Sudan University of Science & Technology

College of Graduate Studies

Foaming Fractionation and Physicochemical Characterization of *Azadrakhta indicia Gum*

التجزئه الرغويه والخصائص الفيزيوكيميائيه لصمغ النيم

A Thesis Submitted in Partial Fulfillment for the Requirements of a Master Degreein Chemistry

By: Nedal Ahmed Saeed Ahmed

(B.Sc. Hons Chemistry)

Supervisor of: Mohammed El Mubarak Osman

August 2016

الآية

قَالَ تَعَالَىٰ:

﴿ يَرْفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُواْ مِنكُمْ وَٱلَّذِينَ أُوتُواْ ٱلْعِلْمَ دَرَجَٰتٍ ۚ وَٱللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿١١﴾ ﴾

سورة المجادلة: ١١

Dedication

I dedicate this work to:

The soul of my father,

my lovely mother,

my brothers and sisters.

Acknowledgment

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

A deep gratitude goes to my supervisor Prof. Mohammed Elmubark Osman for the support he offered me in during this research project. I have learned a lot from him and hope to learn more. I was lucky to have a chance to work with him. I am very proud of being one of his students.

My thanks go to the department of chemistry, the technical staff and the library of the Sudan university, also all my friends and colleagues whom helped ,supported, directed me to acquire knowledge during my academic journey to obtain this research.

Abstract

In this research from *Azadirachta Indica* (Wild) , neem gum was fractionated by foaming method into two fractions, high protein fraction (HPF) that represents 65% of the weight gum and a low protein fraction (LPF) which represents 35% of the gum.

A comparative study between the crude gum and the two fractions was conducted in the scope of their physiochemical properties.

Proximate analysis of the crude gum and fractions HPF and LPF respectively revealed that, the moisture content 7.56 and 7.54, ash% 8.41and 8.31 .The nitrogen 1.41 and 1.31and the protein content 2.85, 0.91 ,specific rotation +57.8and +55.8, the pH 4.5 and 4.3, equivalent weight and molecular weight of the neem gum in two fractions HPF and LPF respectively revealed that (equivalent weight = 4.87×10^5 and 4.57×10^5; molecular weight = 7.31×10^5 and 7.01×10^5 were insignificantly different.

However the intrinsic viscosity of the Fraction was 17.6 and 6.6 cm^{-3}/g were highly significantly different.

المستخلص

فى هذه الدراسة، تمت التجزئه الرغويه لصمغ النيم ، وتم الحصول علي جزيئين الاول يحتوي علي كميه كبيره من البروتين تمثل (65%) من وزن الصمغ (HPF) والجزي الثاني يحتوي علي كميه قليله من البروتين تمثل (35%) (LPF) من الوزن الكلي .

أظهرت الخصائص الفيزوكيميائية لجزيئي صمغ النيم عالى البروتين ومنخفض البروتين النتائج التالية على التوالى :محتوى الرطوبة = 7.56%0 و 07.54%، الرماد = 8.41%0 8.31%، الناتروجين = 01.41 و 1.31، البروتين 02.85 و 0.91الدوران الضوئى النوعى °57.8 و °55.8والآس الهيدروجينى = 4.5 و 4.3 وايضا أظهرت نتائج الوزن المكافئ والوزن الجزيئى لصمغ النيم فى الجزيئين عالى البروتين ومنخفض البروتين القيم التالية على التوالى: الوزن المكافئ = 4.87 * 10^5 و 4.57 * 10^5، والوزن الجزيئى = 7.31 * 10^5 و 7.01 * 10^5واوضح انه ليس هنالك اختلاف كبير.

اما بالنسبه للزوجة 17.6 و 6.6 فقد أظهرت القيمتان أختلافاً كبيراً.

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Introduction

1. INTRODUCTION

1.1 Plant gum

Plant gums are adhesive substances that are carbohydrates in nature and are usually produced as exudates from the bark of trees or shrubs. Some plant gums, such as gum Arabic are soluble in water, dissolving to give clear solutions. Others include gum tragacanth produce mucilage by absorption of large quantities of water (Mejanelle et al, 2002).

Plant gums are Arabinogalactan-protein (AGP) which are higher plants secretions.

They are a group of macromolecules characterized by a high proportion of carbohydrate in which galactose and arabinose are the predominant monosaccharide sub-units.Plant gums originating from many countries have been an important item in international trade for centuries in food, pharmaceutical, paper textile and other industries. Depending upon their major use, plant gums may be broadly classified as 'food' and 'non-food' or 'technological grade' gums. The former can be used as food additives in various kinds of confectioneries, foods and beverages and include gum arable, gum tragacanth, gum karaya and gum carob. The latter category finds its major use in non-food industrial applications and includes 'gum ghatti', 'gum talha' and a variety of other gums (Bleton et al, 1996), Chemically, plant gum is a complex mixture of macromolecule of different size and composition (mainly carbohydrates and proteins). Today the properties and the feature of Acacia gum have been widely explored and developed and it is being used in a wide range of industrial sectors such as textiles, ceramics, lithography, cosmetics (Singh et al, 2006).

Plant gums have been used in a variety of applications such as in food emulsifiers, stabilizers, and thickeners, pharmaceuticals, cosmetics, textiles, and in art. Plantgums have been used for centuries as binding media, topaint, write and

illuminate manuscripts and to apply metallic leaf decorations (Andreotti et al, 2008). Gums and other kind of saccharide materials, such as honey, fig milk or starch, are known to have been used as binding media, sizing agents or mummification materials since antiquity. Actually, carbohydrates are contained in a variety of materials used as support, binders and varnishes in painted objects (Mills et al, 1999). Wood and paper are common paint supports, and carbohydrates, both free and bound, can be encountered as minor fractions in a variety of paint materials, such as proteinaceous binders, as well as plant and animal terpenoid resins.

There are many different chemolysis procedures used to study plant gums (Willför et al, 2009), based on: the methanolysis (Mejanelle et al, 2002; Bleton et al, 1996), on the hydrolysis (Pitthard et al, 2006; Pitthard et al, 2006; Schneider et al, 2001; Kharbade et al, 1995; Pitthard et al, 2001), and hydrolysis assisted by microwaves of the polysaccharide (Colombini et al, 2002; Singh et al, 2003 and Singh et al, 2006).Rates of degradation differ for each monosaccharide during hydrolysis.

