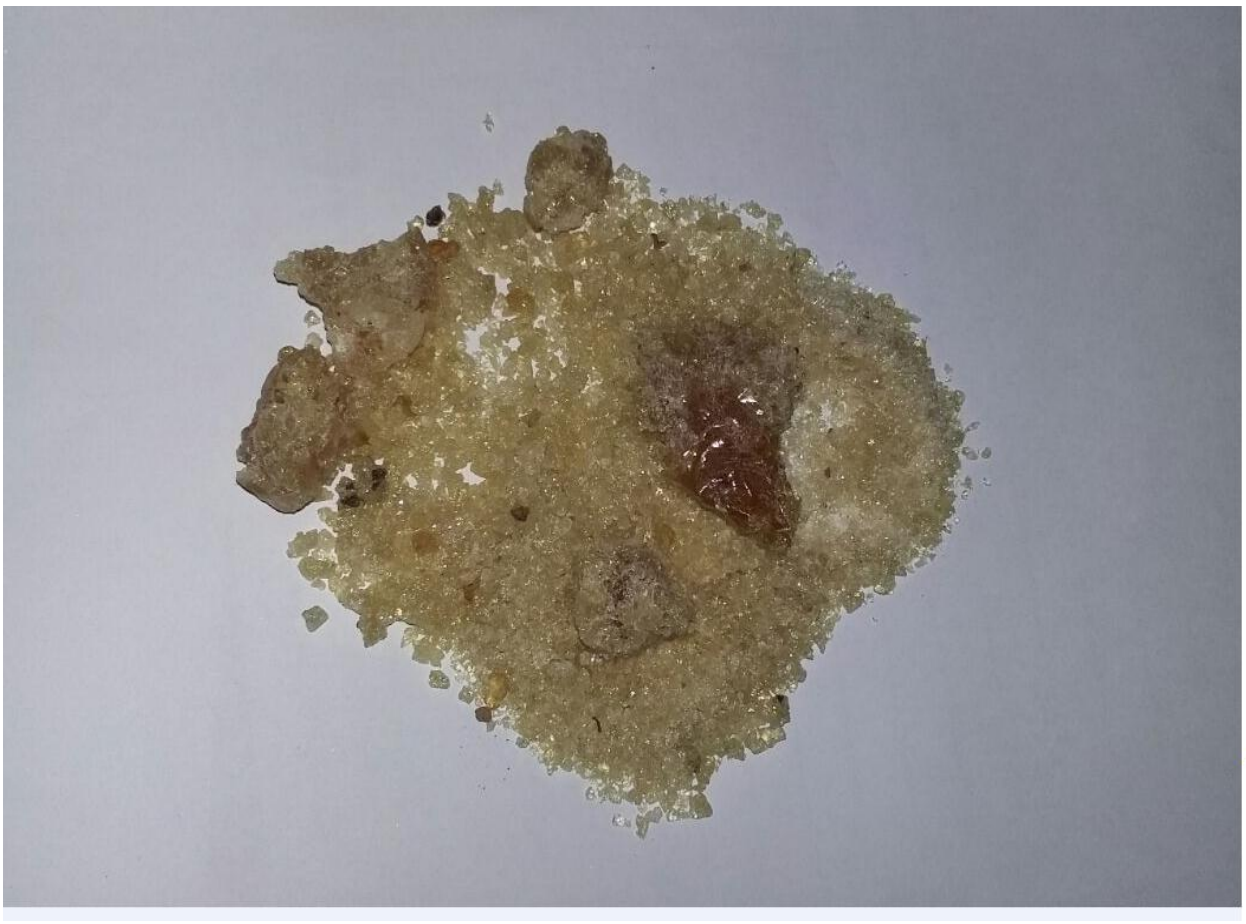

Figure 2.1: *Acacia ehernbergiana* gum

2.1.4 Apparatus

- Porcelain crucible
- Beakers
- Measuring cylinders
- Weighing bottles
- Sensitive balance
- Oven
- Thermostatic water bath
- High Performance Liquid Chromatography system (15950, Shimadzu, Japan).
- Atomic Absorption Spectrometer (6800F, Shimadzu, Japan).
- pH meter (Corning 555)
- Polarmeter (A D P 10,Gallen camp)
- Colloid Osmometer (Osmostat 050-Genotech, Germany).

- UV spectrophotometer (3600,Jenway, U.K).
- Infra Red spectrophotometer (Thermo Nicolet, 300 i.r, U.S.A).

2.2 Methods

2.2.1 Determination of Moisture content

Accurately 0.5 gram of each sample was weighed in a clean preheated and pre weighed dish. Then it was heated in an oven at 105 C for 12hours to constant weight. Moisture content was then calculated as a percentage of the initial weight from the following relation;

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

W_1 = Original weight of sample (g).

W_2 = Weight of sample after drying (g).

2.2.2 Determination of Ash

Accurately weighed 3.0 grams of the dried sample were ignited in a muffle furnace at 550^{o}C for 12 hours and ash % was calculated from the following relation;

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

Where:

W_1 = Weight of the empty crucible.

W_2 = Weight of the crucible + the sample.

W_3 = Weight of the crucible + ash.

2.2.3 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 1g/100 ml gum aqueous solution (w/v) calculated on dry weight basis.

Figure 2.2: pH meter (corning 555)

2.2.4 Determination of Specific optical rotation .

Accurately weighed (3.0 g) of gum samples were dissolved in 100ml of distilled water to give a solution of 3% w/v and mixing on a roller mixer until the sample fully dissolved . The solutions were centrifuged for 20 min at 2500 rpm and the optical rotation was measured against the D-line of Na (589.3nm) using a Perkin-Elmer polarimeter (20 cm path length, 25 C), using distilled water as a blank between each measurement. The specific rotation was calculated according to the relationship:

$$[\propto]_D^T = \frac{\alpha \times 100}{l \times c}$$

Where:

α= Observed angle of rotation in degree.

L = the length of sample holder in decimeters.

