Sudan University of Science and Technology

College of Graduate Studies

The Preparation Of Magensium Glucourantes From *Acacia Seyal* Var. Seyal Gum

تحضير جلايكويورانات الماغنزيوم من صمغ الطلح

A dissertation Submitted in Partial Fulfillment for the Requirements Of a Master Degree in Chemistry

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الآيـــــــة

قَالَ تَعَالَىٰ:

﴿ فَتَعَٰلَى ٱللَّهُ ٱلۡمَلِكُ ٱلۡحَقُّۗ وَلَا تَعۡجَلۡ بِٱلۡقُرۡءَانِ مِن قَبۡلِ أَن يُقۡضَىٰٓ
إِلَيۡكَ وَحۡيُهُۥۖ وَقُل رَّبِّ زِدۡنِي عِلۡمٗا ﴾

سورة طـــــــه الايه 114

DEDICATION

I dedicate this work to:

My father

The soul of my mother,

And my brothers

Acknowledgment

I would like express my deep thanks to Almighty Allah for the great support I got during my whole life and especially in this study.

Iam greatly indebted to my supervisor Prof. Mohammed El MubarkOsmanfor his keen guidance,encouragement and concern. Also I wish to thank him for never failing to be therewhen needed. It was through his invaluable advice I was able to present this dissertation.

My thanks are extended also, to everybody who helped and encage me during the research period.

Abstract

In this investigation,physicochemical properties of *Acacia seyal*(Talha) sample was studied [moisture content (10.79%), ash (1.1294%), nitrogen (0.15%), specific optical rotation (+46.1°), pH (4.5)].

Mineral contents of Ca and Mg in two samples (S1gum, S2 magnesium glucurnates and S2) has been analysizedby using Atomic Absorption Spectroscopy (AAS), the results were (Ca in sample S1 = 1934.4, Ca in sample S2 = 65.22, Mg in sample S1 = 19.86 and Mg in sample S2 = 1264.8).

المستخلص

في هذه الدراسة تمت دراسة الخصائص الفيزوكيميائية لصمغ الطلح (محتوى الرطوبة = 10.79%، الرماد = 1.1294%، النايتروجين = 0.15%، الدوران الضوئى النوعى = +46.1° والآس الهيدروجينى = 4.5). المحتوى المعدنى لعنصرى الكالسيوم والماغنزيوم للعينتين (الصمغ S1 و S2جلويكويورانات الماغنزيوم) تم حسابه بإستخدام جهاز مطياف الأمتصاص الذرى، وكانت النتائج (الكالسيوم فى العينة S1 = 1934.4، الكالسيوم فى العينة S2 = 65.22، الماغنزيوم فى العينة S1 = 19.86 و الماغنزيوم فى العينة S2 = 1264.8).

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CHAPTER ONE

INTRODUCTION

1. INTRODUCTION

1.1 Gum

Gums have been for thousands of years and remains to be an important article of commerce to the present day. Natural gums obtained from incisions of stems and branches of several *Acacia* species growing in arid and semi-aridagro ecologies are used for making different beverages, medicines, and water-soluble glues (EFAP, 1994; FAO, 1995a). In the food industry, gums are used as thickening, stabilizing, emulsifying and suspending agents, besides their applications in making foods and drinks. In pharmaceutical industry, gums are used as a binding agent in tablets and as a suspending and emulsifying agent in creams and lotions (FAO, 1995b). Some of the technical applications of gums are in printing and textile industries where advantage is taken of their filming and sizing properties, respectively (Cossalter, 1991). Local medicinal uses have been claimed to serve as smoothening and softening agent, taken internally for cough, diarrhea, dysentery, hemorrhage; and externally in the treatment of local inflammations and nodular leprosy (FAO, 1995a, 1995b).

Africa is the world's leading producer and exporter of gum arabic from *A. senegal*. Sudan accounts for 80% of the world's gum arabic production, followed by Chad, Nigeria, Senegal, Mauritania, Mali, Ethiopia, Chad, Tanzania and Niger, according to their importance (Seif el Din and Zarroung, 1996). Total gum production in Ethiopia is approximately 3000 tons per annum and only an estimated 50% of the produce is exported through formal trading channels (EFAP, 1994).

Gum represents a group of non-wood forest products, which have played and continue to play a significant role in the economies of the Sudan people in Ethiopia. Though the Sudan Region is one of the major gum-producing regions of

Ethiopia, the actual production and trade data on gum from this region is hard to come by. Information on the production and marketing of these products is inadequately documented.

Furthermore, the extent of variations in the nature, quality, characteristics and use of the products compounds the situation. Nevertheless, gum collection plays an important role in the livelihood of the rural communities of Sudan Region (Mulugeta*et al*., 2003).