The sugars are released in the order of ease of bond fission: furanosidic > pyranosidic,6-deoxyhexosidic > hexosidic > and neutral hexosidic > uronosidic (Stephen et al, 1990). Hydrolysis is complicated if there are proteins or polyphenols in the gum sample, and therefore interaction with the reducing sugars may take place. If the polysaccharide has a limited solubility, this also increases the difficulties. Derivatisation is fundamental in the GC-MS analysis of saccharides, due to the high number of polar moieties present in each molecule (Ruiz-Matute, et al, 2011; Molna´r-Perl et al, 2000; Harvey et al, 2011).

1.1.1 Origins of plant gums

The origin of the plant gum is still uncertain, but it is thought by some authorities to be the starch granules present in the cells.

Great many plants exude viscous, gummy liquids, which when exposed to air and allowed to dry, clear, glassy masses, gums are worthy of investigation in their own right and as an aspect of plant biochemistry in bacterial polysaccharides. They occur naturally as salts (especially of calcium and magnesium) and in some cases a proportion of the hydroxyl groups are etherified, most frequently as acetates. The site of esterification is, however, entirely unknown at present structurally gums are related to other less complex plant polysaccharides (Omer, 2004).

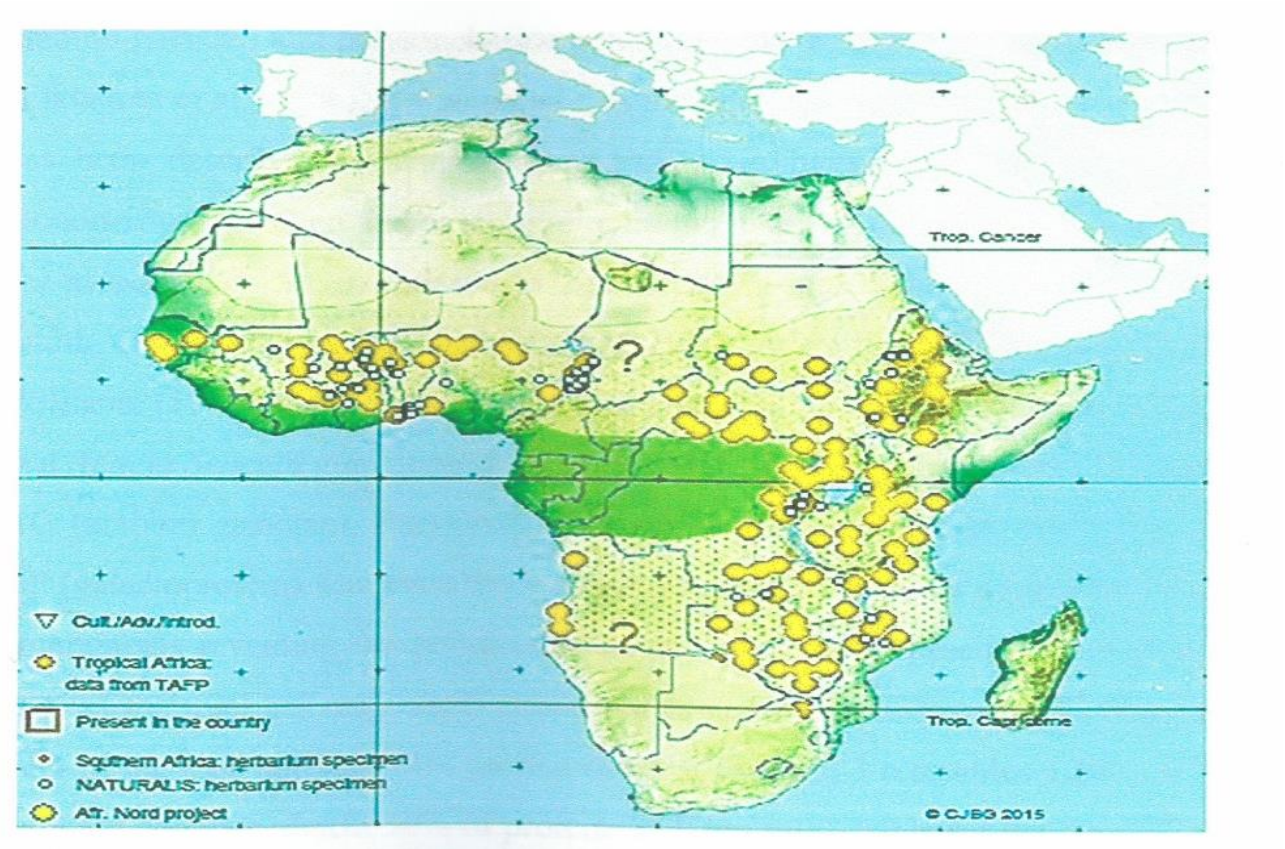


Figure (1.1): Distribution of Azadirachta Indica tree in the African gum belt

1.1.2 Structure of plant gums

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

that D-galactose and uronic acid residues generally constitute the backbone of gum polysaccharide with 1-3 and 1-6 linkages predominating side chain are characterized by the presence of D-xylopyranose, L-arabinose, and L-arabino-furanose linkage (Elnour, 2007).

1.1.3 Chemical Structure of Gums

Gum Arabic is branched, neutral or slightly acidic, complex polysaccharide obtained as a mixed calcium, magnesium and potassium salt. The backbone consists 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. Both the main and the side chains contain units of a-1-arabinofuranosly, a-1-rhamnopyranosyle, b-d-glucuronopyranosyl, and4-O-methyl-b-d-glucuronopyranosyl, the latter two mostly as end-units (Anderson, 1986). They further analyzed the product by methylation and gelpermeation chromatography and found that the uronic acid and the rhamnose residues were eliminated first which proved that they are located at the periphery of the molecule and the core was consisted of a β1,3-galactopyranose chain with branches linked through 1,6 position. They also found that the protein component was associated with the high molecular weight fraction and lower molecular mass fraction was virtually exclusively polysaccharides. Figure (1.3) shows the polysaccharides in gum Arabic.(Street et al.,1983) used computer modeling to analyze the previous data and proposed the structure illustrated in Figure1.4, (Churms et al.,1983) subjected the gum to smith degradation leaving the reaction to reach completion after each stage of degradation procedure. They obtained different values for the composition and size of the molecule of each degradation product than those previously obtained by Anderson, 1966b, and proposed amore regular structure than the previous one proposing that the galactan core

consisted of 13β-, 3-D-galactopyranosyl residues having two branches, which give single repeating subunits having molecular mass of 8× 103 within the molecule. As the whole gum was found to have molecular weight of 560,000 thus it was proposed that the molecule consists of 64 of these subunits and that they were symmetrically arranged. (Defye and Wang, 1986).