C = the concentration of sample per 100 ml of solution.

T = Temperature(25).

D = wave length of sodium light (589.3nm).

Figure 2.3: Polarimeter (Model; ADP10)

2.2.5 Determination of total glucouronic acid.

A glass column was packed with an Amberlite Resin IR ($120H^{+}$). HCl 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 chloride free. 50 ml of 3.0 % gum solution was passed through the column, followed by the distilled water until a volume of 250 ml of the eluent and washing were collected. This was titrated against 0.1N NaOH. The apparent equivalent weight of the acid (A.E.W) was calculated by:

$$\text{A.E.W} = \frac{\text{Weight of sample}}{\text{Volume of titrate X molarities of alkali}} \times 1000$$

% 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.

2.2.6 Determination of Intrinsic Viscosity

Measurement of dilute solution viscosity (resistance to flow) provide the simplest and most widely used technique for routine determination of 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 a certain volume of solution through a capillary viscometer at constant temperature .The viscosity of a solution may have a complicated 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.

(1) $\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots = \frac{\eta_{solution}}{\eta_{salvent}} = \frac{t}{t_o}\eta_{rel}$

Where $\eta_{solution}$ and $\eta_{solvent}$, refer to solution and solvent viscosity respectively, in poise units or Pascal seconds, t and t0 the flow time of the solution and pure solvent through capillary viscometer respectively ,and η_{rel} is relative viscosities.

The specific viscosity η_{sp} is the relative viscosity minus one:

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

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

$\eta_{rel} = \frac{\eta_{sp}}{C}$ ..... (3)

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

$\eta_{inh} = \ln\frac{\eta_{rel}}{C}$ ..... (4)

Intrinsic viscosity (limiting viscosity number) [η] is determined by extrapolating a plot of either reduced or inherent viscosity versus concentration to zero.

$\lim_{C\to 0}[\frac{\eta_{sp}}{C}] = \eta$ or $= [\ln\frac{\eta_{rel}}{C}]$ ..... (5)

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

$[\eta] = KM^{a}$ ..... (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 allot of log [η] versus log molecular weight of a series of fractionated polymer samples whose molecular weight have been determined by on absolute methods (i.e .light

scattering).

2.2.7 Determination of Nitrogen content

Nitrogen content was determined using a semi – micro Kjeldahl method according to (AOAC, 1984). Hence protein was determined by multiplying Nitrogen content by 6.25 as factor (Anderson *et al*., 1986).

The procedure used is a two stage; process in which the gum samples are (1) digested in hot concentrated sulphuric acid, and(2) the ammonia released using sodium hydroxide is neutralized using standard acid.

Digestion:

Sample + H_2SO_4 conc. + (Mercuric oxide as catalyst) +heat $\rightarrow (NH_4)_2SO_4$

$(NH_4)_2SO_4$ + NaOH ─steam $\rightarrow$ $2NH_3 + Na_2SO_4 + 2H_2O$

ii) Neutralization:

$NH_3 + H_3BO_3 \rightarrow NH_4^+ + H_2BO_3$

The Borate anion equivalent to the ammonia produced is back titrated with standard Hydrochloric acid (0.02 M)

$H_2BO_3 + HCl \rightarrow H_3BO_3 + Cl^-$

Accurately weighed samples of the dry gum 0.2g were transferred to the digestion tubes to which a catalyst tablet and 10 cm^3 of nitrogen free concentrated sulphuric acid were added. The tube was placed in the digestion heating system which was previously set to 240 C . Complete digestion was attained when the heated solution turned into clear yellowish – green coloration. The tubes were allowed to cool to room temperature. Blanks containing 10 cm^3 of sulphuric acid and catalyst were digested in the same way as the test samples. The blanks and the samples were analyzed by the addition of sodium hydroxide (40%) followed by steam distillation. Ammonia released was absorbed in a known volume of boric acid and the borate anion generated is back titrated with 0.02 M hydrochloric acid. The volume required to neutralize the borate anion is determined.

The nitrogen content of the samples is calculated as follows:-

$$N\ (\%) = \frac{14.01 \times 0.02 * (volume of titrat - blank)}{weight of gum sample\ in\ gram}$$

Protein content =N% × 6.6

2.2.8 Determination of cationic composition.

Dry ashing method was used in sample preparation. One gram of gum sample was placed in a well-glazed porcelain dish. The porcelain dish was placed in the furnace, and then heated to 550^{o} C, maintaining temperature for 4 hours. Then the sample was cooled and 10 ml of 3N HCl were added. The sample was covered with watch glass, and boiled gently for 10 minutes. Then it was cooled, filtered into a 100cm^3volumetric flask and diluted to volume with deionized water and then the elements were determined using Atomic Absorption spectophotometry.