The degradation and loss of ecosystem and the gradual destruction of natural resources, on which the collection of gum depends, appear to be among the biggest threats to the development of this sector in the Sudan Region. The major problems affecting production and trade of gum in Sudan Region are related to inadequate knowledge of botanical sources, lack of proper market information, poor infrastructural development, and poor production and handling of the products. The information on the ecology and distribution of the species is scanty. Sound and sustainable management of this natural resource is required to ensure socio-economic benefits, processing and product development of gum arabic (Mulugeta *et al*., 2003). This can be achieved by undertaking investigations targeted at understanding of the ecology of the species, the socio-economic impacts of the products as well as their potentials for investment, employment and income opportunities for the rural population in Sudan Region. The data generated from such study should enable to create awareness of issues on the part of policy-makers, to gain policy and legislative support in the development, management and conservation of this natural resource to ensure sustainability of gum production, trade and marketing in the Sudan Region. Therefore, the objectives of this study were to identify gum producing species and their distribution in the Sudan Region;

assess the ethno-botanical and cultural values of the gum producing species; and assess gum tapping procedures used by the local community.

Gum arabic is the dried gummy exudate 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 *A. Seyal* (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 exudate that is rich in nonviscous soluble fiber (Williams and Phillips, 2000). Clinically, it has been tried used as supportive treatment for 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 2.79×10^5 and is deficient in protein. Fraction 2, which represents 10.4% of the total, is an arabinogalactan protein complex with a molecular mass of 1.45×10^6, 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 $\sim 2.8 \times 10^5$ 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 protein aceous 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, either neutral or 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 gum arabic; *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 gum arabic, but a wide range of other tree exudates are 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 the 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 rain fed areas of North Kordofan in western Sudan (El-Dukheri, 1997). *A. senegal* trees are managed in the Sudan in an agroforestry system known as the bush-fallow system (Obeid and Seif El Din, 1970). However, the recent disruption of this traditional agroforestry 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 Eldin, 1995). It has been reported that rainfall and

temperaturehave 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 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 (Greenwood and Morey, 1979; Ghosh and Purkayastha, 1962; Luckner, 1990).

The community inhabiting the gum producing areas in the Sudan, believe that high gum yield by *A. Senegal* is directly correlated with the abundance of certain beetles locally known as *Al Garraha* (Injector) that is in years when the occurrence of these beetles was high, gum production was maximum (Osman, 1993). Several gum producers interviewed in Kordofan State (western Sudan), firmly believe that *Al Garraha* (Injector) pierces holes into the tapped branches of *A. Senegal* (El Khalifa et al., 1989).

1.1.1 Definition and nomenclature

Although different regulatory organizations have defined gum Arabic of commerce somewhat differently, they all acknowledge it as a dried gummy exudate obtained from the stems and branches of *A. senegal* (L.) Willdor closely related species (US pharmacopoeia 1985; FAO, 1990). The name gum arabic derives from the fact that it formerly was shipped to Europe from Arabian ports (Obeid and Seif El Din, 1970). There are also different local names depending on the area of origin. Ballal Siddig (1991) gave a comprehensive list of local names from different countries. In Kenya, it is known by the following names: Ekunoit (Turkana), Babito Buradima or (Boran), Iderikes (Samburu), Idado (Sudan), Mongoli (Kamba), munshuin (Maasai), Chepkomon (Kipsigis), Matengewa (Bajuni) and Kikwata (Swahili) (Coe and Bentjee, 1991).

1.1.2 Formation and function

According to a hypothesis on the mechanism of formation proposed for gum arabic, the gum acid has as its precursor, some highly branched arabinogalactan of a hemicellulosic type, to which is added rhamnose, glucuronic acid and 4-0-methyl glucuronic acid (arising from oxidation related mechanisms) terminated side chains in the final stages of gum production (Anderson and Dea, 1968). It is envisaged that enzyme systems probably differ at different parts of the tree and the dark brown (Hennawi) gum formed on the main trunk is thought to be manufactured by a different enzyme.

The site of formation has been, comprehensively, studied (Ghosh and Purkayastha, 1962). In a study of the anatomy of wood and bark of *A. Senegal* Ghosh and co-worker observed that gum comes from gum cysts which develop in the inner bark of some trees that, naturally, exude it. The cysts are developed in the tangential rows of the axial parenchymastrands of the phloem adjacent to the cambial zone. They are first developed schizo genousily but later on enlarged considerably (lysi genousily) due to the breakdown of the surrounding cells. The cysts do not have a particular shape or size but appear as vertically aligned, sinuous and sometimes interconnected passages ending abruptly. The development is preceded by certain widespread changes like profuse development of parenchymatous tissues, disappearance of starch etc in both xylem and phloem. This observation is supported by work of Anderson and Dea (1968) who also reported lack of starch in the wood tissues of excised *A. Senegal*. A recent study by Joseleau and Ullman (1990) provides further biochemical evidence for the site of formation of gum arabic in *A. senegal*. By comparison of the carbohydrate analysis of the tissues from the inner bark, cambial zone and xylem of the gum producing branch with corresponding tissues of a none producing branch, they found comparable molar proportions of the sugars (galactose, arabinose, rhamnose and glucuronic acid) in the inner bark of the former branch as in gum arabic.

