Shendi University

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Physicochemical Characterization of *Acacia tortilis var spirocarpa* Gum of Sudanese Origin

A Thesis Submitted to Faculty of Graduate Studies, Shendi University in Fulfillment of the Requirements for the Degree of M.Sc in Chemistry

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الآستهلال

بسم الله الرحمن الرحيم

(اللّهُ لاَ إِلَهَ إِلاَّ هُوَ الْحَيُّ الْقَيُّومُ لاَ تَأْخُذُهُ سِنَةٌ وَلاَ نَوْمٌ لَّهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الأَرْضِ مَن ذَا الَّذِي يَشْفَعُ عِنْدَهُ إِلاَّ بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ وَلاَ يُحِيطُونَ بِشَيْءٍ مِّنْ عِلْمِهِ إِلاَّ بِمَا شَاء وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالأَرْضَ وَلاَ يَؤُدُهُ حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيمُ)

سورة البقرة (255)

DEDICATION

I dedicate this work to:

My parents

My brothers and sisters

ACKNOWLEDGMENT

My deep thanks and prayers to Almighty Allah for giving me health and desire to successfully complete this work.

I cannot express enough thanks to my supervisor, Dr. **Elfatih Ahmed Hassan**, not only for offering me the opportunity to work with him but also for his endless patience, support and guidance. It has been an honor and a privilege to work with him, and above all I am deeply indebted to him for his kind character. I hope that one day I will become as good an advisor to my students as Dr. **Elfatih** has been to me.

My thanks would be extended to all those who helped, taught me during the achievement of this work.

ABSTRACT

Characterization and physicochemical study on *acacia tortilis var spirocarpa* gum samples was undertaken studied. Results showed that moisture content (7.64%), ash content (2.35%), refractive index (1.34), nitrogen (2.31%), protein (15.48%), pH (5.64), acid equivalent weight (4000), specific optical rotation (+64.67°), viscosity (6.71 cm^3g^{-1}). Number average molecular weight (2.5 x 10^5 g/mol)]. The minerals were in the order: K > Ca > Na > Mg > Fe > Mn > Zn > Cu > Ni > Pb]. The sugar contents of *Acacia tortilis var. spirocarpa* gum were measured using HPLC technique. The results showed that arabinose had a highest percentage (48.99%) than galactose (20.40%) and rhamnose (4.93%). *Aacacia tortilis var spirocarpa* gum sample has good stabilizing properties for emulsifiers when used as a stabilized material, the highest value of emulsification is one the seventh day, the emulsifier is completely stable at the eighth day and the following days.

المستخلص

تمت دراسة الخصائص الفيزوكيميائية لصمغ السمر و كانت النتائج كالآتى: [(محتوى الرطوبة = 7.64%) ، (الرماد = 2.35%)، (معامل الانكسار = 1.34)، (النتروجين = 2.31%)، (البروتين = 15.04%)، (الآس الهيدروجينى = 5.64)، (الوزن المكافىء = 4000)، (مجموع حمض اليورينك = 4.85)، (الدوران الضوئى النوعى = +64.67°)، (اللزوجة = 6.71 سم^3 جم$^{-1}$)، (متوسط الوزن الجزيئى = 2.5×10^5)].

وجدت المعادن على النحو الآتى: K > Ca > Na > Mg > Fe > Mn > Zn > Cu > Ni > Pb . تم تقدير المحتوى السكرى لصمغ السمر بواسطة جهاز الكرموتغرامى ذو الفصل العالى، وأظهرت النتائج أن الأريابونوز له أعلى نسبة (48.99%) مقارنة بالجلاكتوز (20.40%) و راحمنوز (4.93%).

أظهر صمغ السمر خواص تثبيتية جيدة للمستحلبات عند استخدامه كمادة مثبتة، أعلى قيمة لثبات المستحلب تكون عند اليوم السابع ثم يكون المستحلب ثابت تماماً عند اليوم الثامن والأيام التى تليه.

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Chapter One

1. Introduction

1.1 Gums

Gums have been used by man for millennia 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-arid agro ecologies are used for making different beverages, medicines, and water-soluble glues (EFAP, 1994; FAO, 1995a). In food industry, gum is used as thickening, stabilizing, emulsifying and suspending agent, besides their applications in making foods and drinks. In pharmaceutical industry, gum is 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 the 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, Ethiopia, Mali, Mauritania, Niger, Nigeria, Senegal and Tanzania, according to their importance (Seif el Din and Zarroung, 1996). Total gum production in Ethiopia is approximately 3000 tones per annum and only an estimated 50% of the produce is exported through formal trading channels (EFAP, 1994).

1.2 Gum Arabic

Gum arabic is the dried gummy exudate from the stems and branches of *Acacia Senegal* (L) Wild, or of other related species of *Acacia* (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 internally 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 used 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 (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 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 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, 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 arabic gum, but a wide range of other tree xudates 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 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, 1973; 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 (Awouda, 1973; Abdel, 1978; 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 low 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 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 (Ghosh and Purkayastha, 1962; Greenwood and Morey, 1979; Luckner, 1990).

1.2.1 Definition and nomenclature

Although different regulatory organisations have defined gum Arabic of commerce somewhat differently, they all acknowledge it as a dried gummy exudate obtained from the stems and branches of gum Willd or closely related species (US pharmacopoeia 1985; FAO, 1990).

The name gum Arabic is derived from the fact that it is formerly, 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 Bura dima or (Boran), Iderikes (Samburu), Idado (Somali), Mongoli (Kamba), munshuin (Maasai), Chepkomon (Kipsigis), Matengewa (Bajuni) and Kikwata (Swahili) (Coe and Bentjee, 1991).

1.2.2 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 gum 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 parenchyma strands of the phloem adjacent to the cambial zone. They are first developed schizo genously but later on enlarge considerably (lysi genously) due to the breakdown of the surrounding cells. 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 gum. A recent study by Joseleau and Ullman (1990) provides further biochemical evidence for the site of formation of gum arabic. 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 much to 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.

1.2.3 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 2-4 cm wide from branches of the tree using either a traditional tapping axe (modified spear like equipment designed for tapping). Gum exudes from the wounds as droplets of clear viscid fluid which increase in viscosity as they lose water 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 6 weeks to harvest, the exact period depending on climatic conditions. Subsequent crops are harvested at shorter intervals of 2 weeks.

1.2.4 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 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 colour.

“Siftings and dust” the waste from other grades, particularly the cleaned and

sifted grades.

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. Also, smaller quantities come from Mali, Niger and Mauritania. About six grades are recognised (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 colour.

-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 colour).

1.2.5 Properties of gum arabic

1.2.5.1 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 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 of a molecular weight value from an expression (Mark Houwink) of the form $(\eta) = K.Mw^a$ (Anderson, 1967) where:

(η) = 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 $K= 1.3 \times 10^{-2}$ 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 0.1×10^6 to 1.18×10^6. A value of 0.58×10^6 has been considered as most representative. Vandevelde and Fenyo (1985) found values from 0.44×10^6 to 2.2×10^6 for gum arabic based on laser light scattering technique. Because of the broad distribution exhibited, the term heteropolymolecular 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 proteinaceous 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).

1.2.5.2 Molecular structure and properties

The first elaborate description of the structure of gum 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 modelling and stepwise reconstruction of structures of precursors has led to a possible structure (Osman, 1993). In the structure arabinose occurs partly as short arab in of uranosyl side chains and partly as arab in opyranosyl 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-glucuroniceither 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 Arabin ogalactan proteins (Fincher *et a*l, 1983; Akiyama *et al*, 1984). Confirmation that it is indeed an arabin ogalactan protein complex came from the work of Vandevelde and Fenyo (1985) who used size exlusion

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 describing the structure of gum Arabic as an Arab in ogalactan Protein (AGP) have been proposed, the "wattle blossom" and the "twisted hairy rope".

1.2.6 Uses of gums

Gums are used in food and nonfood industrial applications (Sandford and Baird, 1983).

1.2.6.1 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 amino acids. 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 makes it 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 diving -not more than 15% (105°C, 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 –*salmonella sp*. negative in 1gm; E. coli negative in 1gm.

1.2.6.2. Industrial applications

1.2.6.2.1 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.

1.3 *Acacia tortilis*

1.3.1 Taxonomy status

Kingdom : *Plantae*

Phylum : *Tracheophyta*

Class : *Magnoliopsida*

Order : *Fabales*

Family : *Fabaceae*

Genus : *Acacia*

Species : *tortillis* (Sunita, 2016)

Subspecies: *A. tortilis var. spirocarpa, A. tortilis var. raddiana*

Figure (1): *Acacia tortilis var. spirocarpa* tree (Sunita, 2016)

1.3.2 Description and distribution of *Acacia tortilis*

Acacia tortilis is a thorny legume tree, usually about 4 to 8m high but can grow up to 20m (Orwa *et al.,* 2009). The crown is dense, umbrella-like

and flat-topped; hence it is called Umbrella tree in some areas. Leaves are compound and the leaflets (6- 22 pairs) are very small (1- 4 mm long x 0.6 – 1 mm broad), glabrous to pubescent. Flowers are white, cream or yellow and highly aromatic. Fruits are distinguished by twisted shape; hence the epithet "tortilis" (Ecocrop, 2009). *Acacia tortilis* is native to semi-arid areas of Africa and Middle-East. It is found between 15 and 30ºN and between Sea level and 1000m altitude (Araya *et al.,* 2003). *Acacia tortilis* is tolerant to severe drought due its deep taproot system. *Acacia tortilis* is nitrogen fixing species and thus a soil improver (FAO, 1985). The tree is used in controlling soil erosion since it can grow fast and stabilize shifting dunes or hill slopes. If introduced in humid areas where fire wood and grazing land are inadequate, the specie can grow out of control and become nuisance (Ecocrop, 2009).