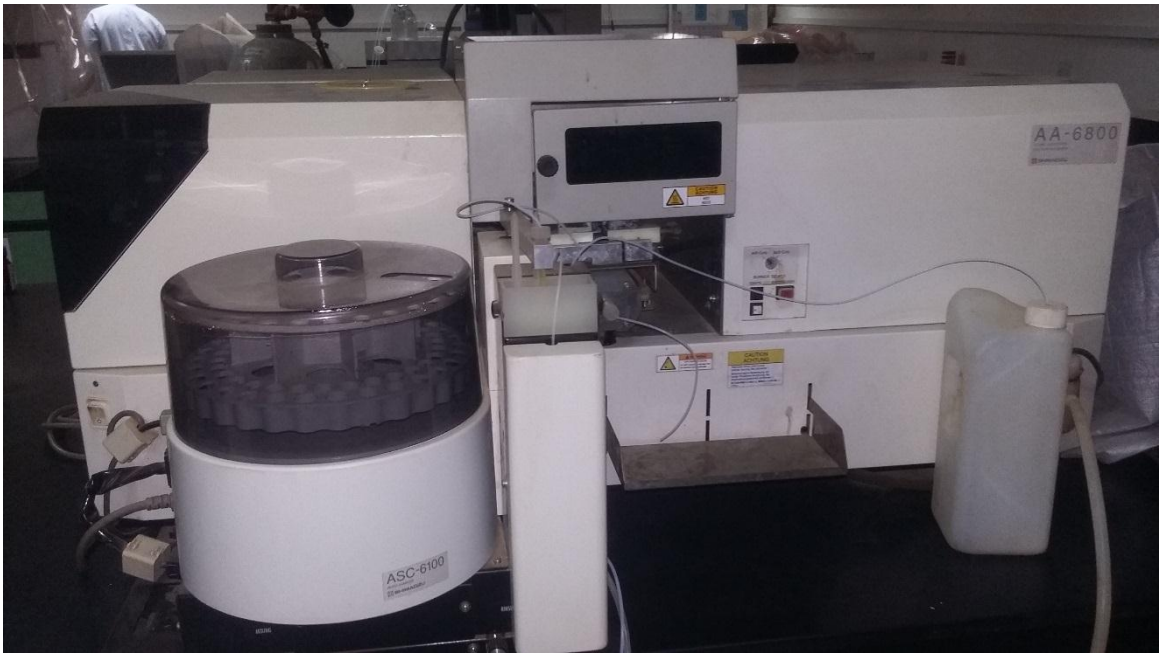

Figure 2.4: Atomic Absorption spectrometer

2.2.9 Determination of Sugar composition

The samples were Acid hydrolyzed to liberate the sugar residues .Sample (100mg) was accurately weighed, including allowance for moisture content, added to 10cm^3 of 4% H_2SO_4 and incubated at 100C for 6 hours. Following this ,1g of $BaCO_3$was added to the solution and left overnight (minimum of 12 hours) to neutralize the solution. After $BaCO_3$ treatment, universal indicator strips were used to ensure that the sample was neutral before proceeding to the next stage.

The solution was then centrifuged at 2500rpm for 10 minutes to allow the barium sulphate (formed from neutralizing the H_2SO_4) to settle. The supernatant was removed and filtered through a 0.45µm whattman nylon filter and then diluted 1:1with 70/30 Acetonitrile /buffer. This constituted the final solution of which 1ml was analyzed using HPLC (15950, Shimadu, Japan). Fig 2.5

to determine the relative concentration of each sugar residue present in the sample, namely rhmnose(Rha), arabinose (Ara) ,galactose (Gal) and glucuronic acid (GlcA). Before analysis of the hydrolyzed gum samples, calibration curves of these sugars were prepared. Stock concentration of 5 mg cm^3 for each sugar were made up by hydrating in 70/30 acetonitrile /buffer for 2 hours. Dilutions of the stock solution achieved six different concentration for each sugar over a range of 2.5 - 0.5 mg cm^3. This allowed six levels for the calibration curve and an average of replicates for each level was used to ensure accuracy. This calibration allowed the determination of the unknown sugar content for the gum samples. The concentration of each sugar was calculated by peak height and expressed as % of the total sugar content.

Figure 2.5: High Performance Liquid Chromatography

2.2.10 Determination of Density of solid gum

The density and the specific volume of the gum give good idea about the distance between the molecules. Gradient tube method may be used to determine the

density of the polymer (Tager, 1978).

2.2.11 Determination of the Number average molecular weight

2.2.11.1 Method.

The colloid -osmotic pressure is measured by means of an osmotic cell (Osmomat 050). The lower half of the osmotic cell, which is closed off to the outside, is filled with electrolyte containing ringer's solution. The upper half of the cell, which is open to the outside, is filled with a colloid-containing solution.

The two halves of the cell are separated from each other by a semi membrane. This membrane possesses defined pores, through which only water molecular migrate . Due to osmotic pressure differential of the two solutions, solvent permeates from the lower into the upper half of the measuring cell until equilibrium is reached between the pressure in lower half of the cell and the osmolal concentration. An electronic pressure measuring system, which I mounted into the lower half of the cell, transduces the under pressure into an electronic signal, which is shown on a digital display.

Figure2.6: Osmomat050

2.2.12. Determination of emulsion stability

Three types of refined oil groundnut, sesame and corn oil and 2% aqueous gum solution were used to prepare stock emulsions. Emulsions were prepared by blending a measured amount of the gum solution (20%) and the oil (2:1v/v)for

one minute at 1800 rpm using kitchen blender (triplicate preparations were used Karamalla *et al.*, 1998). Aliquot (1Ml) of the stock dilution of 1/1000dilution.

The absorbance was then read at 520nm in spectrophotometer. Another reading of absorbance was recorded after an hour Emulsion stability was calculated as:

$$\text{Emulsion stability} = \frac{\text{First reading of absorbance}}{\text{Reading of absorbance after one hour.}}$$

Test for stability under influence of emulsion factors: these tests were done with the objective of studying effect of emulsification factors such as storing time, concentration and gum grade on emulsion stability.

Storing time: This test was done to study the effect of the length of storing on emulsion stability. One ml of the stock emulsion was diluted with distilled water to a concentration of 1/1000 and then stirred for different time (1, 2, 3, 4, and 5 days). Emulsion stability was determined following the previous procedure.

Concentration: distilled water diluted concentration of stock emulsion of 1, 2, 3 and 4/1000 were prepared and examined for emulsion stability under a fixed storing time of one minute at room temperature. Emulsion stability was measured as before.

Gum grade: Emulsion was prepared by blending 20% agueous gum solution and corn oil in 2:1water determination of emulsion stability was done following the method described by Karamalla *et al.* (1998).

Figure 2.7: Jenway UV/VIS spectrophotometer

2.2.13 Infrared spectroscopy:

Dried powder of gum samples were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The disk of each gum specimen was loaded in FTIR spectroscopy (Thermo Nicolet 300 ir), with a scan range from 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1}, was used to record the spectrogram for each gum sample.