Gum is believed to be formed by a tree in order to seal off the injured parts, not so mucht o prevent infection, but to prevent loss of water (Smith and Montgomery, 1959). This suggestion is supported by Obeid and Seif El Din (1970) who noted that gum is exuded naturally from lesions caused by drought, sun scorch and fire or from wounds caused by animals as a defence mechanism to avoid dehydration. That it is produced under conditions of stress or disturbance when in stress is further reported by Awouda (1973) though his suggestion of defence against infection has been rejected.

1.1.3 Gum production

Gum is produced either naturally (spontaneous exudation) or after an injury. In countries like Sudan where gum production is an established activity, tapping is the common source of the commercial product. The process is carried out by cutting and peeling off pieces of bark, 10-20 cm long by 2-4cm wide from branches of the tree using either a traditional tapping axe or a sonke (modified spear like equipment designed for tapping). Gum exudes from the wounds as droplets of clear viscid fluid which cleverer in viscosity as to water loss by evaporation and harden from the outside. Continuous exudation forces the outer skin to break repeatedly allowing the droplet (nodule) to increase in size until the flow rate declines and the outer skin becomes too thick and hard. The size of the nodule is variable and ranges from 2-10 cm in diameter (Obeid and Seif El Din, 1970). The first crop of nodules takes 3-6 weeks to harvest, the exact period depending on climatic conditions. Subsequent exudates are harvested at shorter intervals of 1-2 weeks.

1.1.4 Gum grading

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 colour. 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 a sit 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 colour.

"Siftings and dust" the waste from other grades, particularly the cleaned and sifted grades.

At present, the Gum Arabic Company 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 colour in solution. French speaking countries in West Africa appear to export their gum under more or less same conditions. Principal producers are Chad and Senegal 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.

-gomme blonde darker colour.

-gomme petit blonde-small pieces of the same.

-gomme vermicelle -a whitish to pale yellow gum

-gomme fabrique–rejected pieces of gum (because of their dark colour).

1.1.5 Properties of gum arabic

1.1.5.1 Physical and chemical properties

Gum Arabic readily dissolves in water forming a slightly acidic solution with a pH range between 4.2 and 4.6. The acidity is due to glucuronic acid and its 4-0-methyl ether (Smith, 1939). Some of the free carboxyl groups are partly neutralized by calcium, magnesium, sodium, potassium and other cations in smaller amounts notably, iron, copper, zinc and manganese (Adamson and Bell, 1974; McDougal, 1987). It can thus be also referred to as the part neutralized salt of an acidic polysaccharide. Good quality gum arabic dissolves in water to give colourless or pale yellow solutions with a sweet smell. Gums that give coloured solutions, are less soluble or have a distinct rotten or irritating smell are considered to be of poor quality. In solution, gum arabic gives a negative optical rotation. Typical values for Sudanese and Nigerian samples have specific rotation values between -27° to -33° (Anderson et al, 1990). It is long known that optical rotation is influenced by the composition and nature of the sugars present (Stoddart, 1971).

One of the important physical properties of gum arabic is its ability to dissolve in water to yield solutions of very high concentrations (up to 55%). At 5% (w/v) it forms solutions of low viscosity in comparison to other naturally occurring hydrocolloids. This property makes the gum very useful commercially (Whistler, 1959). Analytical studies on a wide range of samples of gum Arabic gave mean intrinsic viscosity values of 16 ml/g for Sudan and 18 ml/g for Nigeria with a range from 13-22 ml/g for a 1% concentration (Anderson et al, 1990). The viscosity of gum arabic has been shown to be closely related to molecular weight (both being dependent on molecular size distribution and shape). Measurement of intrinsic viscosity allows the estimation f a molecular weight value from an expression (Mark Houwink) of the form $(n)_i$ = K.Mwa (Anderson and Rahman,1967) where:

$(n)_i$ = intrinsic viscosity (ml/g)

K = a constant characteristic of the polymer and solvent at a specified temperature.

Mw = weight average molecular weight.

a = a constant related to the shape of the polymer.

Anderson and Rahman (1967) deduced the values as K= 1.3X 10^{-2} and a = 0.54 for gum arabic. It has long been established that the molecular weight distribution is broad and skewed and has values ranging from 0.1 x 10^6 to 1.18 x 10^6. A value of 0.58 X 10^6 has been considered as most representative. Vandevelde and Fenyo (1985) found values from 0.44 X 10^6 to 2.2 x 10^6 for gum arabic based on laser light scattering technique. Because of the broad distribution exhibited, the term heteropoly molecular is also applied to such a gum i.e. a polymer system having either a variation in monomer composition and/or a variation in the mode of linking and branching of monomer units in addition to a distribution in the molecular weight (Anderson and Stoddart, 1966).