1.3.2 Chemical composition of *Acacia tortilis*

Acacia tortilis is considered as one of the valuable sources of protein for herbivorous (Dube *et al.,* 2001; Ngambi *et al.,* 2009). Its leaves contain high levels of crude protein (CP), ranging from 140 to180 g/kg. It has moderate levels of detergent fibre (Table 1.1). The levels of minerals in *A. tortilis* leaves are also favorable (Table 1.2) (Abdulrazak *et al.,* 1999). Nutrient composition depends on various factors such as soil condition, season and stage of leaf growth (Nyamukanza and Scogings, 2008).

Table (1.1): Chemical composition (g/kg) of *Acacia tortilis* leaves

Chemical composition	Mean	SEM
Dry matter	955	24.2
Ash	41	16.7
Crude protein	189	2.4
Crude fiber	184	6.8
Neutral detergent fiber	195	94.3
Acid detergent fiber	169	80.4
Acid detergent lignin	82	1.3
Hemicellulose	45	1.6
Crude fat	43	1.4
Gross energy (MJ/kg)	18	-
Digestible energy (MJ/kg)	12	-

Sources: Abdulrazak *et al*. (2000); Dube *et al.* (2001); Rubanza *et al.* (2005) and Heuzé and Tran, (2011).

Table (1.2): Minerals content (g/kg) of *Acacia tortilis* leaves

Mineral	Concentration (g/kg)
Calcium	6.1
Phosphorus	1.8
Magnesium	1.5
Potassium	11.4
Sodium	0.5
Zinc	21.6
Copper	17.2
Manganese	12.3
Iron	22.3

Source**:** Abdulrazak *et al.* (1999); Heuzé and Tran (2011).

1.3.3 *Acacia tortilis* Gum

Acacia tortilis var spirocarpa, is one of the most wide spread trees in, seasonally, dry areas of Africa and the Middle East. The umbrella thorn is the dominant tree in many savanna communities and provides an important source of browse for both wild and domesticated animals (Nuha *et al*., 2016).

1.3.3.1 Chemical constituents

Fatty acid: 19% oleic acid, 72% linoleic acid, 60% linoleic acid from the seed.

Tannin: The leaves, and to a lesser extent the bark, of many species contained between 1 and 8% hydrolyzable tannins (Sunita, 2016).

Gum : *A. tortilis* contins Uronic acid (8%), Galactose (23%), Arabinose (66%), Rhamnose, nitrogen 0.99%, protein content 6.18%, pH 6.46 (Sunita, 2016).

Flavenoid : Apigenin-6,8-bis-C-β-d-lucopyranoside (vicenin)8 Rutin (quercetin 3-O-rutinoside) (Sunita, 2016).

1.3.3.2 Chemical Composition and Structural features of *Acacia* Gums

Gums are complex copolymer of polysaccharide obtained as mixed calcium, magnesium and potassium salt, with high molecular mass and a complex structure. However all Acacia gums are Arab inogalactan-proteins (AGP) and it was described as heterogeneous. The heterogeneous nature of the gum has been studied extensively using different techniques: hydrophobic affinity chromatography, anion-exchange chromatography (Nuha *et al*., 2016).

1.3.3.3 Physical properties of *Acacia tortilis* gum

Colour: The color of the gum nodules is pale yellow to brown.

Shape: The shapes of the gum nodules, as exuded naturally, are irregular or tear shaped.

Solubility: *Acacia tortilis* gum is highly soluble in water forming transparent solution, and classified as a soluble gum (Nuha *et al*., 2016).

1.4 Physicochemical properties of gums

The identification of a particular gum from a series of different gum exudates needs an extensive number of analytical tests to perform as shown in Table (1.3). This approach enables "a chemical finger print" of each gum to be determined. The five most important parameters that can be used to identify raw gums and distinguish them from other *Acacia* gums are: (1) Specific optical rotation, (2) Nitrogen content, (3) Ash content, (4) Moisture content and (5) Tannins contents (Karamallah, 1999). The most fundamental properties of a gum which makes it unique amongst polysaccharide generally are its solubility and viscosity. The majority of gums dissolve in water at different concentration, gums readily dissolves in cold and hot water in concentrations up to 50%.

1.4.1 Moisture content

Moisture content of the gum determines the hardness of the gum and hence the variability of densities and the amount of air entrapped during nodule formation. It can be determined by measuring the weight lost after evaporation of water. Reducing the moisture content of the natural gum can be readily used as a tenable method of reducing the microbial counts (Karamallah, 1999).

Anderson *et al*., (1963) reported the moisture content of gum in the range from 11% to 16.1%. Randall *et al*., (1988) found that the moisture content of gum in Kordofan was 15.5%. Osman (1993) reported the moisture content of gum was range between 12% to 15%. Osman (1993) reported the moisture content of gum in the average of 13.0%.

Karamallah *et al*., (1998) reported the mean value of moisture content for 803 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 gum samples collected in season 1995/1996, the minimum value was 9.15% and the maximum value was 14.3%. Moisture content for 100 commercial samples of gum collected between 1992 and 1996 in the same study had a mean value of 14.16%. The moisture content of gum samples collected from trees of various ages and different locations by Idris *et al* (1998) was found to be in the range of 12.5%-16%. Karamallah (1999) measured the moisture content in gum and gum collected between 1960 and 1999 in Sudan, it was found equal to 10.75% and 9.4% respectively. Hassan (2000) in the study of gum from different locations of Sudan reported an average of 8.5% moisture content.

Table (1.3): Analytical data of the gum exudates from different *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	1.3	+74.16
A. sieberana var .*vermesenii*	4.90	2.10	0.35	02.19	3.88	1.4	+77.16
A .nubica	4.60	0.03	0.35	02.19	3.50	0.5	+64.16
A. tortilis subsp. raddiana	4.40	1.80	1.84	11.50	3.60	0.7	+71.33
A. tortilis subsp. spirocarpa	6.40	2.03	1.40	07.50	3.85	0.7	+68.66
A. tortilis subsp. tortilis	6.10	1.90	1.20	08.75	4.15	0.8	+69.00
A. drepandolobium	6.10	0.01	0.87	05.44	4.05	1.0	+75.83
A. grrardii	5.90	3.10	2.31	14.44	4.40	2.7	+48.50
A. ehrenbergiana	7.90	2.60	0.22	01.37	3.45	0.3	+5.66
A. nilotica subsp. nilotica	6.10	0.03	0.06	00.37	4.10	0.6	+97.66
A. nilotica subsp .tomentosa	5.80	0.04	0.10	0.62	4.48	0.9	+80.16
A.nilotica subsp. astringen	5.60	0.06	0.06	00.37	3.75	0.6	+75.16
A. laeta	3.20	2.80	0.51	03.19	3.70	1.1	-37.50
A. polyacantha	6.50	2.70	0.34	02.12	4.25	0.6	-19.10
A. seyal var. *seyal*	7.20	2.30	0.10	00.63	4.35	1.2	+50.50

1.4.2 Ash content

The ash content indicates the presence of inorganic elements existing in salt form. Anderson *et al*., (1968) and Karamallah (1999) showed that the type of soil (clay or sand) affected the ash content significantly.

Anderson *et al*., (1963) reported the ash content of gum in the range from 1.94% to 3.55%. Anderson (1977) reported the ash content of 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 the mean value of ash content 3.6% on samples provided by importers in 1990/1991. Jurasek *et al* (1993) found that the ash content 3.0%.

Osman (1993) reported an ash content in the average of 3.6%. The mean value of ash content had been determined for 803 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 gum.

Hassan *et al*., (2005) in the study of gum from different locations of Sudan reported an average of 0.21% ash content. Omer (2006) reported the ash content in the average of 3.27% and in the average of 2.61% for gum. The mean value of ash content reported by Abdelrahman (2008) in in the average of 3.32% and 2.43%. Younes (2009) reported the mean value of ash content for gum was 4.89% in the range of 4.0% – 5.23%.

1.4.3 pH value

The hydrogen ion concentration plays great importance in the chemistry and industry of the gums. The change in the concentration of hydrogen ion may determine the solubility of gum and the precipitation of protein, therefore functional properties of a gum may be affected by change in pH for example viscosity and emulsifying power. Crude gum Arabic is slightly acidic because of the presence of few free carboxyl groups of its

constituent acidic residues, D-glucuronic acid and its 4-O-methy1 derivatives.

Karamallah *et al*., (1998) reported the pH mean value of 4.66 for the 755 authentic 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 and 1996, also they reported an average value of 4.4 for gum samples, collected between 1960 and 1995. The pH value had been determined by Younes (2009), he reported values of 4.78 and 5.16 for gum.

1.4.4 Specific optical rotation

The optical activity of organic molecules (saccharrides and carbohydrates) is related to their structure and is a characteristics property of the substance, and thus the specific rotation is considered as the most important criterion of purity and identity of any type of gum.