CHAPTER THREE

Results and discussions

CHAPTER THREE

3. Results and Discussion

The characterization of gum and determination of its physicochemical properties is very important whenever its use in the industrial applications is intended. In this study, the chemical, physical and emulsion-stability properties of *A. ehernbergiana* gum samples were studied to set up the defining characteristics of the gum in order to avoid used of mixed or adulterated samples and to know the specifications of the samples under study. Tables (3.1, 3.2 and 3.3) summarize the results of analytical data of exudates gum of *A. ehernbergiana* gums collected from River Nile state, Sudan from two locations namely Elnagaa and Elshagalwa.

3.1 Moisture content

The moisture content of the gum is usually affected by the season of collection, the prevailing climate condition and storage condition. The moisture content of *Acaica ehernbergiana* gum samples studied was found to ranged between 6.4- 7% with an average value of 6.7%.This result is less than that reported in literature for *acacia seyal* (Anderson et al.,(1963), Karamallah *et al*.,(1999), Hassan(2000), siddig *et al.*,(2005),Omer(2006), Abdelrahman (2008),and Younes(2009)).Also it is less than those of *A. nilotica var. nilotica* reported satti (2012). It is expected that low moisture content shall prevail, considering the dry and hot climatic conditions prevailing in northern part of Sudan where the samples of the study were collected.

3.2 Ash content

The ash content value of *A. ehernbergiana* gum samples ranged between 3.1%-3.8% with an average value 3.4%.This result is higher than that reported in literature for *A. seyal var. seyal* (Anderson et al .,(1963), Karamallah (1999), Hassan et al .,(2005) but is typical for *Acacia seyal* reported by (Omer (2006),and Abdelrahman (2008). The ash mean content value of *Acacia ehernbergiana* gum sample is less than that of *A. nilotica var. nilotica* reported satti (2012).The ash content of gums usually reflects the gum freeness of contaminant like , sand particles and pieces of bark etc. Since that sample of *A. ehernbergiana* were

collected intentionally for the purpose of research they were clean from possible natural contaminant that might have contributed to lager value of ash content if they were present .

Table 3.1 Physicochemical properties of *A.ehernbergiana gum*

	Samles collecyed frm tow locations		
Samples	*A.ehernbergiana* (Elnagaa)	*A.ehernbergiana* (Elshagalw)	average value
Ash %	3.1	3.8	3.4
Moisture%	7.0	6.4	6.7
pH	6.0	5.8	5.9
Nitrogen %	0.278	0.487	0.382
Protein%	1.74	3.04	2.39
Refractive index	1.3393	1.3383	1.3388
Acid equivalent	1224.5	1158.3	1191.4
Uronic acid%	15.8	16.7	16.3
Tannin%	0.0133	0,0167	0.015
Density of solid gum	0.97	0.95	0.96
Specific optical rotation	1.06	4.0	2.53
molecular weight	7.4×10^5	6.42×10^5	6.9×10^5
Arabinose	31.9	36.6	34.25
Galactose	27.6	22.7	25.15
Rhamnose	9.7	11.8	10.75

3.3 pH of *A. ehernbergiana* gum solution

The pH of *A. ehernbergiana* gum samples solution was fond to be in the ranged between 5.8- 6.0 with an average value of 5.9.This result is typical to that reported for *A. seyal var. Seyal* (Younes, 2009). Also is similar to the one reported by Satti (2012). For *A. nilotica var. nilotica*. In this regard *A. ehernbergiana* is not different from all the *Acacia* gums studies so for as. They all, show mild acidic nature.

3.4 Acid equivalent weight and uronic acid content

The acid equivalent weight values of the *A. ehernbergiana* gum collected from two different locations samples, shown in Tables 3.1.were found to be1158,3 and

1224 respectively. Uronic acid content values were found to be 15.84 - 16.74 respectively. In comparison to other *Acacia* gums this result is not very much different from that of *seyal var. seyal* reported by (Abdelrahman, 2008) and Younes (2009), but it is less than that of *A.seyal ver. seyal* reported by Hassan et al .,(2005), and also less than that of *A. nilotica ver. nilotica* reported by Satti (2012).

3.5 Viscosity:

The intrinsic viscosity of *A. ehernbergiana* was found to be 6.41$cm^3 g^{-1}$ as shown in Figure (3.1). This result was less than the reported in literature for *A. seyal var. seyal* (Karamallah (1999), Hassan *et al*, (2005,), Omer (2006), siddig *et al*.,(2005), Abdelrahman (2008) and Younes (2009). It is also less than the value of [η] of *A.nilotica var. nilotica* reported by Satti (2012). It is the lowest among the *Acacia* gums of Gummiferae series. Low intrinsic viscosity reflects more compact ,or globular molecular structure.

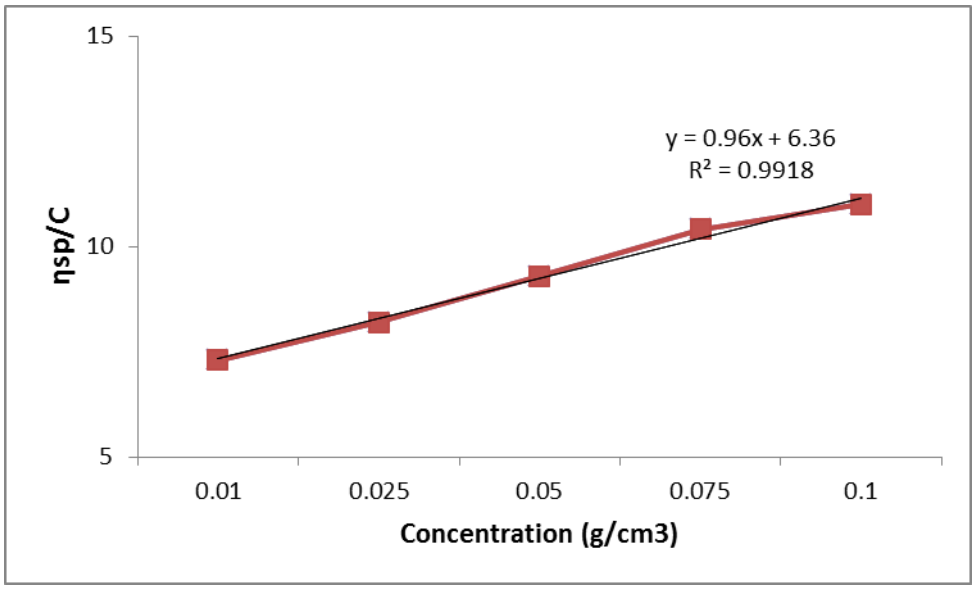

Figure 3.1:variation of Intrinsic viscosity.With concentration (Elnagaa)