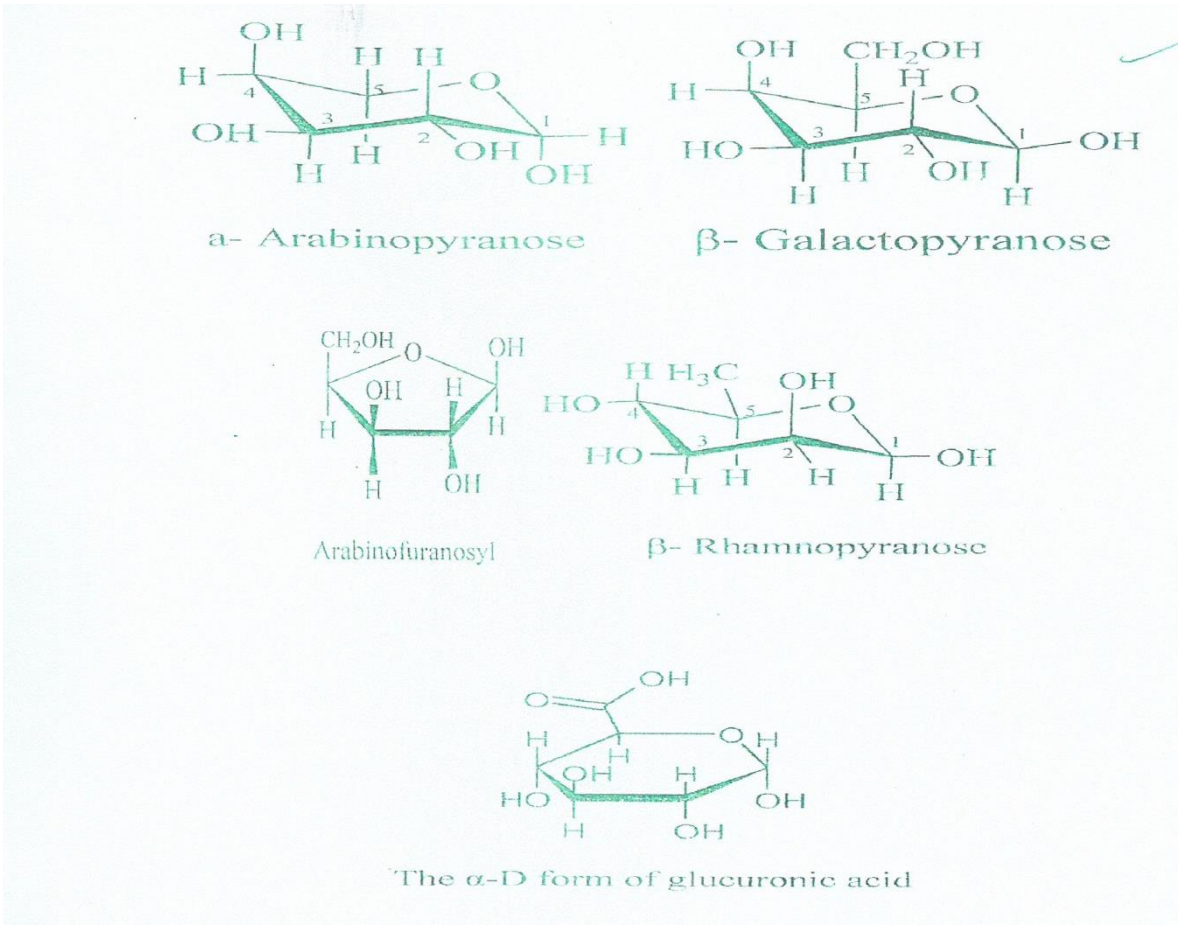


Figure (1.2): The structure of carbohydrates units of gum molecule (street et al. 1983)

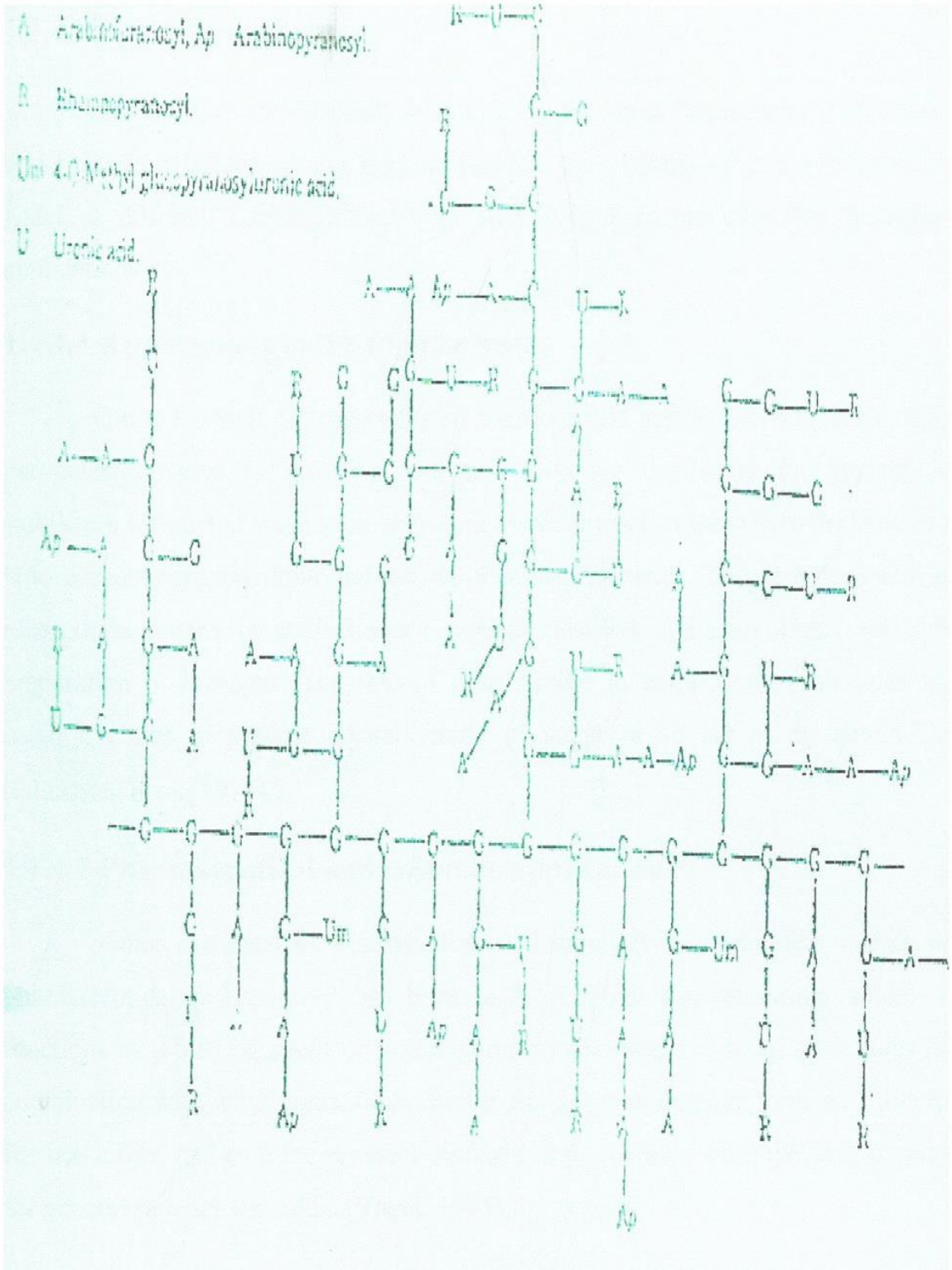


Figure (1.3): The structure of gum as proposed (street et al., 1983)