Anderson *et al*., (1963) reported the specific optical rotation of *A. seyal* gum in the range from +44° to +56°. Anderson (1977) reported a value of +30° and +51° specific optical rotation for *A. senegal var senegal* gum. Vandevelde and Fenyo (1985) reported specific optical rotation of *A. senegal* gum to be ranging between -29° to -34.4°. Anderson (1991) reported the mean value of specific optical rotation -30.5° on *A. senegal* gum samples provided by importers in 1990/1991. Jurasek *et al*, (1993) reported a range of -20° to -32° for *A. senegal* gum, and a value of +51° for *A. senegal* gum. Osman (1993) reported specific optical rotation of *A. senegal* gum to be ranging between -29° to - 31°. Karamallah *et al*., (1998) reported the specific optical rotation for the 789 authentic *A. senegal var senegal* gum samples, between -26° to -34°. Specific optical rotation of *A. 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° to -

36^{o}. Hassan (2000) reported that *A. seyal var seyal* gum exhibit dextrorotatory optical rotation ranging from $+40^{o}$ to $+62^{o}$. Hassan (2005) reported $+53^{o}$ mean value of specific optical rotation of *A. seyal* gum. Optical rotation of *A. seyal* gum had been determined by Siddig *et al*., (2005) and found to be $+45^{o}$. Omer (2006) reported that an average values of specific optical rotation equal of *A. senegal var senegal* gum are $+32^{o}$, and $+49.4^{o}$. Abdelrahman (2008) reported the average value of optical rotation of *A. tortilis var. raddiana* gum -31.5^{o} whereas equal to $+61^{o}$ for *A. tortilis var. raddiana* gum. Younes (2009) reported a value of -30^{o} specific rotation and $+52^{o}$ for *A. seyal* gum.

1.4.5 Viscosity

The viscosity of a liquid is its resistance to flow, shearing, stirring or to flow through a capillary tube. Viscosity was considered as one of the most important analytical and commercial parameters, since it is a factor involving the size and the shape of the macro – molecule (Anderson *et al*., 1969). Viscosity can be presented in many terms such as relative viscosity, specific viscosity, reduced viscosity, inherent viscosity and intrinsic viscosity. It is also presented as kinematic or dynamic viscosity.

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

The stiffness of the polymer can be known from the relationship between intrinsic viscosity and changing ionic strengths of gum solutions.

Anderson (1977) reported a value of 13.4 cm^3g^{-1} intrinsic viscosity for *A. senegal* gum. Duvallet *et al*., (1989) reported that the intrinsic viscosity of *A. senegal* gum had a value of 21.8 cm^3g^{-1}. Jurasek *et al*., (1993) found that the intrinsic viscosity ranged between 13.4-23.1 cm^3g^{-1} for *A. senegal* gum. Idris *et al*., (1998) studied the intrinsic viscosity of *A. seyal* gum of different ages and different locations and concluded that the intrinsic viscosity varies with age of the trees but no affect was seen from trees in different locations. They found that the intrinsic viscosity was in the range from 10.4 to 19.8 cm^3g^{-1}. Karamallh *et al*., (1998) reported that the mean value of intrinsic viscosity of 1500 *A. senegal var senegal* gum samples was 16.44 cm^3g^{-1}. The intrinsic viscosity had been determined by Flindt *et al*., (2005), they reported that the intrinsic viscosity value of *A. seyal var seyal* gum samples in the range from 11.6 to 17.7 cm^3g^{-1}.

Al-Assaf *et al*., (2005) reported an intrinsic viscosity of sixty seven *A. senegal* gum samples in the range 9.7-26.5 cm^3g^{-1}. Abdelrahman (2008) reported the average value of intrinsic viscosity of *A. tortilis var. raddiana* gum 15.4 cm^3g^{-1}. Younes (2009) reported a value of intrinsic viscosity were 18.9 cm^3g^{-1} and 15.5 cm^3g^{-1} for *A. seyal var seyal* gum samples.

1.4.6 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 (Dickinson *et al.,* 1988, Randall *et al.,* 1989). Dickinson (1991) studied the emulsifying behavior of gum arabic and concluded that there was a strong correlation between the proportion of protein in the gum and emulsifying stability. Anderson *et al*., (1963) reported that nitrogen content of *A. senegal* gum ranged from 0.09 – 0.19% w/w. Nitrogen content of gum had been determined by Anderson (1977) and was found to be 0.29% and 0.14%. Jurasek *et al*., (1993) reported 0.28 to 0.35% nitrogen content for *A. senegal var senegal* gum samples. Osman (1993) reported that nitrogen content for the *A.*

senegal gum to be 0.31% and protein content 2.4%. Idris *et al*., (1998) studied the nitrogen content of *A. seyal* from trees of different ages and different locations and they found the range between 0.22- 0.39%, hence protein content ranged between 1.5-2.6%.

Karamallah *et al*., (1998) reported the mean value of nitrogen content for 642 *A. senegal var senegal* gum samples were collected in season 1994/1995 as 0.33%. Also they reported the mean value of nitrogen content for authentic 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* gums collected between 1960–1999 in Sudan to be 0.33% for *Acacia* gum, and 0.11% for gum. Hassan *et al*., (2005) reported protein content of *A. seyal var seyal* gum 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 *Acacia* from different locations, the values were 0.35% and 0.14%, whereas protein content had a value of 2.3% and 0.93. Abdelrahman (2008) reported the average value of nitrogen content of *A. tortilis var. raddiana* gum 0.37%, he found that protein content of *A. tortilis var. raddiana* gum were 2.4% and 0.95%. Recent study by Younes (2009) determined nitrogen content for *A. Senegal var senegal* gum was 0.35% and protein content 2.3%.

1.4.7 Acid equivalent weight and uronic acid

Titrable acidity represented the acid equivalent weight of gum, from which the uronic acid content, could be determined (Anderson *et al.,* 1977, Vandevelde *et al*., 1985, Jurasek *et al.,* 1993). Gums were found to differ widely in their equivalent weight and uronic acid content (Anderson *et al.,* 1977).

Anderson *et al*., (1963) reported that the uronic anhydride of *A. senegal* sample after electrodialysis 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 a value of 16% and 12% uronic acid for *A. senegal* gum. Jurasek *et al*., (1993) reported uronic acid for *A. senegal* gum was found to be in the range between 12-28.3% and 6.5%. Hence acid equivalent weight found to be in the range between 1430- 1125 and 1470. Osman *et al*., (1993) reported a value of 21% uronic acid for *A. senegal* gum. Karamallah *et al*., (1998) reported the mean value of uronic acid for 115 *A. Senegal var senegal* gum samples collected in season 1994/1995 as 13.7% and a mean value of 1436 acid equivalent weight. Idris *et al*., (1998) found the uronic acid of *A. seyal* gum from trees of different ages and different locations in the range of 15-16%, hence acid equivalent weight ranged between 1118-1238. Karamalla (1999) calculated that the glucuronic acid percentage for *A. senegal* gum in the range 16-17% and 11-12%.

Hassan *et al*., (2005) study seventy four authenticated different samples of *A. seyal* gum 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* gum 16%. Omer (2006) reported that the acid equivalent weight was to be 1161 in average, and glucouronic acid was to be 15.2% in average for *A. senegal* gum whereas acid equivalent weight was to be 1107.9 in average and glucouronic acid was to be 15.9%. Abdelrahman (2008) reported the value of 16.8% uronic acid of *A. tortilis var. raddiana* gum and 1153.8 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 1620 and uronic acid 11.89% for *A. seyal* gum, also he reported a value of 1180 acid equivalent weight and 16.34% uronic acid.

1.4.8 Tannin content

One of the most important tests that can be used to identify *acacia*

gum 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 *A. senegal* 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, 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).

1.4.9 Cationic composition

The most four abundant cationic elements present in gum arabic are calcium, potassium, magnesium, and sodium.

It had been cited in the final report of the safety assessment of different *Acacia* gum that Anderson *et al*., (1990) reported the cation composition of Sudanese *A. senegal var senegal* gum samples between 1904 and 1989. In the same report United States Pharmacopoeia reported the specifications grade of *Acacia* as arsenic (3 ppm), lead (0.001%) and heavy metals (0.004%). The specifications for food grade *Acacia* include arsenic (3 mg/kg maximum), heavy metals (0.002% maximum) and lead (5 mg/kg maximum) had been cited in the same report.

1.4.10 Amino acid composition

The most abundant amino acids exist in gum arabic are hydroxyproline and serine. (Qi *et al*., 1991, Osman *et al*., 1995).

Randall *et al*., (1989) fractionated gum using hydrophobic affinity chromatography, they found that while hydroxyproline and serine were the major amino acids in fractions 1 and 2, the amino acid composition

of fraction 3 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 hydroxyproline-rich polypeptide chain consisting of ~400 amino acid residues.

1.5 Spectroscopy analysis of some *acacia* gum

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 NMR

Figures (1.2) and (1.3) show ^{1}H and ^{13}C NMR spectra of *Acacia* polysaccharide gums (*Acacia Senegal* var. *senegal*, *Acacia mellifera*, *Acacia seyal var. seyal*, and *Acacia tortilis var. raddiana*. Typical NMR spectra were reported for ASG (Nie *et al*., 2013b) and for ASY (Nie *et al*., 2013a).

Figure (1.2) shows crowded signals in the ^{1}H NMR spectrum between 3 and 6 ppm which is typical of polysaccharides and reflects the presence of similar sugar residues, for each gum an upfield peak at around 1.4 ppm assigned to the methyl group of rhamnose sugar which on high resolution shows triplet of triplets. The chemical shift at 2.13 ppm in the *Acacia seyal var. seyal* and *Acacia tortilis var. raddiana* gums spectra indicates the presence of an acetyl group ($COCH_3$). Signals at 3.3–3.8 ppm were due to the presence of $AOACH_3$. The non-anomeric protons (H2–H6) were assigned between 3.3 and 4.6 ppm. The high intensity peak at 4.6–4.80 ppm is partially due to the presence of H_2O. In the anomeric region (4.8–5.8 ppm), more than ten peaks were observed, clearly, in the ^{1}H NMR spectra of the gums (Nep and Conway, 2010).