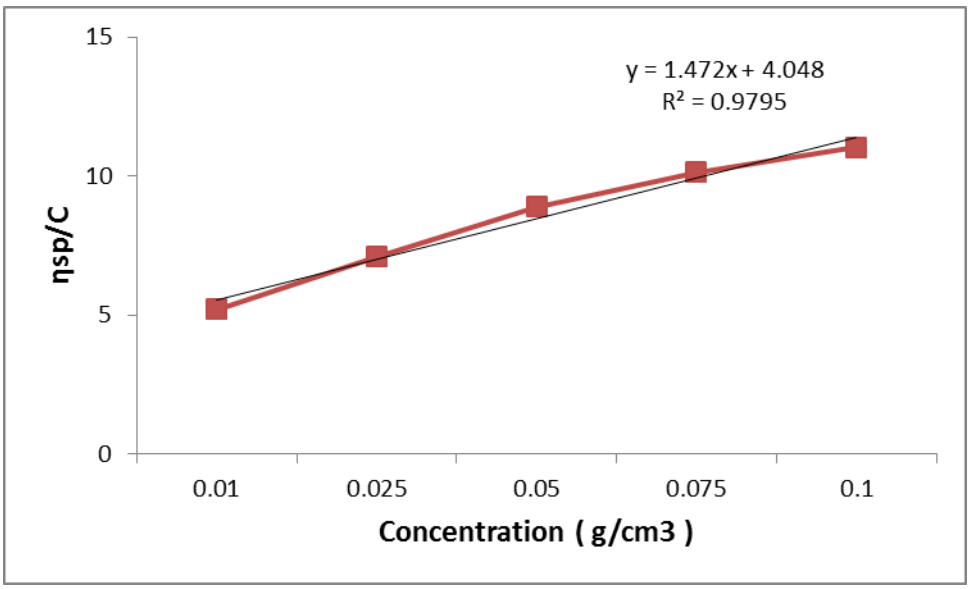

Figure3.2: variation of Intrinsic viscosity.With concentration (Elshagalwa)

3.6 Refractive index:

The refractive index values for *Acacia ehernbergiana* gum samples were found to be 1.3393 and 1.3388 for samples collected from Elnagaa and Elshagalwa respectively. These results show that there is no effect of location in values of the refractive index.

3.7Nitrogen content and protein content:

The nitrogen content of *Acacia ehernbergiana* gum was measured using kjeldahl method . The percentage of nitrogen content ranged between 0.2778 and 0.487. The protein content was calculated from the Nitrogen content using nitrogen conversion factor of 6.25. The protein content of *A. ehernbergiana* gum percentages ranged between 1.833 and 3.2168.This result it far higher than *A. seyal var seyal* reported by(Anderson(1963), Karamallah (1999),Hassan *et al* .,(2005,), Omer (2006), siddig *et al*., (2005), Abdelrahman (2008) and Younes (2009). and higher than that *A. nilotica var. nilotica* reported by Satti (2012). This result suggests better emulsification performance by *Acacia ehernbergiana* gum compare to *A. seyal* and *A. nilotica*. It might approach *A. sengal* in this regard.

3.8 Sugar composition:

The sugars contents of *Acacia ehernbergiana* gum after acid hydrolysis was measured using HPLC technique and illustrated in Table 3.2 and (3.3). The results showed that arabinose had a higher percentage (31.8725%) followed galactose (27.6422%) and rhamnose (9.684%). These result are not very much different from these reported in literature for *A. seyal var. seyal* (Jurasek et al.,(1993), Karamallah (1999),William et al.,(2000), siddig et al., (2005), and Abdelrahman (2008). It is not far from those of *A.nilotica var. nilotica* reported by Satti (2012). The major mono saccharide in *Acacia ehernbergiana* are similar to the group of sugars found in most of *Acacia* exudate gum studied so far.

3.9 Specific optical rotation

The specific optical rotation is regarded as one of the analytical parameters by means of which an *Acacia* species gums can be distinguished from other *Acacia* species gums. *Acacia ehernbergiana* has a positive specific optical rotation and it belongs to Gummiferae series which contains *A. syeal*, *A. siberiana, A. tortilis*, *A. Oerfota, Acacia nilotica and some other less studied species.* Whereas *A. senegal* has negative specific optical rotation and belong to Vulgares series that contains *A. Leata, A. polyacantha, A. mellifera*….etc. *A. ehernbergiana* gum specific optical rotation value was found to be +2.53 in average as shown in Table 3.1. This value (+2.53) is the lowest value for specific optical rotation reported for all the members Gummiferae series so far. Anderson (1963), Jurasek *et al*.,(1993), Karamallah (1999), Hassan (2000), Hassan et al.,(2005), siddig et al., (2005), Omer (2006), Abdelrahman (2008) , Younes (2009) and Satti (2012).

3.10 Density of solid gum:

The Density of solid gum of the *A. ehernbergiana* gum samples studied ranged between 0.95 g$cm^{-3}$and 0.97gcm^{-3} with an average value of 0.96 gcm^{-3} .

3.11 Number average molecular weight:

The number average molecular weight of *A. ehernbergiana* samples, understudy, was determined by osmometery measurement as shown in Table (3.1).The number average molecular weight of *A. ehernbergiana* samples ranged between

6.42×10^5– 7.4×10^5 Dalton With an average value of 6.9×10^5 Dalton.This result is higher than that of *A. seyal* reported by Abdelrahman (2008),and it is less that of *A. nilotica var. nilotica* reported by Satti (2012). The order of magnitude is in the same range for other gummiferae series member e :10^5. It worth noting here that the low intrinsic viscosity of *Acacia ehernbergiana* gum and value of its number average molecular weight suggest that it possesses more compact structure or more branched and globular molecule compared to *A. seyal* and *A. nilotica* .