1.1.4 Applications of gums

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

1.1.4.1 Applications in the food industry

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

1.1.4.2 Pharmaceutical and cosmetic applications

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

1.1.4.3 Other industrial uses

Due to its adhesive properties gums have been used in the manufacturing of adhesives for postage stamps and also in the formulations of paints and inks. Gums may serve as a source of monosaccharide, as e.g. mesquite gum (family prosopis) serve as a source of L-arabinose (51%) because of its easier hydrolysis, and availability of the gum in large quantities. The mesquite gum can be dialyzed by addition of ethanol (White,

1947 and Hudson, 1951), oral ternatively, isolated by crystallization from methanol after removal of acidic saccharides on ion exchange resin or precipitated by barium salts. Gums are widely used in textile industries to impart luster to certain materials (silk), as thickeners for colors and mordant in calico printing (Omer, 2004).

1.2 Neem tree (Azadirachta Indica)

Neem, a member of the family Meliaceae, is a botanical cousin of mahogany.

Family Meliaceae consists of near about 50 genera and 800 species. The members of this family are found to be distributed in tropical regions. In India the family is represented by Azadirachta indica Juss and some other plants. Azadirachta indica is a small to medium-sized tree, usually evergreen, up to 15 (30 max.) m tall, with a round, large crown up to 10 (20 max.) m in diameter; branches spreading; bole branchless for up to 7.5 m, up to 90 cm in diameter, sometimes fluted at base; bark moderately thick, with small, scattered tubercles, deeply fissured and flaking in old trees, dark grey outside and reddish inside, with colorless, sticky foetid sap (Salve, 2014). Neem (Azadirachtaindica) is one of the very few trees known in the Indian subcontinent (Puri, 1999). This tree belonged to Meliceae family, and grows rapidly in the tropic and semi-tropic climate. It is also observed that this tree could survive in very dry and arid conditions. (Puri, 1999). The Neem Tree is an incredible plant that has been declared the Tree of the 21st century by the United Nations (Puri, 1999). In India, it is variously known as ‘Divine Tree’, ‘Life giving tree’, ‘Nature’s Drugstore’, ‘Village Pharmacy’ and ‘Panacea for all diseases’. It is one of the major components in Ayurvedic medicine, which has been practiced in India since many centuries.

Extracts from the Neem tree (Azadirachtaindica) also called ‘Dogonyaro’ in Nigeria are most consistently recommended in ancient medical texts for gastrointestinal upsets, diarrhoea and intestinal infections, skin ulcers and malaria (Schmutterer, 1995). All parts of Neem plant such as leaves, bark, flower, fruit, seed and root have advantages in medical treatment and industrial products. Its leaves can be used as drug for diabetes, eczema and reduce fever. Barks of Neem can be used to make toothbrush and the roots has an ability to heal diseases and against insects. (Puri, 1999). The seed of Neem tree has a high concentration of oil. Neem oil is widely used as insecticides, lubricant, drugs for variety of diseases such as diabetes and tuberculosis (Puri, 1999; Ragasaet al., 1996).

Neem is well-known for its durable wood. In addition, the non-wood productsof neem like flowers, fruits, bark and gum also find various uses. The antifungal, insecticidal and other versatile biological varieties of these products are well known (Salve, 2014).

Neem is also used as a bio-control agent to control many plant diseases. This plantmay usher in a new era in pest control, provide millions with inexpensive medicines, cut down the rate of human population growth and even reduce erosion, deforestation, and the excessive temperature of an overheated globe." Neem's other descriptions, such as "nature's gift to mankind," "the tree for many an occasion," " the tree that purifies," " the wonder tree," "the tree of the 21st century," and "a tree for solving global problems," are its recognitions. The ingredients of Neem tree have remarkable pest control properties besides medicinal values. Neem tree is known for rich pesticide properties, even though the neem trees are infested by insect, taking it into consideration present investigation is undertaken. During present investigation it is observed that

Neem trees from Maharashtra, India are infested by the insect Aeolesthesholosericea fabricius. Neem plant is found as a host plant for insect Aeolesthesholosericea fabricius first time from Maharashtra, India. No previous records are available from Maharashtra, India for such type of infestation caused by Aeolesthesholosericea fabricius on Neem plant (Salve, 2014). India encouraged scientific investigations on neem tree as part of his program to revitalise India tradition and also increase commercial interest on neem (Stix, 1992) and presently some authors believe that no other plant or tree in the world has been so extensively researched or used in all possible capacities so far. In Africa, extracts from neem leaves have provided various medicinal preparations (Ekanem, 1971; Udeinya, 1993). Neem plant (Azadirachta indica) has been of great benefit in human health due to its biochemical, pharmacological, and medicinal properties.

1.2.1 Scientific classification

Kingdom : Plantae

Division : Magnoliophyta

Order : Sapindales

Family : Meliceae

Genus : Azadirachta

Species : A. indica

Binomial name : Azadirachta indica

Figure (1.4) Azadirachta Indica tree

1.2.2 Description of the Neem tree

The neem tree (Azadirachta indica), is a tropical evergreen with a wide adaptability, native to India and Burma, it has been transplanted to Africa, the Middle East, South America and Australia. It is especially suited to semi-arid conditions and thrives even in the poorest soil with rainfalls as little as 18 inches (450 mm) per year and temperatures up to 50° C (120° F). Neem can grow into a big tree to a height of about 20 to 35 m. Its canopy of leaves makes it a useful shade tree. It is planted along roads and avenues in the towns and villages of India The lifespan of the Neem tree is described to be anywhere between 150 to 300 years. Its blossoms are small, white flowers with a very sweet, jasmine-like scent. Its edible fruit is about 3/4 of an inch (2 cm) long, with white kernels. A neem tree generally begins bearing fruits at three to five years of age, and can produce up to 50 kg of fruit annually when mature. The pinnate leaves have a very bitter taste and a garlic-like smell Trunk: The trunk is relatively short, straight and may reach a diameter of 1.2 m (about 4 feet). It is classified as a bush.