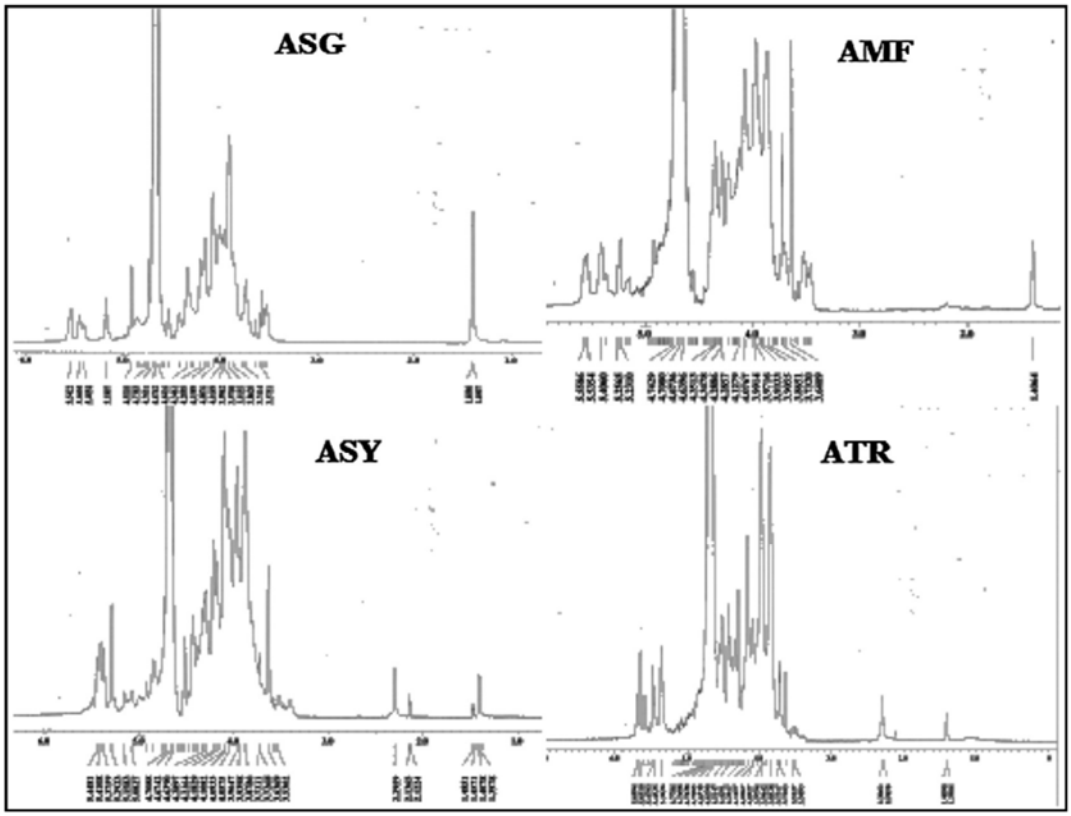


Figure (1.2): ^{1}H NMR spectra of *Acacia Senegal* var. *senegal* (ASG), *Acacia mellifera* (AMF), *Acacia seyal var. seyal* (ASY), and *Acacia tortilis var. raddiana* (ATR) gums

From ^{13}C spectra of *Acacia Senegal* var. *senegal*, *Acacia mellifera*, *Acacia seyal var. seyal*, and *Acacia tortilis var. raddiana* gums, Figure (1.3), the peaks at 16.96, 16.91, 16.21, and 16.95 ppm respectively, belong to the carbon of methyl group of rhamnose, and this indicates that gums contain deoxygenated sugars (Cui, 2005). While the signals due to non-anomeric carbons C2–C5 appear between 60 and 85 ppm (Nep and Conway, 2010), signals from anomeric carbons of the monosaccharide components appear in the 90–110 ppm (Cui, 2005), and for each gum the peak at around 175 ppm arises from uronic acid typical of C6 signal.

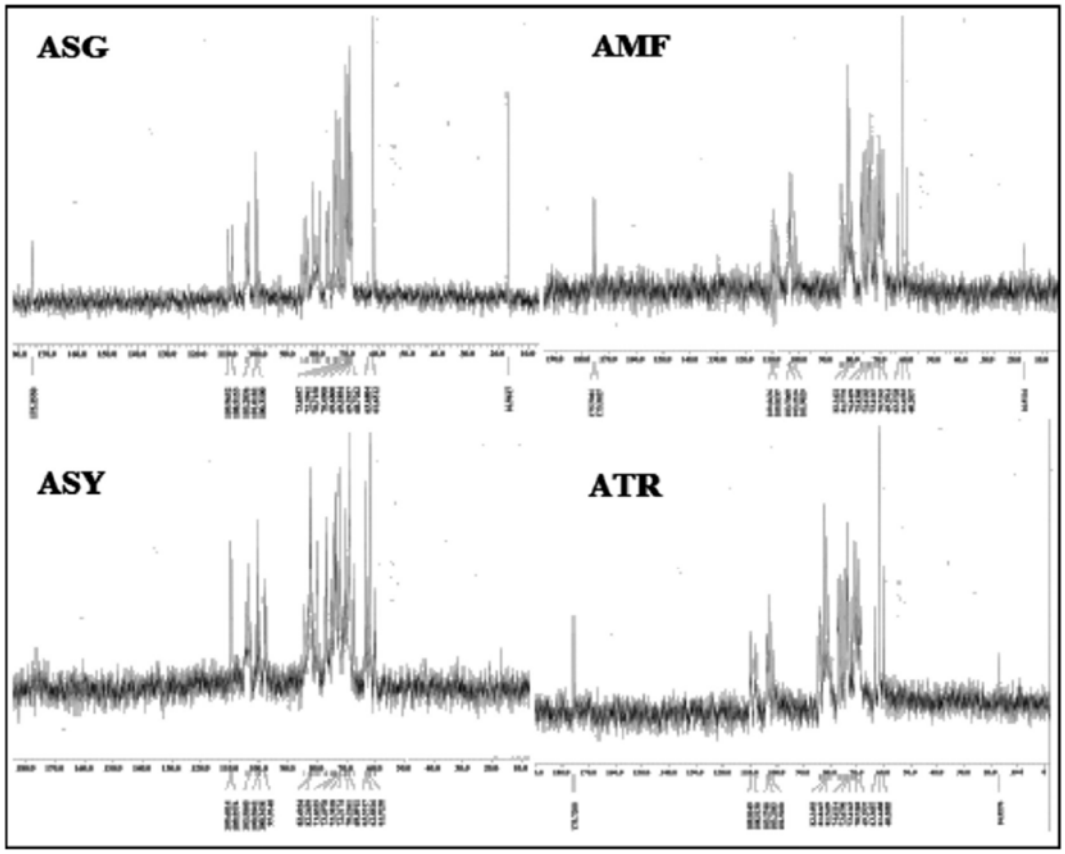


Figure (1.3): ^{13}C NMR spectra of *Acacia Senegal* var. *senegal* (ASG), *Acacia mellifera* (AMF), *Acacia seyal var. seyal* (ASY), and *Acacia tortilis var. raddiana* (ATR) gums

1.5.2 FTIR spectroscopy

In the FTIR spectra of *Acacia Senegal* var. *senegal*, *Acacia mellifera* , *Acacia seyal var. seyal*, and *Acacia tortilis var. raddiana* gums, Figure (1.4), a characteristic absorption band at 3290–3305 cm^{-1} representing the presence of hydrogen bonded OH group was observed. The characteristic absorption band in the region of 3400–3500 cm^{-1} for amino group must have been masked by the broad O-H group absorption band. The bands at 2926 cm^{-1} indicate the presence of sugars, galactose, arabinose, and rhamnose, also the presence of alkane C-H stretch and aldehyde C-H stretch. The polymers also showed the characteristic band of C-C stretch, amide NH bend, NO_2 from both aliphatic and aromatic galactoproteins, and amino acids around 1602

cm^{-1}. The glucuronic acids have specific vibrations such as the band at 1411 and 1363 cm^{-1} due to C-O symmetric stretching and -OH bending, respectively. Alkane CH_3 bend, Aromatic C-C stretch, ketone C-C stretch, carboxylic acid C-O stretch, Anhydrides C-O stretch, Amine C-N stretch from Polysaccharides and Galactoproteins were observed at 1377 cm^{-1} band. The band at 1264 cm^{-1} represents alkane CH_3 bend, alcohol C-O stretch, ether C-O-C stretch, carboxylic acid CO stretch, amines C-N stretch, and alkyl due to sugar backbone showing alkane bend, alcohol stretch. Ether stretch is due to attachment of two galactose sugars, and CO and CN stretches from galactoproteins. A distinct band at around 1029 cm^{-1} represents alkene C-H bend from polysaccharides for all gum samples.