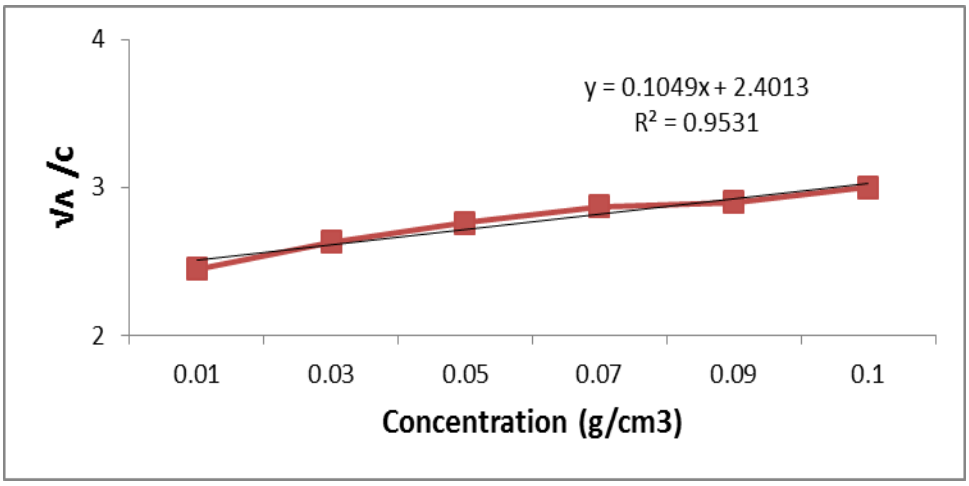

Figure 3.3: Number average molecular weight of Acacia ehernbergiana gum (Elnagaa)

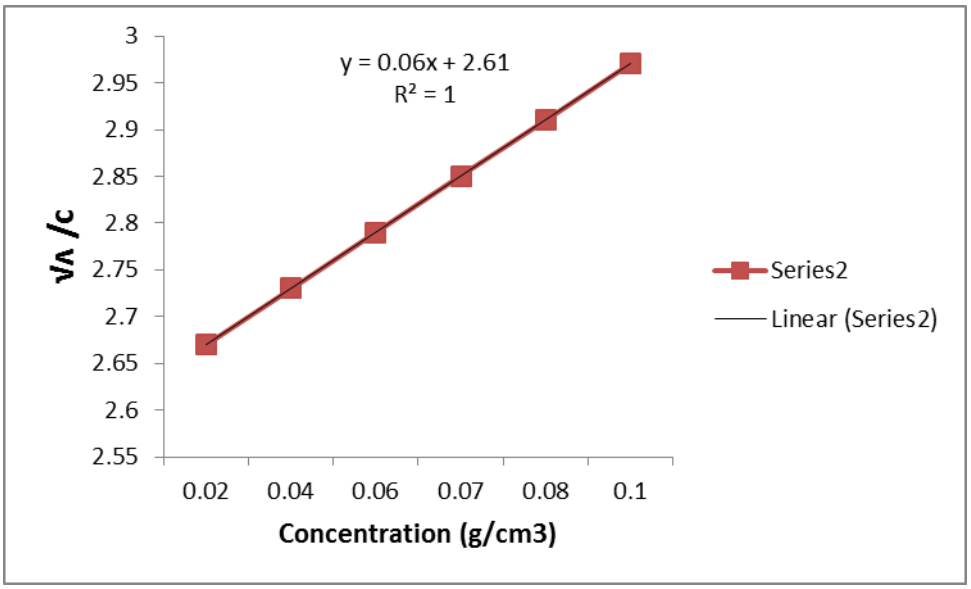

Figure3.4: : Number average molecular weight of Acacia ehernbergiana gum (Elshagalwa)

3.12 Cationic composition

Minerals composition of the samples was determined using atomic absorption spectrophotometer and the average values of the studies cation were shown in Table (3.4). The major element in the order were : Ca, Mg, K, Fe, Mn, Na, Cu ,Pb, Ni, Zn and Cr . Calcium, magnesium, potassium and Iron recorded highest values, indicating that the gum is a salt of calcium, magnesium, potassium and Iron .The most striking observation in these result is the high Fe content compared to other members of Gummiferae series where usually Fe is of low concentration. This high value of Fe content makes *Acacia ehernbergiana* gum more superior compared to other Gummiferae series from nutritional point of view.

Table 3.4: Cationic composition of *A. ehernbergiana* Gum

Eelement	Ppm
Ca	32550
Mg	6100
K	310
Fe	264.67
Mn	48.23
Na	23
Cu	9.55
Pb	7.18
Ni	5.65
Zn	2.9
Cr	No detected

3.13 Infra-red (IR) spectral analysis

The infra red (IR) spectra for samples of *A. ehernbergiana* gum collected from Elnagaa and Elshagalwa are shown in Fig. 15, 16, respectively. The IR spectra showed six peaks with broad one at about 3426.02cm^{-1} most likely for hydroxyl groups (OH); and two peaks at 2923.18cm^{-1} probably for aliphatic groups (C-H), and two peaks also at 1615.72$cm^{-1}$and 1418.56cm^{-1} probably for ketone (C=O) groups, the remaining peaks (at 1070.57 – 657.71cm^{-1}) were specific for *A. ehernbergiana* gum. Similarly the FT.IR spectra for *A. senegal* (fig 1.6) gum sample donated by Gum Arabic Company and *A. seyal* (fig 1.5) gum sample collected from South Kordofan showed clear peaks with broad one at about 3401.60$cm^{-1}$and 3398.29cm^{-1} most likely for hydroxyl groups (OH)

respectively.