Leaves: The opposite pinnate leaves are 20-40 cm (8 to 16 inch) long, with 20 to 31 medium to dark green leaflets about 3-8 cm (1 to 3 inch) long. The petioles are short. Very young leaves are reddish to purplish in colour. The shape of mature leaflets is more or less asymmetric and their margins are dentate with the exception of the base of their basiscopal half, which is normally very strongly reduced and cuneate or wedge-shaped (Ganguli, 2002).

Flowers: The (white and fragrant) flowers are arranged axillary, normally in more-or-less drooping panicles which are up to 25 cm (10 in.) long. The inflorescences, which branch up to the third degree, bear from 150 to 250 flowers. An individual flower is 5-6 mm long and 8-11 mm wide. Protandrous, bisexual flowers and male flowers exist on the same individual. Flowers are used to make a curry called ugadipachadi.

Fruit: The fruit is a smooth (glabrous) olive-like drupe which varies in shape from elongate oval to nearly roundish. The fruit skin (exocarp) is thin and the bitter-sweet pulp (mesocarp) is yellowish-white and very fibrous. The mesocarp is 0.3-0.5 cm thick. The white, hard inner shell (endocarp) of the fruit encloses one, rarely two or three, elongated seeds (kernels) having a brown seed coat (Ganguli, 2002). Seeds usually fall to the ground and might stay there or be carried away with rain water. Occasionally they are dispersed away from the parent tree by birds which give them a greater chance of growing into a healthy new plant. Neem oil is obtained from the seeds.

1.2.3 Neem oil

Neem oil is a vegetable oil pressed from the fruits and seeds of neem plant (Azadirachtaindica).Neem oil is generally light to dark brown, bitter and has a rather strong odour that is said to combine the odours of peanut and garlic. It comprises mainly triglycerides and large amounts of triterpenoid compounds,

which are responsible for the bitter taste. It is hydrophobic in nature and in order to emulsify it in water for application purposes, it must be formulated with appropriate surfactants (Rajeev Seenappa, 2009).

Neem oil also contains steroids (campesterol, beta-sitosterol, stigmasterol) and a plethora of triterpenoids of which azadirachtin is the most well-known and studied. The azadirachtin content of neem oil varies from 300ppm to over 2500ppm depending on the extraction technology and quality of the neem seeds crushed (Puri,1999).

1.2.4 Neem gum

Neem Gum is a clear, bright and brown-coloured gum obtained from the trunk of neem. This is as a result of certain metabolic mechanism of plants and trees. The gum is a multipurpose by product either water soluble or absorbs water to form a viscous solution. Neem gum is obtained from the trees of Azadirachta indica belongs to the family Meliaceae. Each and every part of the tree (bark, leaves, root and fruit) serves a certain purpose. Neem gum contains mannose, glucosamine, arabinose, galactose, fucose, xylose and glucose(Anderson and Henrie, 1971).
Neem (Azadirachta indica) gum occupies a special position among plant gums in that, it contains about one-thirdof its weight as proteins (Anderson and Henrie, 1971), the highest concentrationreported for any plant gum.

Neem gum used as a binder in pharmaceutical dosage forms. Sustained release matrix tablets of nimesulide using the fruit mucilage of Azadirachta indica was studied (Anderson and Henrie, 1971).

1.2.5 Chemical compounds in neem

The late Pakistani scientist Salimuzzaman Siddiqui was the first scientist to bring the plant to the attention of phytopharma cologists. In 1942 while working at the Scientific and Industrial Research Laboratory at Delhi University, India, he extracted three bitter compounds from neem oil, which he named nimbin, nimbinin, and nimbidin respectively (Ganguli, 2002). The seeds contain a complex secondary metabolite azadirachtin. Several chemical compounds have been identified and scientists feel that there are many more compounds yet to be identified in neem. Other than sodium, potassium, salts, it contains chloriphyle, calcium, phosphorus, iron, thiamine, riboflasium, nicocin, vitamin C, carotene, and oxalic acid. The chemicals classified are:

• Nimbin: anti-inflammatory, anti-pyretic, anti-histamine, anti-fungal

• Nimbidin: anti-bacterial, anti-ulcer, analgesic, anti-arrhythmic, anti-fungal

• Ninbidol: anti-tubercular, anti-protozoan, anti-pyretic

• Gedunin: vasodilator, anti-malarial, anti-fungal

• Sodium nimbinate: diuretic, spermicide, anti-arthritic

• Quercetin: anti-protozoal

• Salannin: insect repellent

• Azadirachtin: insect repellent, anti-feedant, anti-hormonal.

Other chemicals that form its therapeutic value are:

• Limonoids

• Terpenoids and steroids

• Tetranortarpenoids

• Fatty acid derivatives like margosinone and margosinolone

• Coumarins like scopoletin, dihydrosocoumarins

• Hydrocarbons like docosane, pentacosane, hetacosane, octacosane etc.

• Sulphur compounds

• Phenolics

• Flavonoglycosides

• Tannins

The highest concentrations of the active ingredients are found in the seed and oil, however the active ingredients are also found in lesser amounts in the bark and the leaves.

1.2.6 Medicinal properties of neem

For thousands of years the beneficial properties of Neem (Azadirachta indica A. Juss) have been recognized in the Indian tradition. Each part of the neem tree has some medicinal properties (Biswas et al., 2002).

Since time immemorial, Indians are aware of medicinal properties of neem. Neem has been extensively used in Ayurveda, Unani and Homeopathic medicine. Traditionally, manydisorders like inflammation, infections, fever, skindiseases, dental disorders and others have beentreated with different parts of neem tree such as leaves, flowers, seeds, fruits, roots and bark (Girish et al, 2008). Neem leaf exhibits a wide range of pharmacological activities viz., anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal,

ntibacterial ,antiviral, antioxidant, antimuta genicanti carcinogenic and immunology dilatory (Girish et al, 2008). Ayurvedic literature lists various medicinal uses of neem. It describes neem bark to be cool, bitter, as tringent, acrid and refrigerant and useful intiredness, cough, fever, loss of appetite, worm infestation (Girish et al, 2008). The bark is reported to heal wounds and vitiate conditions of kapha, vomiting, skin diseases, excessive thirst and diabetes. Neem leaves are reported to be beneficial for eye disorders andinsect poisons and to treat vatic disorder. It is reported to be antileprotic. Neem fruits are bitter, purgative, antihemorrhoid and antihelminthic (Girish et al, 2008).