Complete hydrolysis of gum arabic with dilute acid yields D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid and its 4-0-methyl ether (Cree, 1966). Cree gave a detailed review of the properties of gum arabic which revealed that the molar proportions of sugar residues in the gum are of the order of 3: 3: 1: 1 for galactose, arabinose, rhamnose and glucuronic acid respectively. However, values seem to vary from 3: 2: 1: 1 to 3:3: 1: 1 to 4: 2: 1: 1 (Anderson et al, 1990, 1991) which appear to reflect variation in regions and variety. The acidic component in the gum is usually expressed as uronic acid anhydro sugar. Analysis shows that it consists of glucuronic acid and its 4-0-methyl ether with values ranging from 13-25% and 0.24-1.5% respectively (Cree, 1966; Anderson et al, 1990).

Gum arabic also contains protein aceous material covalently bonded to the polysaccharide. Values of protein content vary from 1.9-2.3% though higher values of 4.7% have been observed in some gums (Anderson et al, 1990; 1991). The peptide/protein part contains eighteen amino acids of which hydroxyproline, proline, serine, threonine and leucine account for 82% (Anderson and McDougal, 1987). Most of the amino acids are contained in the internal structure of the gum (i.e. in the branched galactancore) with only a smaller part associated with the periphery. This partly explains why the amino acid content of the gum cannot be readily reduced by mild chemical treatments or by action of enzymes. Further, gum cannot be completely deprotenised without gross degradation of the gum molecules and destruction of its functionality (e.g. emulsification) and surface activity.

As earlier mentioned, gum arabic contains cations which exist as part neutralized salts of acidic polysaccharides. A total of fourteen cations have been detected in the gum though calcium, potassium, sodium and magnesium are considered as most abundant (Douglas, 1989). The actual amount depends on the relative abundance of the elements in the soils at different locations. Higher occurrence of some elements, particularly the heavy metals for which upper limits are specified by regulatory authorities can lead to rejection of such gums for food uses (Anderson and Weiping, 1991).

1.1.5.2 Molecular structure and properties

The first elaborate description of the structure of gum from *Acacia Senegal* was by Anderson and Stoddart (1966) based on the results of sequential Smith degradation (Goldstein et al, 1965). They showed that the gum molecule consists of Q-D-(1-3) galactan core and ß-D-(1-6) linked galactan branches ramified with side chains of arabinose, rhamnose and glucuronic acid as terminal groups. Subsequent work on whole and partially hydrolyzed gum subjected to second Smith degradation followed by gel permeation chromatography showed that the gum consist of uniform subunits of fl-D-(1-3) galactopyranose residues of about 8000 molecular mass (Churms et al; 1983) while further work by Street and Anderson (1983) revealed that the lowest Smith degradation has 116 ß-D-(1-3) linked product about galactopyranose blocks with (3-D-(1-6) linked branch units. Re-interpretation of the revised Street and Anderson structure (1983) using computer modeling and stepwise reconstruction of structures of precursors has led to a possible structure (Osman, 1993). In the structure arabinose occurs partly as short arabin of uranosyl side chains and partly as arabin of pyranosyl end groups of (3-D-(1-3) linkage. Rhamnose is present entirely as end group and is said to be attached to carbon 4 of glucuronic acid. The linkage is thought to be (1-4) ß-D-glucuronic either a-L-rhamnopyranosyl acid or its j3-D glucuronic acid (4-ome).

Gum arabic is also known to contain small amount of protein (Anderson and Stoddart, 1966) and hence belongs to a group of proteoglycans known as Arabinogalactan proteins (Fincheret al, 1983; Akiyama et al, 1984). Confirmation that it is indeed an arabinogalactan protein complex came from the work of Vandevelde and Fenyo (1985) who used size exclusion chromatography to separate the gum into two components; a major component comprising 70% of the total gum but deficient in protein and a minor protein rich component consisting of 30% of the gum. On the basis of this information, two models describingthe structure of gum Arabic as an Arabinogalactan Protein (AGP) have been proposed, the "wattle blossom" and the "twisted hairy rope".

1.1.6 Uses

Gum arabic is used in food and nonfood industrial applications (Sandford and Baird, 1983).

1.1.6.1 Food and allied applications

The food industry is the main consumer of world production of gum

arabic. The main areas are bakery, noncarbonated beverages, confectionery, soft drinks and brewing. Its major function in the bakery industry is to act as an adhesive (bakery glaze) while in confectionery it is used as a crystallization inhibitor (retard crystallization of sugar), emulsifier (assists in attaining a fine dispersion) and stabilizer i.e maintains the dispersion (Adamson and Bell, 1974). Emulsifying property is believed to be due to the gum containing hydrophobic protein moieties and hydrophilic carbohydrate residues (Randal et al, 1988).Good emulsifiers have high content of hydrophobic aminoacids. The properties of emulsification and stabilization enables the gum to be used in other areas like foam stabilization. Its high solubility in water, nontoxic, colourless, odourless and tasteless nature make sit a suitable additive to various formulations to provide "body", "mouth feel" and texture to foods. It has been awarded the status "Acceptable Daily Intake (ADI) Not Specified" by FAO (1982) following extensive toxicological studies.