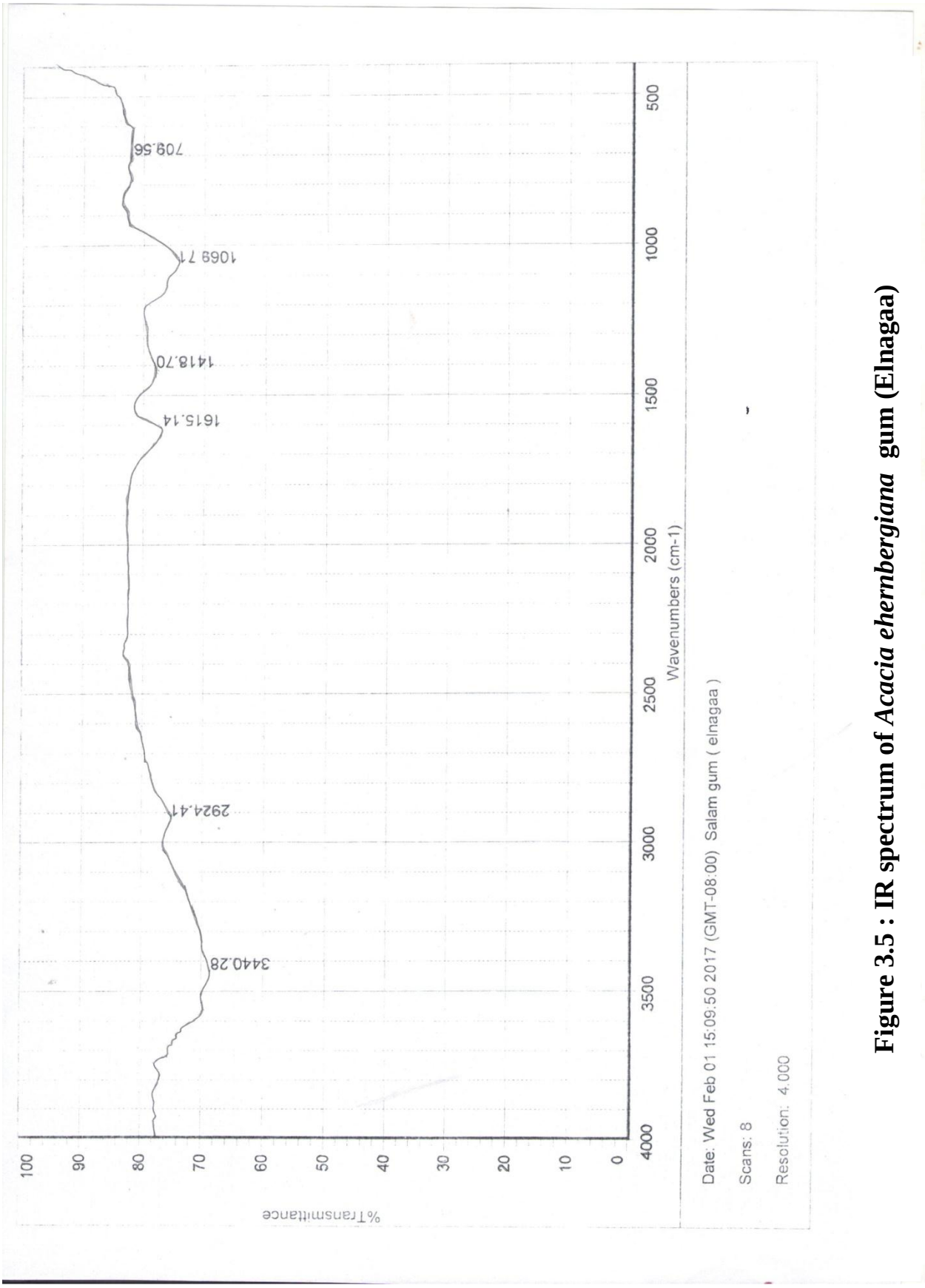

Figure 3.5 : IR spectrum of *Acacia ehernbergiana* gum (Elnagaa)