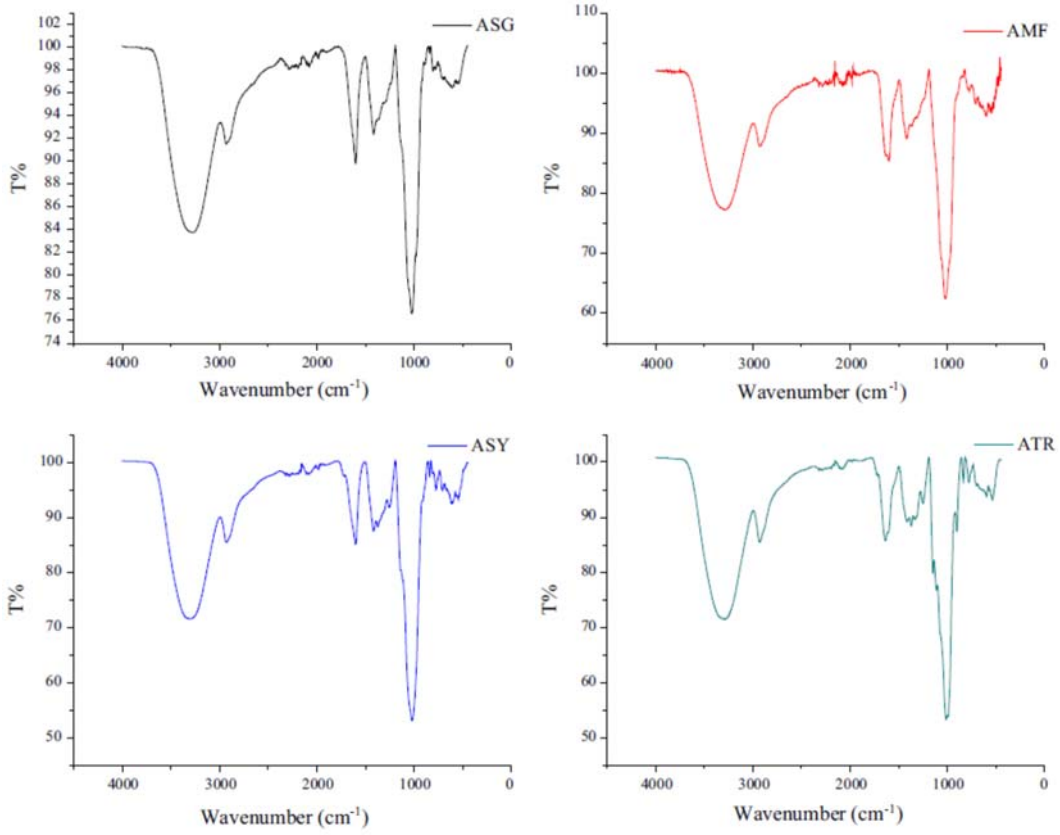


Figure (1.4): FTIR spectra of *Acacia Senegal* var. *senegal* (ASG), *Acacia mellifera* (AMF), *Acacia seyal var. seyal* (ASY), and *Acacia tortilis var. raddiana* (ATR) gums

1.6 Objectives

The aims of this work are,

- To characterize *Acacia tortilis var. spirocarpa* gum.
- To evaluate the emulsification qualities of gum, *Acacia tortilis var. spirocarpa* gum in comparison to that of *Acacia tortilis var. raddiana*.

Chapter Two

2. Materials and Methods

2.1 Materials

Acacia tortilis var. spirocarpa gum sample was collected from EL-Nagaa and EL-Moswarat area, (Nahr Elneel State), in March, 2016. Gum samples were in form of whole and broken nodule of different sizes.

2.1.1 Sample preparation and treatment

The gum sample, after being left to dry in the shade, were cleaned by hand from any extraneous materials like pieces of leaves and bark. The cleaned gum sample were grounded using pestles and mortar into fine powder and kept in containers.

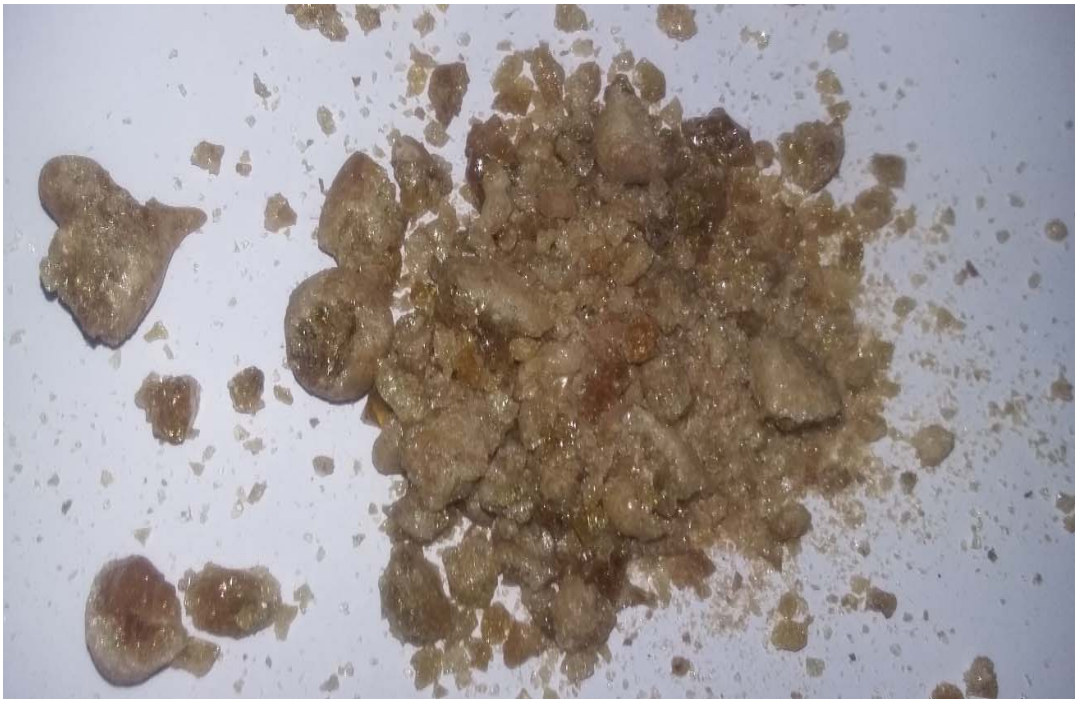


Figure (2.1): *Acacia tortilis var. spirocarpa* gum

2.1.2 Chemicals and materials

- *Acacia tortilis var. spirocarpa* gum
- Distilled water.
- Copper sulphate – Potassium sulphate catalyst.

- Sulphuric acid (nitrogen free).
- HCl.
- Barium carbonate.
- Methyl red.
- Ammonia.
- Boric acid.
- Phenolphthalein.

2.1.3 Apparatus and Instruments

- Porcelain crucible
- Beaker
- Measuring cylinder
- Weighing bottles
- Sensitive balance
- Hot air oven
- Thermostatic water bath
- pH meter.
- Polarimeter.
- Mortar and pestle
- Colloid Osmometer (Ganatec, 050, Germany).
- FTIR Spectrometer (Thermo Nicolet, 300 i.r, U.S.A).
- Atomic absorption spectrophotometer (6800F, SHIMADZU, Japan).
- HPLC system (15950, SHIMADZU, Japan).
- UV/VIS spectrophotometer (Jenway, model 6300, U.K).

2.2 Methods

2.2.1 Determination of moisture content

Accurately 0.5 gram weighed from each sample were weighed in a weighed pettri dishes and heated at 105°C in oven for 6 hours or to a constant weight. The loss on drying heat was found from the following relation;

Moisture % = $(w_2/w_1) \times 100$

Where w_2 = weight of sample after heating

w_1 = weight of sample before heating

2.2.2 Determination of Ash content

The total ash of each sample was determined according to the method cited in FAO paper No, (44). A crucible was heated at 550°C, cooled in a desiccators and weighed, accurately one grams of sample was weighed in the crucible and ignited in muffle furnace at 550°C for 6 hours, and cooled in desiccators and reweighed. Total ash % was calculated from the following relation (AOAC, 1990).

Ash % = (weight of Ash in grams / weight of sample in grams) × 100

2.2.3 Determination of total Nitrogen and estimation Protein

0.5 gram of each sample (in duplicate) were weighed, and transferred to Kjeldahl digestion flasks, one Kjeldahl tablet (copper sulphate – potassium sulphate catalyst) was added to each. Then 10 ml of concentrated (nitrogen free) sulphuric acid were added. The flask was then mounted in the digestion heating system which was heated at 245°C, and capped with aerated manifold. The solution was then heated at the above temperature until a clear pale yellowish – green color was obtained which indicates the completion of the digestion. The flasks were then allowed to cool to room temperature, this content was quantitatively transferred to Kjeldahl distillation apparatus, and the steam distillation of the ammonia was commenced the released ammonia was observed in 25 ml of 2% boric acid. Back titration of the generated borate was then carried out versus, 0.02 M, HCl using methyl red as indicator. Blank set of experiment was carried in the same way.

The nitrogen content percentage in the samples was calculated from the relation;

N% = 14.01 × M × (volume of titrant – volume of blank) / weight of sample (grams)

Where M is the molarity of HCl.

Protein content of sample was calculated using nitrogen

Conversion factor (NCF) of 6.7

Protein % = N% × 6.7 (Mona, 2011)

2.2.4 Determination of intrinsic viscosity

An aqueous solution (1%) was prepared from each sample of the whole gum sample and the rate of flow recorded for successive dilutions using a capillary viscometer (shott Gerate type 50 120/11) in a constant temperature bath at 30°C. The viscosity was obtained by extrapolation of reduced viscosity against concentration back to zero concentration.