Neem is called 'Sarvaroganivarini' meaning'the curer of all ailments'. In rural India, delivery chambers are fumigated with burning bark of neem.

Dried margosa leaves are burnt to repel mosquitoes. In India several viral diseases are treated with neem (Girish et al, 2008). Neem leaf paste has been used to treat small pox, chicken pox and warts. Neem twigs are used as tooth brushes in rural India and Africa. Dental gel containing neem leaf extract reduces the oral plaque index and bacterial count (Girish et al, 2008). Methanolic extract fraction of neem leaves when tried against Coxsackie 'B' group viruses, produced in vitro antiviral and virucidal effect (Girish et al, 2008). Neem is used to treat malarial fever in ayurvedic medicine system. Neem oil treated mosquito nets and mosquito-repellent tablets are now available in the North-east India. Gedunin (a liminoid) obtained from neem has activity similar to quinine against malarial pathogen (Girish et al, 2008). The neem liminoids (Azadirachtin, salannin, deacetylgedunin) exhibited high larvicidal, pupicidal and antiovipositional bioactivity against malaria vector –Anopheles

stephensi (Girish et al, 2008).Tablet suspension of the bark and leaf of neem showed moderate effect against malarial pathogen, Plasmodium sp. (Girish et al, 2008). Currently, studies on effect of administration of neem solutions on cancer, diabetes, heart diseaseand AIDS are being carried out. Anticarcinogenic activity of neem leaf extract was observed in murinesystem (Girish et al, 2008).Injection of neem leaf preparation to tumor in mice reduced tumour growth, exhibiting anticarcinogenic activity (Girish et al, 2008). Induction of apoptosis in rat oocytes was seen when treated with neem leaf extract (Girish et al, 2008). Buccal pouch carcinogenesis inhamsters was inhibited by ethanolic leaf extract of neem (Girish et al, 2008).The ethanolic leaf extract of neem also caused cell death of prostate cancer cells (PC-3) byinducing apoptosis (Girish et al, 2008).Good antioxidant activity was observed with neem leaf aqueous extract, flower and stem bark ethanolic extracts (Girish et al, 2008).Administration of aqueous extract of neem along with DOCA salt prevented the development of hypertension in rats (Girish et al, 2008). Neem leaf extracts are antimutagenic. The ethanolic extract of neem leaves exhibited strong antimutagenic activity in Channapunctatus, a fresh water fish model (Girish et al, 2008).Aqueous extract of neem root and leaves reducedblood sugar level in rats exhibiting antidiabeticactivity (Girish et al, 2008).The bark extract completely healed theduodenal ulcers when administered at the dose of 30-60 mg twice daily for 10 weeks. Neem bark extract had potential of controlling gastrichypersecretion, and gastroesophageal andgastroduodenal ulcers (Girish et al, 2008).Acetone-water neem leaf extract showed antiretroviral activity through inhibition of cytoadhesion. The extract increased hemoglobinconcentration, mean CD4+ cell count anderythrocyte sedimentation rate in HIV/AIDS patients(Girish et al, 2008). Enhancement of antibody production andcellular mediated response by neem

components helps in the treatment of AIDS (Girish et al, 2008). Neem leaf and seed extracts exhibited antidermatophytic activity against dermatophytesviz,Trichophytonruberum, Mentagrophytes, Trichophytonviolaceum, Microsporumnanum and Epidermophytonflocosumunder in vitro conditions (Girish et al, 2008). Neem seed oil showed bactericidal activity gainst 14 strains of pathogenic bacteria (Girish et al, 2008).Crudeaqueous and solvent extracts of neem were tried against 20 strains of pathogenic bacteria whereincrude extract produced better results (Girish et al, 2008). The contraceptive property of neem oil has been reported (Girish et al, 2008). Neem leaf extract has spermatotoxic effect. The leaf extracts of neem, showed 100% immobilization and mortality ofhuman spermatozoa at a 3 mg dose within 20seconds (Girish et al, 2008). A new vaginal contraceptive, NIM-76 was developed from neem oil having antimicrobial activity against Escherichia coli, Klebsiellapneumoniae and Candida albicans (Girish et al, 2008).

Traditionally neem was used in Ayurveda for a number of conditions. It is one of the main ingredients in every blood purification formula used in Ayurveda and it appears in most diabetic formulas as well. It is also used for arthritis, rheumatism, the removal of external and internal parasites, including malaria and fevers and as an insect repellent(Sri, 2009).

Leaf: Leprosy, skin problems, skin ulcers, intestine worms, anorexia, eye problems, epistaxis, biliousness Bark: Analgesic, curative of fever Flower: Elimination of intestine worms, phlegm, bil suppression, Fruit: Diabetes, eye problem, piles, intestine worms, urinary disorder, wounds, leprosy, epistaxis Twig: Asthma, cough, piles, intestine worms, obstinate urinary disorder, phantom tumor, spermatorrhoea Gum: Scabies, wounds, ulcer, skin diseases Seed: Intestine

worms and leprosy Oil: Intestine worms, skin diseases and leprosy Root: Refrigerant, diuretics(Sri, 2009).

1.2.7 Modern researches on the effects of neem

Neem leaf extract has been prescribed for oral use for the treatment of malaria by Indian Ayurvedic practitioners from time immemorial. Recently, a clinical trial has been carried out to see the efficacy of neem extract to control hyperlipidemia in a group of malarial patients severely infected with P. falciparum. The lipid level, especially cholesterol, was found to be lower during therapy when compared to non-malaria patients. Reports are available regarding the use of neem to treat patients suffering from various forms of cancer (Kausik, 2002). One patient with parotid tumour and another with epidermoid carcinoma have responded successfully when treated with neem seed oi1 Neem leaf aqueous extract effectively suppresses oral squamous cell carcinoma induced by 7,12-dimethylbenz[a] anthracene (DMBA), as revealed by reduced incidence of neoplasm. Neem may exert its chemopreventive effect in the oral mucosa by modulation of glutathione and its metabolizing enzymes (Kausik, 2002).

NIM- 76, a refined product from neem oil, was studied in 10 human volunteers, where intra-vaginal application before sexual intercourse could prevent pregnancy with no adverse effect on vagina, cervix and uterus. The data suggested that intrauterine treatment is safe (Kausik, 2002).