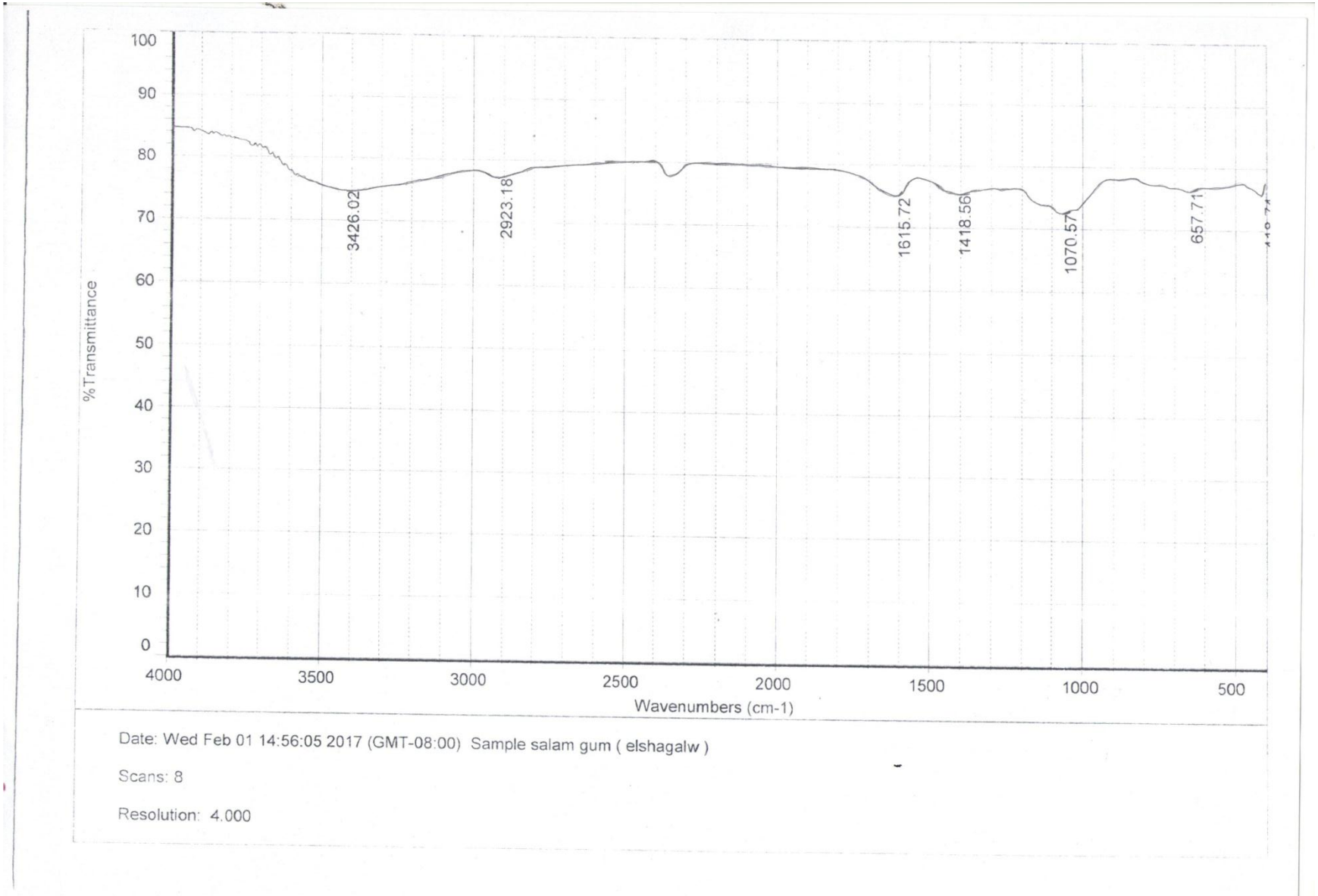

Figure 3.6 : **IR spectrum of *Acacia ehernbergiana* gum (Elshagalw)**

3.14 Emulsifying stability of *Acacia ehernbergiana* gum

The emulsification properties of a number of *Acacia* gum had been subject of studies for many decades. *Aciacia sengal var. sengal* gum (Gum Arabic) is most studied among all known *Acacia* gums. This is not surprising since it's the oldest known plant exudate gum at all and bears the best emulsification properties and the one with widest applications in diverse fields of industry. It is suggested as the standard to which the qualities of all other exudates gums are compared to. Hence it is emulsifying properties are the bench mark to which the functionality of any other gum may be compared. The emulsification quality of an *Acacia* gum can be assessed by following the stability of an emulsion, prepared within a set of controlled condition, with time .One simple method is to follow the optical absorption of a highly diluted emulsion with time. *Acacia ehernbergiana* gum was used as an emulsifier in an oil in water, emulsion following the procedure mentioned in chapter two section (2.2.13). From the variation of emulsion absorbance with time the emulsifying index was calculated, and plotted with time as shown in figure 3.4. The change in absorption index, which is the ratio of absorbance at time (t = 0) and time (t = t) was very small. It was only 10% in 11 days. This may compare well with the emulsifying behavior of *Acacia senegal*, consequently indicating that. *Acacia ehernbergiana* is a good emulsifier for oil in water systems . It performance is better than Acacia *seyal* considering the results published in literature (Hassan (2000). This may be expected since the protein content of *Acacia ehernbergiana* gum is higher compared to that of *Acacia seyal*, and match that of *Acacia senegal*. It worth mentioning here that the molecular weight \of *Acacia ehernbergiana* compare well with that of *Acacia seyal* and far exceeds that of *Acacia senegal* .

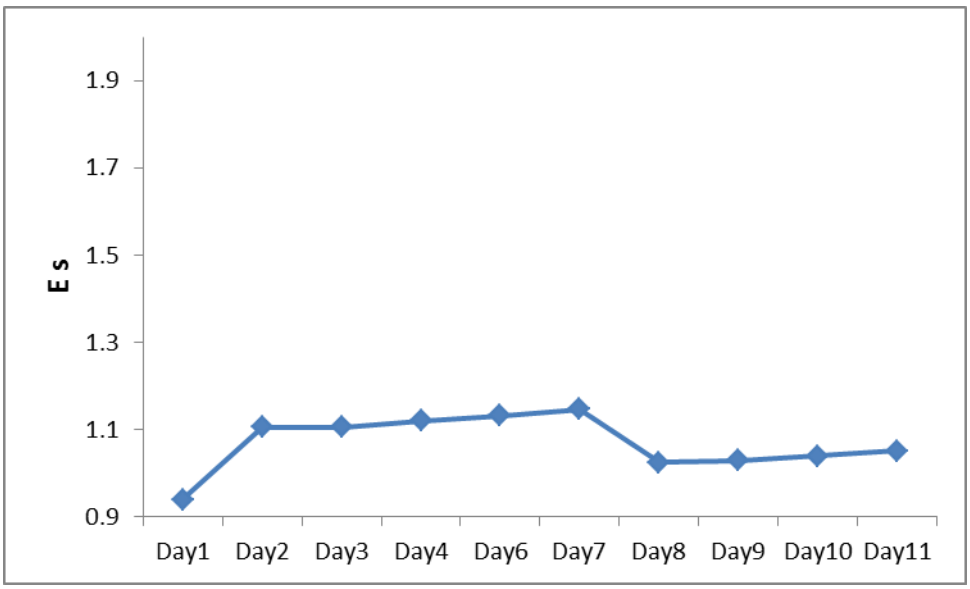

Figure 3.7 : Emulsifying stability of the *Acacia ehernbergiana* gum

3.15 Conclusion

- The physicochemical analysis reviled that ***Acacia ehernbergian*** falls within the gemmifera series with positive optical rotation.
- It possesses good emulsifying property that much *A. senegal* .
- It possesses extra ordinarily high Fe content compared to all *Acacia* exudate gums studies so far.

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