2.2.5 Determination of specific optical rotation

1% solutions (on dry weight basis) were mixed on a roller mixer until the sample, completely, dissolved, filtered through Watmann No.42 filter paper, loaded into the sample holder, without trapping any air bubbles. Optical rotation was measured using digital polarimeter equipped with 250 mm tube filled with the test solution at room temperature. Specific rotation was calculated using the following relationship:

$[\alpha]^{t}_{D} = \alpha \bullet 100/CL$

Where α is the observed rotation of the solution in degrees, C is the number grams of substance per 1 ml of solution, and L is the light pass length in the solution in decimeter.

Figure (2.2): Polarimeter

2.2.6 pH value

The pH was determined for 1% aqueous solution of each sample, using a pH- Meter (Corning 555) at room temperature.

Figure (2.3): pH meter

2.2.7 Equivalent weight

3% aqueous gum solution of each sample were treated with acid washed in Amberlite Resin 120 (H^+) for an hour and then titrated against 0.02 N sodium hydroxide solution using phenolphthalein as indicator and the equivalent weight was determined as follows:

Equivalent weight = weight of the sample × 1000 / volume f titer × molarity of alkali

2.2.8 Determination of number average molecular weight

The colloid osmotic pressure is measured by means of an osmotic cell (Osmostat 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 (0.02%, 0.04%, 0.06%, 0.08%, 0.1%, 0.12%). The two halves of the cell are separated from each other by a semi permeable membrane. The membrane allows only water and electrolyte molecules to pass through its pores. Due to osmotic pressure difference 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 is mounted into the lower half of the cell, transduces the under pressure into an electronic signal, which is shown on a digital display.

Figure (2.4): Osmomat 050

2.2.9 Determination of cationic composition

Dry ashing method was used in sample preparation, two grams of gum sample were placed in a well-glazed, porcelain dish heated to 550 ^{o}C, the temperature was maintained for 4 hours. The sample was cooled and 10 ml of 3N HCl were added. The dish was covered with watch glass, and the sample was boiled gently for 10 minutes. The sample was cooled, filtered into a 100 cm^3 volumetric flask, and diluted to volume with deionized water.

Atomic absorption spectrometer (SensAA-Dual-GBC Scientific Australia equipment) was used to determine the elements.

Figure (2.5): Atomic Absorption Spectrometer

2.2.10 Determination of sugar composition

The samples were hydrolysed to liberate the sugar residues. Sample 100mg/L was accurately weighed, including allowance for moisture content, added to 10cm^3 of 4% H_2SO_4 and incubated at 100^{o}C for 6 hours. 1 g 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 remove barium sulphate (formed from neutralizing the H_2SO_4) to settle. The supernatant was removed and filtered through a 0.45 μm whatman nylon filter and then diluted 1:1with 70/30 Acetonitrile /buffer. 1 ml was analyzed using HLPC to determine the relative concentration of each sugar residue present in the sample, namely rhamnose (Rha), arabinose (Ara) and galactose (Gal).

Before analysis of the gum samples, calibration curves of these sugars were prepared. Stock concentrations 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 concentrations for each sugar over a range of 2.5–0.5 mg cm^{-3}. This allowed six levels of the calibration curve and an average of 3 replicates for each level was used to ensure accuracy. Sugar content from the gum samples was determined. The concentration of each sugar was calculated by peak height and expressed as a % of the total sugar content.

Figure (2.6): High Performance Liquid Chromatography

2.2.11 Emulsifying stability of *Acacia tortilis var. spirocarpa* gum

1.2 ml of oil was homogenized with 1 ml gum solution, then by using ultra turax homogenizer, peanut oil and gum solution were homogenizer for 2 minutes at 24000 rpm. Then the resultant emulsion was diluted in a ratio of 1 : 1000 and its absorbance was read with a spectrophotometer at wavelength of 520 nm. A second reading was taken after one hour. The emulsifying index (EI) was calculated as:

Emulsifying index (EI) = $\frac{A_1}{\quad}$

A_0

Where:

A_1 = is the absorbance measured immediately after one hour

A_0 = is the absorbance preparation of solution i.e zero time

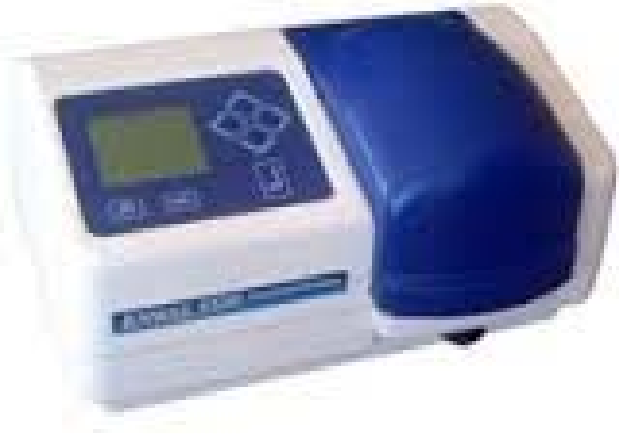


Figure (2.7): Jenway UV/VIS spectrophotometer

2.2.12 Infrared spectroscopy of *Acacia tortilis var. spirocarpa* gum

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 70 sample discs.

The disk of each gum specimen was loaded in FTIR spectroscopy (Thermo Nicolit 300 ir), with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}, was used to record the spectrogram for each gum sample.

Figure (2.8): FTIR spectrophotometer

Chapter Three

3. Results and Discussion

A number of physicochemical and chemical methods were used to characterize *Acacia tortilis var. spirocarpa* gum. The characterization of gums is very important in the industrial applications. The study of chemical and physical properties of gum is used to ensure the purity and to a void mixed samples and to report the specification of the samples under study, Tables (3.1, 3.2, 3.3, 3.4, 3.5 and 3.6) showed analytical data of *Acacia tortilis var. spirocarpa* gum samples.

Table (3.1): Physicochemical properties of the *Acacia tortilis var. spirocarpa* gum

Moisture Content (%)	7.64
Ash content (%)	2.35
Refractive index	1.3383
Nitrogen content (%)	2.31
Protein content (%)	15.477
pH	5.64
Acid Equivalent weight	4000
Total of uronic acid	4.85
Specific optical rotation	+64.67

3.1 Moisture content

The moisture content of the gum is usually affected by the season of collection, the prevailing climate conditions and the storage condition and periods. The average value of moisture content of *A. tortilis var. spirocarpa* samples is given in Table (3.1). It was (7.64%), it was less than the value cited by Mona, 2011) for *A. tortilis var. raddiana*, it was (11.8%).

3.2 Ash content

The ash content of *A. tortilis var. spirocarpa* was (2.35%), it was more than the value reported by (M.O.A, 2011 for *A. tortilis var. raddiana*, it was (1.8%).

3.3 Refractive index

The Refractive index (RI) for the sample of *A. tortilis var. spirocarpa* was shown in Table (3.1). The average value was found to be 1.3383. This result was similar to those reported by Omer (2004) and Elkhatim (2001) (1.3337 and 1.3339, respectively).

3.4 Nitrogen content

The nitrogen content value of *A. tortilis var. spirocarpa* was 2.31%, a value was more than that reported by (M.O.A, 2011) for *A. tortilis var. raddiana*, which was (1.6%).

3.5 Protein content

The protein content value of *A. tortilis var. spirocarpa* sample was found to be 15.477% using nitrogen conversion factor (NCF) of 6.7 as shown in Table (3.1). This result is higher than that reported by Rabeea *et al*, 2016 and Abdel Rahman, 2011 which was (10.378%).

3.6 pH value

The pH value of *A. tortilis var. spirocarpa* was found to be slightly acidic 5.64. Other researcher had reported a values of 4.45 for *A. tortilis var. raddiana* by (Rabeea *et al*, 2016 and Abdel Rahman, 2011).

3.7 Acid equilvent weight

The acid equilvent weight value of *A. tortilis var. spirocarpa* was found to be 4000 as shown in Table (3.1). This value result is higher than that reported by Rabeea *et al*, 2016 and Abdel Rahman, 2011 which was 2.06

3.8 Total of uronic acid

The total of uronic acid value of *A. tortilis var. spirocarpa* sample was found to be 4.85 as shown in table (3.1). This result is less than that reported by reported by (M.O.A, 2011) for *A. tortilis var. raddiana*, which was (6.208%).

3.9 Specific Optical Rotation

A. tortilis var. spirocarpa has been found to possess a positive specific optical rotation of the value +64.67 in average as shown in table 3.1. Rabeea *et al* and Abdel Rahman had reported a value of +86.75 for *A. tortilis var. raddiana*. Specific optical rotation can serve as a simple and cheap discriminatory test to distinguish between varieties of *A. tortilis*.

3.10 Viscosity

The intrinsic viscosity of *A. tortilis var. spirocarpa* was found to be 6.71 $cm^3 g^{-1}$ as shown in Figure (3.1). This result was less to those mentioned in the literature (Anderson *et al*., 1966 and Anderson, 1977) which was 9.5 $cm^3 g^{-1}$, but is far less than that obtained by an FAO study for Nigerian gum which was reported as 35 $cm^3 g^{-1}$ (Al-Assaf *et al*., 2005).