To prevent gum from botanical sources other than that from *A. Senegal* being used in the food industry without having been subjected to positive toxicological evidence of safety, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recently published a revised specification for gum Arabic (FAO, 1990).

The following specifications must be met:

- Loss on drying -not more than 15% (105°, 5h)

-Specific rotation between -26° to -34° (calculated on dry weight basis).

- Total ash-not more than 4%.

- Acid insoluble ash-not more than 0.5 %

- Acid insoluble matter not more than 1%

- Starch and dextrin- absent

- Tannin bearing gums -absent

- Nitrogen 0.27% to 0.39%

- Arsenic not more than 3 mg/kg

- Lead not more than 10mg/kg

- Heavy metals not more than 40

- Microbial criteria –*salmonellasp*. negative in 1gm; E. coli negative in 1gm.

1.1.6.2. Industrial applications

1.1.6.2.1 Pharmaceutical and cosmetic industry

The properties of gum arabic as an emulsifier/stabilizer and binding agent lead to its use in the above industry. It is used in coating sugar coated pills and drug encapsulation. It has been used in the preservation of vitamin A in margarine and stabilization of vitamin C in aqueous solutions. Moreover, it produces a smooth viscous syrup and prevents crystallization of sugar in the preparation of cough syrup (Adamson and Bell, 1974).Its use in the cosmetic industry arises from the nontoxic nature and low tendency to produce allergic reactions. It is thus used in lotions and protective creams where it stabilizes the emulsion and forms a protective coating.

1.2 *Acacia seyal*

Acacia seyal Del (family *Leguminosae*, subfamily *Mimosoideae*). Combines tolerance of periodically, inundated heavy clays with major roles in fuel and fodder production in countries at the southern edge of the Sahara desert, especially Mali, Chad and Sudan (Abdel Nour, 2008).

Figure (1.2.1): *Acacia seyal* (Talha Gum) tree

Acacia seyal is evidently Sudan's number one tree. Its potential has never been fully explored, but they are promising. The emerging global demand for Talha gum is still to be noted (Eltayb et al, 2013). Trees are 3-17m high, bark powdery, smooth or sparsely flaking, whitish, greenish yellow or orange red, sometimes green and red bark occur on the same tree. Flowers Nov.-April; peak in Jan, fruiting Jan.-May, peak in March Stands of var. *seyal* has been established in Sudan, often by direct sowing of pretreated seeds to prepare planting spots. Sowing seed in batches ensures that a high proportion of the spots become occupied. Competition from weed growth is overcomes by using Taungya, with mechanized site preparation and sowing. Sesame or sorghum is intercropped among widely spaced. *A. seyal* on favourable sites produces fast juvenile growth (more than one m per year), maximum height reached after 8-10 years (Eltayb et al, 2013).

In the Sudan the Gum Arabic is cultivated form mainly two trees: *Acacia Senegal* which is called Hashab and *Seyal* which is Talha. In Nigeria, Gum Arabic is classified into grade 1 (*Acacia Senegal*), grade 2 (*Acacia seyal*) and grade 3 (Combretum and other sources). In Zimbabwe, Gum Arabic is derived from A. Karroo. Although there are over 1,100 *Acacia* species worldwide, *Acacia Senegal* and *A. seyal* remain the most commercially exploited species (Elrafie, 2009**)**.

In Sudan, *Acacia seyal* is mainly, managed for the production of fuel wood (Elsidding, 2003) as it produces good and dense firewood (Orwa*et al*., 2009). The species is found to produce a significant amount of gum (locally named as gum Talha) and contributes to about 10 percent of Sudan’s gum production (Gum Arabic Company, GAC, 2008). Gum Talha, now, account for chuck more than 70% of Sudan production and almost 100% of the world market need (Gum arabic board, 2015) The development of production and yield of gum from *Acacia* *senegal* overtime was analyzed by Ballal (2002) for eight years from 1993-2000, and IIED and IES (1990) over a period of 1958 to 1988. An analysis of data from 1949-2008 indicates that production of gum Talha in Sudan is variable overtime (GAC, 2008); however, the amount of produced gum is generally increasing with an average annual production of 3100 metric tones. The variation in gum production could be attributed to environmental factors, such as rainfall and temperature, (Ballal, 2002; Chiveu *et al*, 2009), deforestation (Rahim, 2006) and price polices (Elmqvist *et al*., 2005). Ballal (2005b) revealed a positive relationship between yield of gum hashab and rainfall. However, for the case of gum Talha, El Wasila (1994) reported that gum Talha is collected when there is a short fall in production of gum hashab. This reason might explain the huge variation in production of gum Talha over time beside the factors mentioned above (Ballal, 2002; Chiveu *et al*., 2009; Elmqvist *et al*., 2005; Rahim, 2006). Little is know about reaction of *Acacia seyal* trees to different management regimes. Several scholars have assessed some managerial factors such as stand density, tapping techniques and time of tapping on productivity of gum Talha (Ali, 2006; Fadl and Gebauer, 2004; Mohammed and Röhle, 2011). However, analysis of gum Talha yield and pick-to-pick yield has given little attention.