Table (3.2): Viscosity of the *Acacia tortilis var. spirocarpa* gum

***Tortili* gum sample**	**Concentration C**	$\frac{\eta}{\eta_o}$	$\frac{\eta}{\eta_o} - 1 = \eta_{sp}$	η_{sp}/C
X_1	0.01	$\frac{105}{96} = 1.09$	1.09 – 1 = 0.09	9
X_2	0.025	$\frac{130}{96} = 1.35$	1.35 – 1 = 0.35	14
X_3	0.050	$\frac{202}{96} = 2.1$	2.1 – 1 = 1.1	22
X_4	0.075	$\frac{304}{96} = 3.17$	3.17 – 1 = 2.17	29
X_5	0.1	$\frac{432}{96} = 4.5$	4.5 – 1 = 3.5	35

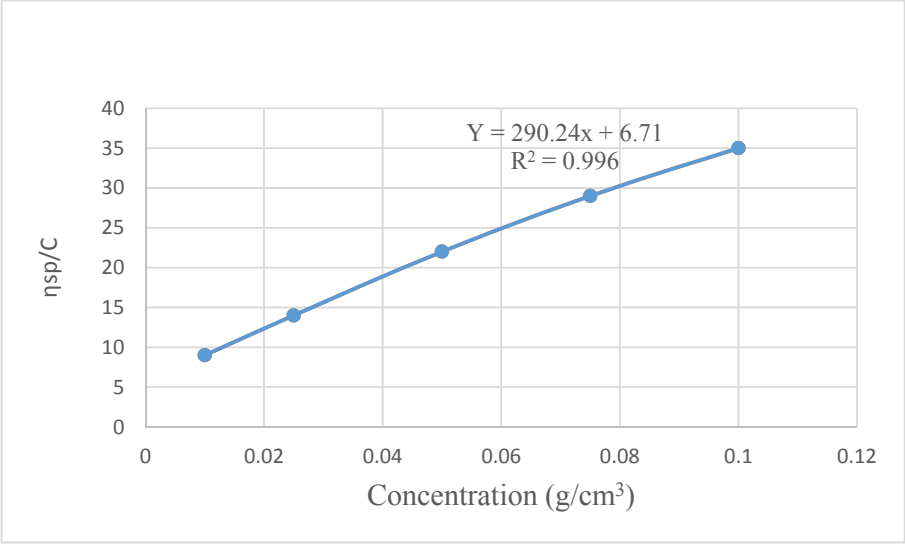


Figure (3.1): Intrinsic viscosity of *Acacia tortilis var. spirocarpa* gum

3.11 Determination of number average molecular weight by Osmometery

Table (3.3) and Fig 3.3 show the osmotic pressure and number average molecular weight of *A. tortilis var. spirocarpa* as (2.5 x 10^5 g/mol). This results was similar to those reported by (Anderson *et al*., 1966, Anderson., 1977, and Al- Assaf *et al*., 2003).

Table (3.3): Osmotic pressure of the *Acacia tortilis var. spirocarpa* gum

Concentration (g/cm^3)	ʌ Osmotic pressure (mmHg)	ʌ /c	√ʌ /c
0.02	0.1646	8.23	2.87
0.04	0.4060	10.15	3.19
0.06	0.7404	12.34	3.51
0.07	0.9404	13.44	3.67
0.08	1.1650	14.56	3.82
0.10	1.6780	16.78	4.09

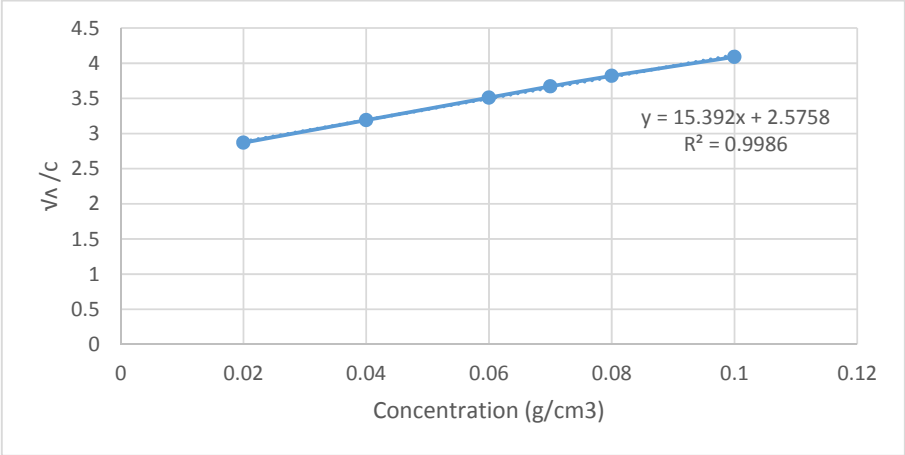


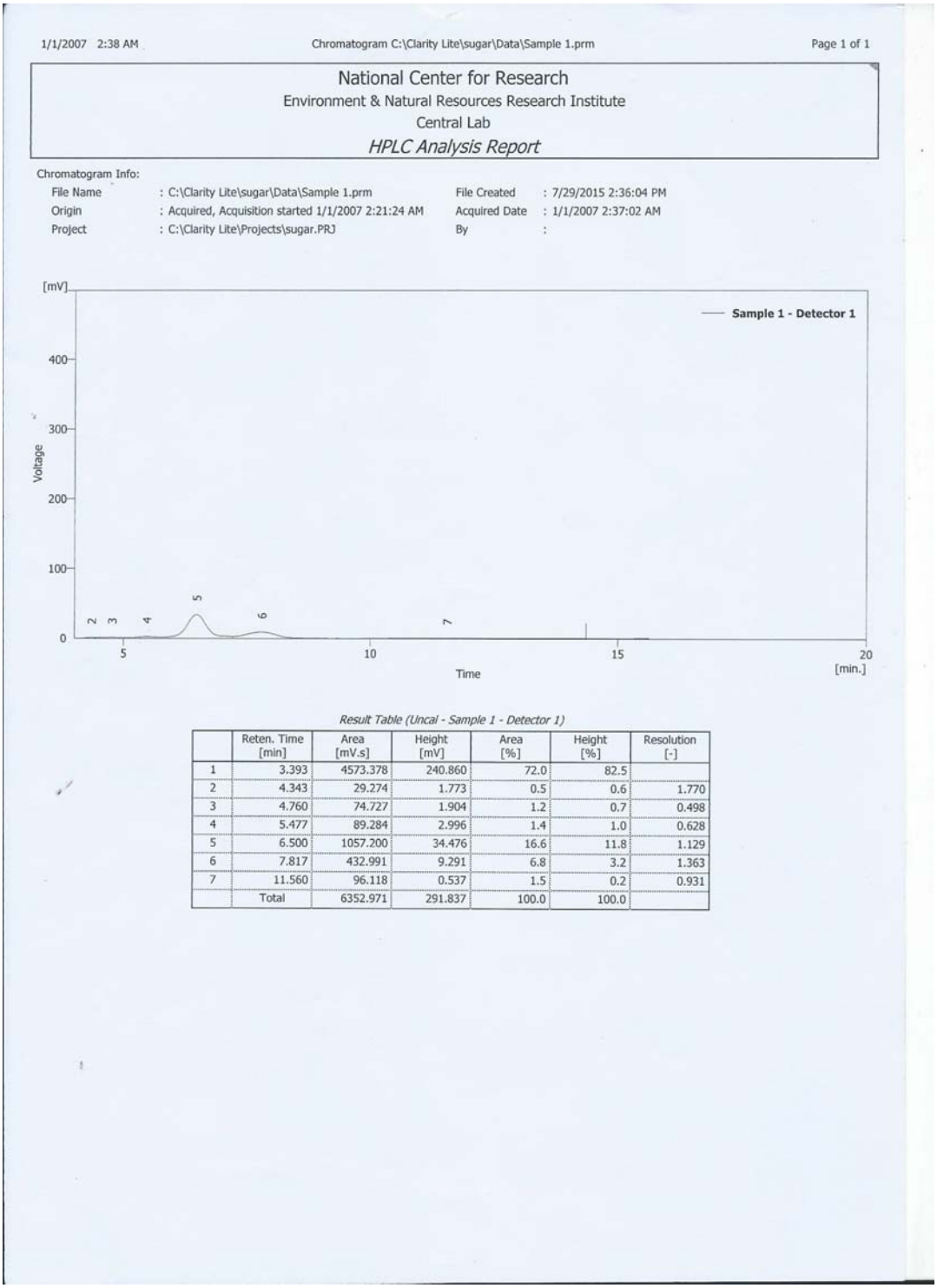
Figure (3.2): Osmotic pressure of of *Acacia tortilis var. spirocarpa* gum

3.12 Sugar composition

The sugar contents of *Acacia tortilis var. spirocarpa* gum after acid hydrolysis were measured using HPLC technique and illustrated in (Table 3.4). The results showed that arabinose had a highest percentage (49%) followed galactose (20%) and rhamnose (5%), these results are similar to those reported by (Yadav *et al*., 2013).

Table (3.4): Sugar composition of the *Acacia tortilis var. spirocarpa* gum

Type of sugar	Amount (%)
Arabinose	49
Galactose	20
Rhamnose	5.0



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National Center for Research
Environment & Natural Resources Research Institute
Central Lab
HPLC Analysis Report

Chromatogram Info:

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Project	: C:\Clarity Lite\Projects\sugar.PRJ	By	:

Result Table (Uncal - Sample 1 - Detector 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	Resolution [-]
1	3.393	4573.378	240.860	72.0	82.5	
2	4.343	29.274	1.773	0.5	0.6	1.770
3	4.760	74.727	1.904	1.2	0.7	0.498
4	5.477	89.284	2.996	1.4	1.0	0.628
5	6.500	1057.200	34.476	16.6	11.8	1.129
6	7.817	432.991	9.291	6.8	3.2	1.363
7	11.560	96.118	0.537	1.5	0.2	0.931
	Total	6352.971	291.837	100.0	100.0	

Figure (3.3): HPLC of sugar contents of *Acacia tortilis var. spirocarpa* gum

3.13 Cationic composition

Cationic composition of *Acacia tortilis var. spirocarpa* gum samples was determined using atomic absorption spectrophotometric technique and the values are depicted in Table (3.5). The minerals were in the order: K > Ca > Na > Mg > Fe > Mn > Zn > Cu > Ni > Pb.

The minerals content results of *Acacia tortilis var. spirocarpa* gum samples were showed similarity to those reported by Siddig *et al*., 2003, Omer, 2006, Abdelrahman, 2008 and Younes, 2009.

Table (3.5): Cationic composition of the *Acacia tortilis var. spirocarpa* gum

Mineral	Concentration (mg/L)
Ni	0.0592
Zn	0.0791
Cu	0.0741
Ca	35.09
Mn	1.3867
Pb	0.0109
Mg	5.8600
Fe	3.4840
Na	23.00
K	56.00

3.14 Infrared of *Acacia tortilis var. spirocarpa* gum

Figure (3.4) shows the IR spectrogram of *A. tortilis var. spirocarpa* gum. A characteristic absorption band at 3223.36 cm^{-1} indicating the presence of hydrogen bonded OH group was observed. The characteristic absorption band in the region of 3400-3500 cm^{-1} for amino group must have been masked by the broad O-H group absorption band. The bands at 2925.22 cm^{-1} indicate the presence of sugars, galactose, arabinose, and rhamnose, also

the presence of alkane C-H stretch and aldehyde C-H stretch. The polymers also showed the characteristic band of C-C stretch, amide NH bend, NO_2 from both aliphatic and aromatic galactoproteins, and amino acids around 1731.51 cm^{-1} and 1602 cm^{-1}. The glucuronic acids have specific vibrations such as the band at 1417.72 cm^{-1} due to C-O symmetric stretching. Alkane CH_3 bend, Aromatic C-C stretch, ketone C-C stretch, carboxylic acid C-O stretch. The band at 1249.37 cm^{-1} represents alkane CH_3 bend, alcohol C-O stretch, ether C-O-C stretch, carboxylic acid CO stretch, amines C-N stretch, and alkyl due to sugar backbone showing alkane bend, alcohol stretch. Ether stretch is due to attachment of two galactose sugars, and CO and CN stretches from galactoproteins. A distinct band at around 1027.40 cm^{-1} represents alkene C-H bend from polysaccharides for all gum samples.

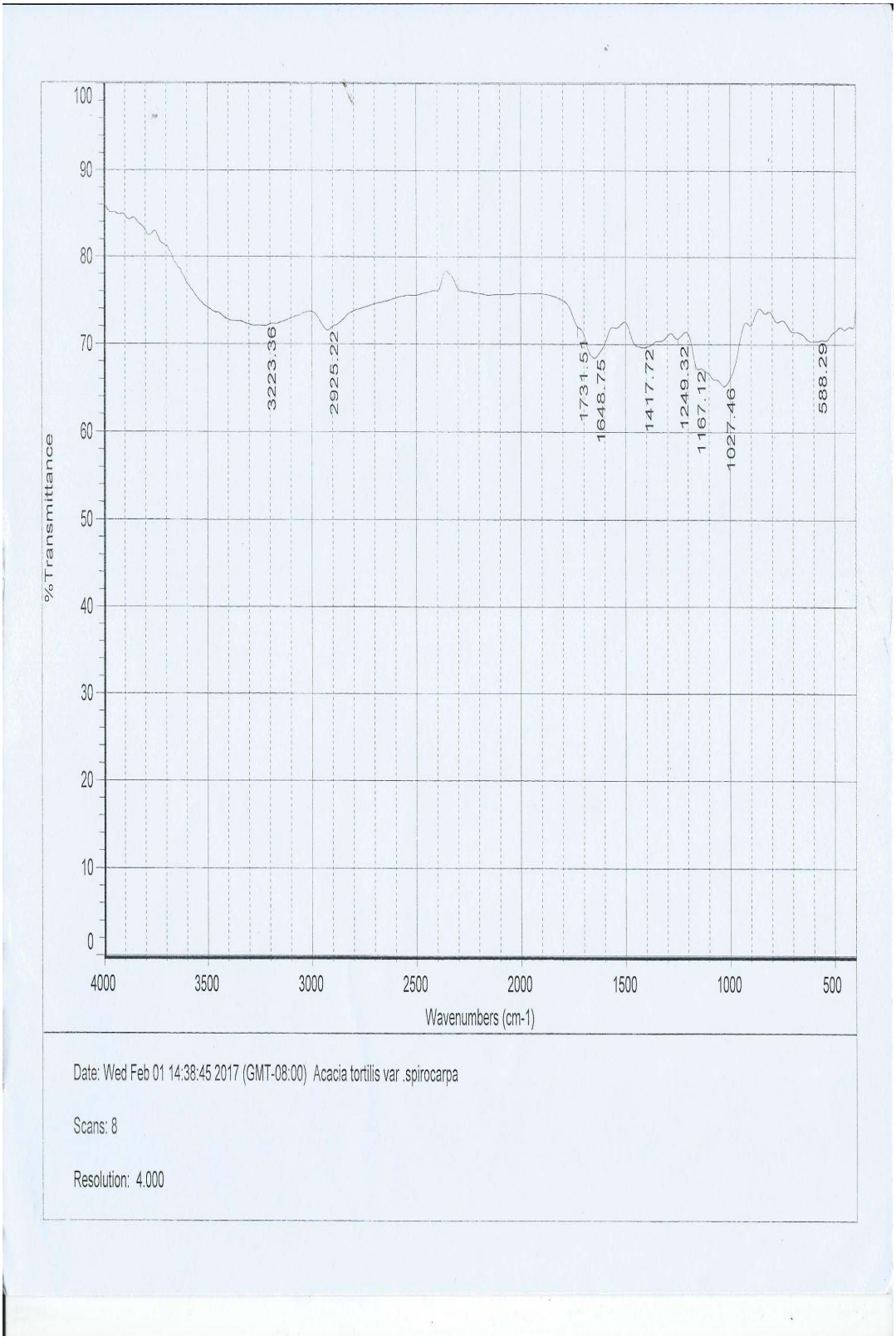

Figure (3.4): IR spectrum of *Acacia tortilis var. spirocarpa* gum

3.15 Emulsifying stability of *Acacia tortilis var. spirocarpa* gum

Emulsifying stability of *A. tortilis var. spirocarpa* gum samples was determined using relative light absorption of a dilute solution of an emulsion when *A. tortilis var. spirocarpa* was used an emulsifier. The absorption at wavelength 520 nm was measured using Jenway UV/VIS spectrophotometer. The average value of emulsifying stability was found to be 1.2 as shown in Table (3.6) and Figure (3.5).

Table (3.6): Emulsifying stability of the *Acacia tortilis var. spirocarpa* gum

Day	Day1	Day2	Day4	Day7	Day8	Day9	Day10	Day12	Day13	Day14
Amount	1.03	1.75	1.10	1.20	1.42	1.22	1.09	1.10	1.09	1.10

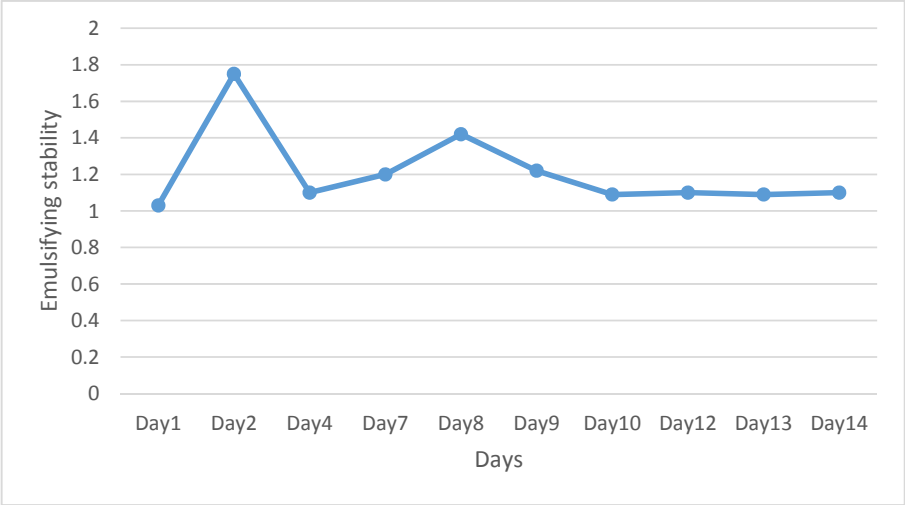

Figure (3.5): Emulsifying stability of the *Acacia tortilis var. spirocarpa* gum

From Figure (3.5) above, the day 2 has the highest value compare to others Days. The stability of the emulsion seems to be established gradually after a process of Ostwald ripening, which is completed by the 8th day of the emulsion preparation. It remained almost unchanged for the next seven day where the experiment ends.

3.16 Conclusion

- The physicochemical analysis reviled that *A. tortilis var spirocarpa* falls within the Gemmifera group with positive optical rotation
- Although it showed all the features of *Acacia* exudate gums it slightly differed from *A. tortilis var tortilis* in the value of its optical rotation, the ratio of the constituent sugars and the intrinsic viscosity as well as number average molecular weight.
- It possesses higher protein content and good emulsifying property just as the other variant *A. tortilis var tortilis.*

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