Information on gum Talha yield and the analysis of yield determinants strategies is paramount important as it provides useful information for developing good management regimes of *Acacia seyal* tree.

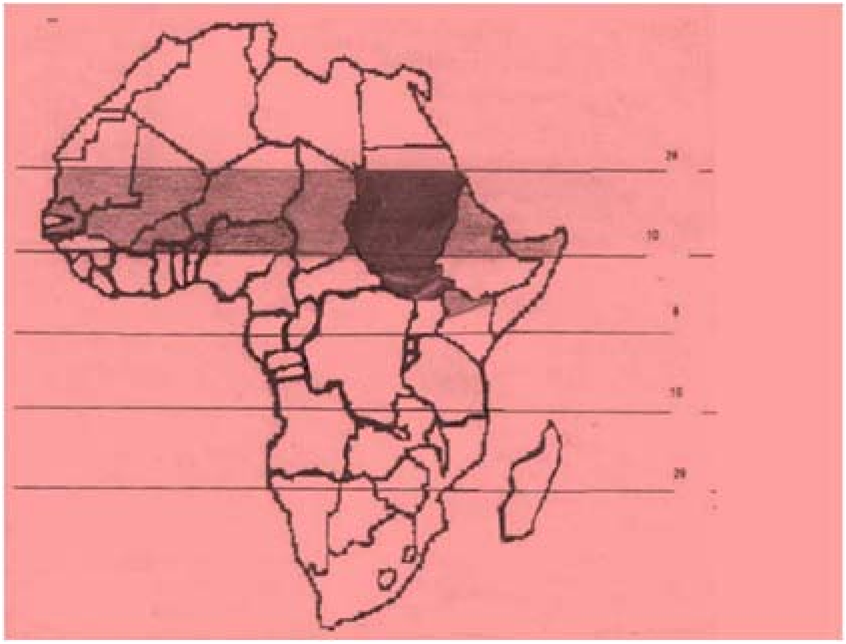

Figure (1.2.2): Map of Africa Showing Gum Arabic and Talha Gum Belts

1.2.1 Management of *Acacia seyal* natural stands in Sudan

In Sudan, both varieties of *A. seyal* are extensively managed for firewood and charcoal production. Historically people used *A. seyal* for generations for different purposes but mainly for the supply of firewood and charcoal (McAllan, 1993). A considerable proportion of fuel-wood derived from natural forests comes from *A.seyal* natural stands. Hence, the sustainable management of these natural stands is mainly undertaken for these purposes (Elsiddig, 2003a).

Generally, in semi-arid savannas the growth rate of *A. seyal*is low; however, early growth rates can be quite fast, trees can reach up to 1 meter in 3 months on favorable sites (McAllan, 1993). As indicated by Mustafa (1997), *A. seyal*trees can reach its reproductive stage rapidly, within 5 years in a natural stand, unless the growth is retarded by local events such as intensive browsing or fire. The periodic increment (PI) of the diameter at breast height and volume, respectively, does not exceed 1.3 cm and 5 m^3/ha in 3 years (Vink, 1990a). In Sudan, the growth and yield of *A. seyal* vary according to region. For example, the mean annual increment (MAI), of *A. seyal*, in Garri forest, Blue Nile, ranged between 1.6-2.4 m^3/ha/year during 1963-1966, where recorded annual rainfall was 657 to 718 mm. However, the MAI ranged between 1-1.5 m^3/ha in the Rawashda forest in eastern Sudan (Vink, 1990a), where annual rainfall ranges between 450-500 mm. Trees managed on a 10-15 year rotation yield 10-35 m^3/ha of fuel-wood per year (Orwa *et al*., 2009).

1.2.2 Volume and height functions for *Acacia seyal*

The volume estimate in forest stands, adequate and reliable allometric functions are needed to be established (Bjarnadottir *et al*., 2007). These functions (formulas 1 and 2) mathematically describe the relationship between the tree volume and diameter at breast height (DBH) and/or the height of the tree (Bjarnadottir *et al*., 2007; Pretzsch, 2009; West, 2004).

As the management of *A. seyal* in Sudan is mainly undertaken for fuel wood, most of the studies were conducted as part of the project "fuel wood development for energy in Sudan". Volume and height functions have been developed, respectively, for predicting volume and the height of *A. seyal* in natural stands (Elsiddig, 2003b).

1.3 Objective

The purposes of this study are:

1- To establish physico - chemical properties and composition of the gum Talha.

2- De-mineralize the gum solution by an ion exchange resin.

3- Magnesium Glucouronate (milk of magnesium) from gum Talha Arabic acid.

CHAPTER TWO

MATERIALS & METHODS

2. MATERIALS AND METHODS

2.1 Collection of samples

Two samples of *Acacia seyal* were collected.

2.2 Samples preparation and treatment

Samples were kept in clean, dry, plastic containers. They were then ground, using mortar and pestles to fine particles and saved in clean, dry glass bottles.

Analysis was carried at the chemistry labs in college of science (Sudan University of science and Technology) and chemical laboratory (Ministry of minerals).

2.3 Chemicals and materials

- Sulphuric acid (2M).

- Barium chloride.

- Sodium hydroxide

- Magnesium bicarbonate.

- Hydrogen peroxide (10%).

- Amberlite cation exchange resin.

- Nitric acid.

- Distilled water.

2.4 Apparatus and Instruments

- Porcelain crucible.

- Beaker

- Measuring cylinder
- Weight bottle
- Sensitive balance
- Hot air oven
- Thermostatic water bath
- pH meter.
- Polarimeter.
- Atomic absorption spectroscopy.
- Mortar and pestle.

Figure (2.1): *Acacia seyal* gum (Talha gum)

2.5 Methods analysis

2.5.1 Moisture content

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

Moisture content (%) = w1-w2/w1 x100

w1 = original weight of sample (g).

w2 = weight of sample after drying (g).

2.5.2 Ash content

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

$$\text{Ash content (\%)} = \frac{(B - C) \times 100}{A}$$

Where:

A = sample weight at grams

B = weight of crucible and dish content in grams.

C = weight of empty crucible in grams.

2.5.3 pH

pH meter was calibrated using two different buffers solutions at pH 4 and pH 11. then after calibration it was used for determination of the pH of the gum fractions of 1g/100ml aqueous solutions (w/v) calculated on dry weight basis in Figure (2.5.1).

Figure (2.5.1): pH meter

2.5.4 Specific optical rotation

100 mg gum was dissolved in 10 ml water (1% w/v) overnight. The solution was filtered, placed in a glass cell of path length 10 cm care being taken to avoid air bubbles getting trapped in the solution and measured in a Perkin Elmer Model 141 polarimeter Figure (2.5.2) set at 589 nm wavelength and 20° ± 2°C. The degree of rotation was displayed and read from the instrument panel and corresponding specific rotation was calculated from the relationship:

$[\alpha]_D = 100a/L.\ C$

Where:

α = the measured rotation

D= wavelength of light (Sodium line, 589 nm)

L= path length in decimeters

C= concentration of solution in gm/ 100 ml

The results were corrected for loss on drying.

Figure (2.5.2): Polarimeter

2.5.5 Total Nitrogen

500 mg of gum samples were transferred into digestion tubes. 15 mlconc. sulphuric acid plus sufficient amount of catalyst (sodium sulphate, copper sulphate and Selenium as catalyst, ratio 20: 2: 1) were added to the tubes and then placed in the "Tecator" digestion heating system (model 1015) preheated to 240°C. Complete digestion is attained when the heated solutions turn to a pale blue colour indicating that gum nitrogen has been converted to ammonium sulphate. The tubes were allowed to cool for 10 minutes and 70 ml of distilled water added. Two tubes containing 15 ml sulphuric acid plus catalyst (as blanks) were included in the digestion. The solutions were made alkaline with 50 ml of 10M (40% w/v) sodium hydroxide in akjeltec distillation "Tecator" unit (model 1002) followed by steam for five minutes.

The total gaseous ammonia liberated was estimated by bubbling through 50 ml of 2% boric acid with bromophenol blue as indicator. The resulting solution was titrated against 0.025M sulphuric acid. Nitrogen content was calculated from the relationship:

1 m10.5M H_2SO_4 = 14 mg N;

20 ml 0.025M H_2SO_4= 14 mg N

Hence N% = (Y ml x 14) x 100

20X

where:

Y ml = volume of 0.025M H_2SO_4 used

X mg = weight of gum sample used (gm).

2.5.6 Uronic acid anhydro and hence glucuronic acid

70g gum was dissolved in 500 ml of water (in a 250 ml conical flask) overnight. Gum solution was filtered and uronic acid anhydride determined by method of cation exchange resin. Amberlite cation exchange resin, IR- 120 (H+ form) was packed half full in a glass column. The column was washed with 2M sulphuric acid and excess acid removed by washing down the column with deionized water until neutral. A sample of gum solution was then passed down the column slowly, the eluent being collected in a 250 ml conical flask.

Three bed volumes of deionized water was washed down the column and collected in the conical flask. The combined volume was titrated against 0.01 M sodium hydroxide using phenolphthalein as indicator.

Thus the acidity of the gum solution was measured and equivalent weight (in grams) of dry salt free gum required to neutralize one mole of sodium hydroxide estimated as:

$$\text{Eq. wt. (gm)} = \frac{\text{sample wt. g x 1000}}{\text{Vol. of titre x Molarity of NaOH}}$$

Hence uronic acid content is calculated from equivalent weight as:

$$\text{UAA} = \frac{\text{molar mass of UAA X 100}}{\text{acid eq. wt.}}$$

Where:

UAA = uronic acid anhydro

Glucuronic acid is expressed as a percentage of uronic acid anhydro because that best quantifies the amount of glucuronic acid present in a polymerised form (Anderson, 1993).

2.5.7 Preparation of magnesium glucournates

50 ml of uorinc solution was neutralized by 16.71g of magnesium bicarbonate, the solution was dried and then the precipitate (A) was ground using mortar and pestle.

2.5.8 Mineral contents

200 mg ash of two samples (S1 and S2, S1: ash of pure gum sample, S2: ash of sample (A) were placed in different volumetric flask, there were dissolved in 10 ml conc. nitric acid and the solution made up to 25 ml in a volumetric flask. About 10 ml was carefully removed and used in the determination of trace elements using Atomic Absorption Spectroscopy (AAS) in Figure (2.5.3).

1 ml was removed from the stock solution (original 25 ml) and 100 ml water added. The resulting solution was used for analysis of calcium and magnesium. For each metal, standard solutions were prepared with pure metal salts and used to calibrate the spectrometer.

Figure (2.5.3): Atomic absorption spectroscopy

CHAPTER THREE

RESULTS & DISCUSSIONS

3. RESULTS AND DISCUSSIONS

Table (3.1): Physicochemical properties of *Acacia Seyal*

Moisture (%)	Ash (%)	Nitrogen (%)	pH	Specific optical rotation
10.79	1.1294	0.15	4.5	+46.1

Table (3.2): Minerals analysis of the *Acacia Seyal* samples

Acacia Seyal	Ca (ppm)	Mg (ppm)
S1	1934.4	19.86
S2	65.22	1264.8

3.1 Moisture content

Table (3.1) showing the physicochemical properties of *A. Seyal* gum. The moisture content value (10.79%) which was in the range of International specifications of quality parameters of gums (10-15%). The moisture content value was comparable to those moistures contents values (9.76%, 9.56%, 8.35% and 8.49%) reported by (Rabeea et al, 2016). Also the value of moisture content was higher than to moisture contents value (5.94%) reported by (Eiman et al, 2016). But it less than moisture content value (13.04%) reported by (Talaat D et al, 2014). Loss of moisture content was observed to drop with storage time.

3.2 Ash content

The ash content value (1.1294%) which was in the range of International specifications of quality parameters of gum Arabic (2-4%). The ash content value was less more than those ash contents values (3.4%, 2.5%, 3.13% and 2.05%) by (Rabeea et al, 2016). Also it less than ash contents values (4.96% and 2.8%) reported by (Eiman et al, 2106 and Talaat D et al, 2014) respectively.

3.3 Nitrogen content

The nitrogen content (0.15%) which was in the range of International specifications of quality parameters of the gum free *Acacia Seyal*. The nitrogen value was less than nitrogen content values (0.327%, 0.630%, 0.243% and 1.549%) by (Rabeea et al, 2016). But are similar to nitrogen content value (0.14%) reported by (Talaat D et al, 2014).

3.4 pH

The pH value (4.5) which showed similarity and difference comparable to pH values (4.94, 4.53, 4.84 and 4.45) reported by (Rabeea et al, 2016).

3.5 Specific optical rotation

The specific optical rotation was +41.5° which was more high and more less comparable to by (Rabeea et al, 2016) (-48.25°, +56°). But the specific optical rotation value of sample showed similarity to specific optical rotation value (45°) reported by (Talaat D et al, 2014).

3.6 Mineral contents

The mineral contents value of *Acacia Seyal* samples (S1 and S2) in Table (3.2). S1 was the gum sample before added $MgHCO_3$, but S2 was the gum sample after added $MgHCO_3$, the value of Ca in S1 (1934.4 ppm) but in S2 was (65.22 ppm), the Mg value in S1 (19.86 ppm) but in S2 (1264.8 ppm), that big variation return to the preparation process in sample S2 by treatment the gum sample by resin and added of $MgHCO_3$.

3.7 Conclusion

Physicochemical properties of *Acacia Seyal* (Talha) samples were studied (moisture, ash, nitrogen, specific rotation, pH and mineral contents). The results were good comparable to other studied and show that the sample used is authentic *A. Seyals* were prepared using Arabic acid from $MgHCO_3$ and gum Talha.

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