1.3 Foam fractionation process Foam

concentration/fractionation is a separation technique in which surface-active solutes are either concentrated from a dilute solution or separated from a mixture by preferential adsorption at a gas liquid interface created by sparking an inert gas through

the solution. These gas bubbles entrain the surfactant solution and form stable foam with a large gas liquid interfacial area. As the foam moves through the column, the surfactant solution tends to drain due to gravity and capillary forces. This results in a decrease in the amount of liquid in the foam. The reduction in the entrained liquid is first associated with the bubbles forming the closest spherical packing, after which they will deform to a dodecahedral shape and then possibly coalesce. Consequently, there is an increase in the gas liquid interfacial area per unit volume of the liquid. The surfactant tends to adsorb preferentially at the gas liquid interface. At the top of the column, the foam is sent to a foam breaker where the foam is broken either mechanically or chemically. This results in either enrichment or concentration of more surface-active protein because of the recovery of adsorbed protein from the gas liquid interface into the bulk entrained liquid. In the case of a dilute solution of a single protein, the extent of enrichment would depend upon the relative amount of adsorbed protein compared to that in the bulk entrained liquid. In the case of a mixture of proteins in solution, the separation of a protein from the mixture would depend upon the extent of preferential adsorption of that protein at the gas liquid interface. Since the adsorption isotherm usually leads to a much higher proportion of adsorbed protein at very low bulk concentrations, foam concentration is very effective for extremely dilute solutions. Because of the presence of hydrophilic and hydrophobic functional groups, proteins are surface active. Therefore, foam-based separations are viable for separation of protein solutions. Foam based separation has been applied to various proteins and enzymes (Narsimhan, 2000).

1.3.1 Performance Characteristics of foam fractionation

To evaluate the performance of the separation the following criteria are considered. Enrichment (Ef) is defined as the ratio of foam concentration to that of initial feed.

Ef = Concentration of protein in the foam Concentration of protein in the initial solution On the other hand, the recovery of protein ratio (PR) is the fraction of feed protein recovered in the foam. It determines the efficiency of the process and is given by: PR = kd x 100 (Kd + Vr ⁄ Vf)

Where Kd is the distribution coefficient, defined as the ratio of protein concentration in foam to that of the residual solution, Vr and Vf are the respective volumes of the residue and foam after separation.
The residual ratio (RR) is also considered as a measure of the residual concentration with respect to the original feed concentration:
RR = Concentration of protein in the residual solution Concentration of protein in the initial solution: PR =

Concentration of protein in the residual solution

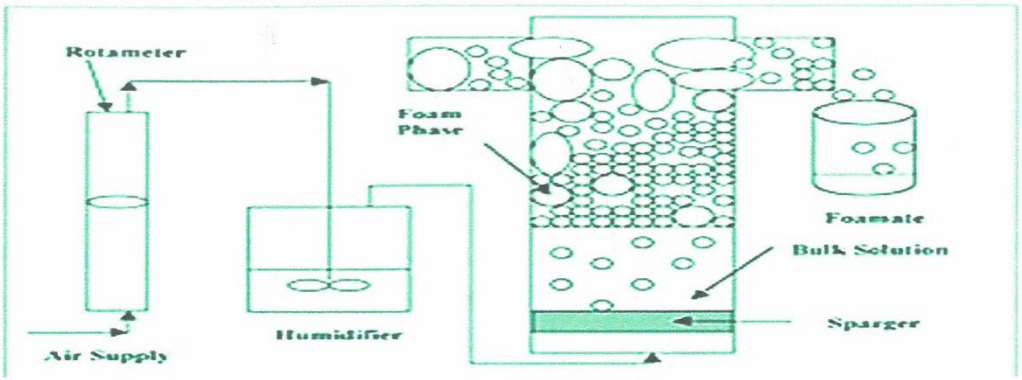
Concentration of protein in the initial solution

The volume of the foam produced is also a measure of the performance as this relates to the loss of liquid from the initial solution

1.3.2 Protein foaming

Protein molecules, in their native conformation, are interconnected polypeptides arranged in specific structures. Generally speaking, both hydrophobic and hydrophilic groups exist at the surface of a protein molecule. At the air-water interface, these hydrophobic groups tend to escape from the water side to spread at the interface, while the hydrophilic groups prefer to stay in the water side, Figures (1.5, 1.6a and 1.6b). The surface pressure increased at a greatly longer time constant than the surface excess, indicating significant unfolding of protein after the surface excess had approached its

equilibrium value. In addition, it was inferred that protein adsorption was diffusion controlled at early stages, but that an energy barrier for adsorption had to be overcome as the surface excess increased. Graham and Philips (1979b) showed that proteins adsorbed in multilayers but that adsorption to anything but the first layer had no effect on the surface tension.



gure (1.5): Diagram of laboratory batch foam - fractionation apparatus

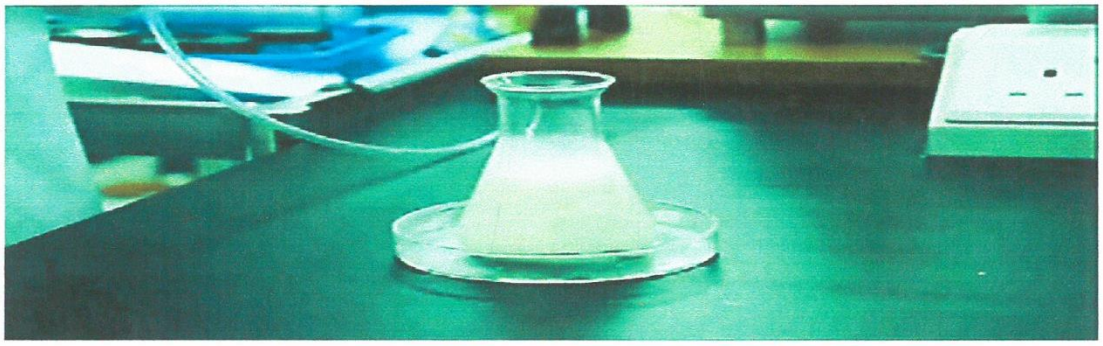


Figure (1.6a): Foam fractionation process when gas start passing

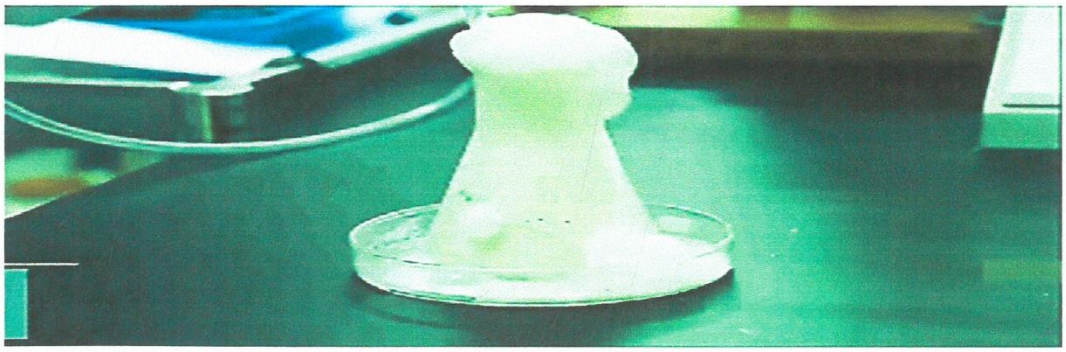


Figure (1.6b): Foam fractionation process after gas passing

1.4 Objectives:

The objectives of this study is:

- Fractionate the Neem gum into two fractions high protein fraction (HPF) or hydrophobic fraction and a low protein fraction (LPF) or hydrophilic fractions using a foaming method.
- To determine the physiochemical properties of the neem gum fractions.

CHAPTER TWO

MATERIALS & METHODS

2. MATERIALS AND METHODS

2.1 Collection of sample

Azadirachta Indica gum collected from neem tree in the Sudan University in March, 2016.

2.2 Sample preparation and treatment

Sample was kept in clean, dry, plastic containers.It was then grinded, using mortar and pestles to fine particles and left to dry at room temperature, and saved in clean, dry glass bottles. Analysis was carried at the chemistry labs in college of science (Sudan University of science and Technology), Central lab in Shambat, University of Khartoum and chemical laboratory atHerbarium of Medicinal and Aromatic Plant Research Institute (MAPRI).

2.3 Chemicals and materials

- Neem gum (AzadirachtaIndica).

- Distilled water.

2.4 Apparatus and Instruments

- Porcelain.

- Beaker

- Measuring cylinder

- Weight bottle
- Sensitive balance
- Hot air oven
- Thermostatic water bath
- pH meter.
- Polarimeter.

- Mortar and pestle.
- Fuming refluxing.

2.5 Methods analysis

2.5.1 Foaming method of neem gum

30 g of neem gum was dissolved in 300 ml of distilled water, the solution was then blown with air using an aerating pump till foaming stops. The foam was collected on a Petri dish and exposed to air to dry. The foam is high protein content fraction (HPF) or hydrophobic Fraction. The Draining was dried using freeze drying, which is the low protein content fraction (LPF) or Hydrophilic fraction. After that the two yieldswere taken to determine the physicochemical properties.

Figure (2.1): Shape and colour of Azadirachta Indica gum

Figure (2.2): HPF or (Hydrophopic fraction) of Azadirachta Indica gum

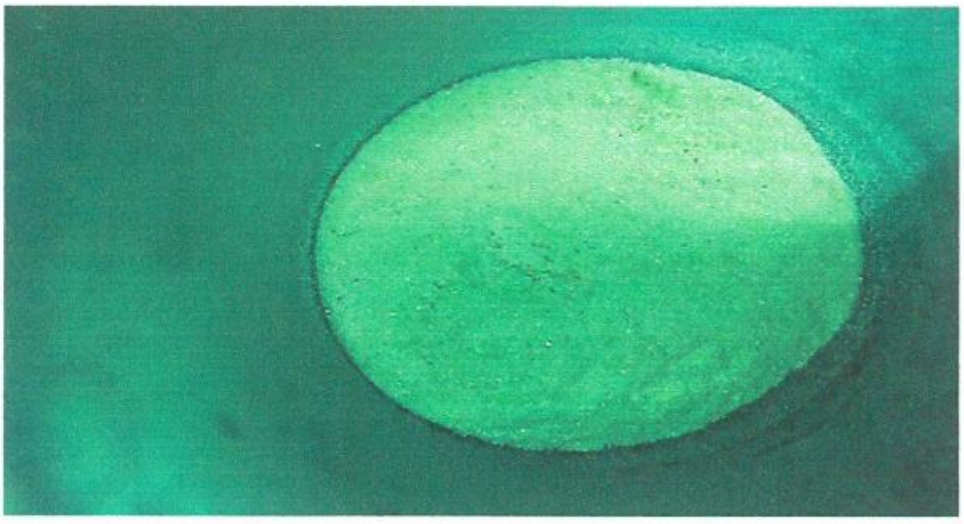


Figure (2.3): LPF or (Hydrophilic fraction) of Azadirachta Indica gum

2.5.2 Determination of moisture content

Accurately 0.5 gram of Azadirachta Indica gum of each sample was weighed in weighed dishes and heated at 105°C in oven for 6 hours to a the initial weight from the following relation; Moisture % = (w2/w1) × 100

Where w2 = weight of sample after heating

w1= weight of sample before heating

2.5.3 Determination of Ash content

The total ash of test sample of each sample was determined according to FAO paper No, (44). A crucible was heated at 550°C, cooled in a desiccators and weighed (w1), accurately one grams of sample was weighed in the crucible (w2) and ignited in muffle furnace at 550°C for 6 hours, and cooled in desiccators and weighed (w3). Then the total ash % s calculated from the following relation.

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

2.5.4 Determination of total Nitrogen and Protein

0.5 gram of each sample (in duplicate) was 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% 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 of 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 as follows
Protein % = N% × 6.25

2.5.5 Intrinsic viscosity

An aqueous solution (1%) was prepared from each sample of the whole gum sample and the fractions 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 intrinsic viscosity was obtained by extrapolation of reduced viscosity against concentration back to zero concentration.

2.5.6 Determination of specific optical rotation1% solution were prepared from the dry samples using distilled water, the gum solutions were mixed on a roller mixer until the sample completely dissolve, and then filtered through watmann No.42 filter paper. Then loaded into the sample holder without trapping air bubbles. Optical rotation was measured using digital polarimeter equipped with 250 mm tube filled with the test

solution t room temperature. Specific rotation was calculated by the following relation;

$[\alpha]tD = \alpha \bullet 100/Cl$

Where α is the observed rotation of the solution in circular degrees, C is the grams of substance per 1 ml of solution, and l is the length of the solution in decimeter.

2.5.7 pH value

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

2.5.8 Equivalent weight

The aqueous gum solution (3%) of each sample was treated with acid washed Amberlite Resin 120 (H+) [2 gms per 10 mls gum solution] 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

2.5.9 Molecular weight

The molecular weight of each sample was calculated from intrinsic viscosity using Mark – Houwink equation.

CHAPTER THREE

RESULTS & DISCUSSION

3. RESULTS AND DISCUSSION

Table (3.1): Physicochemical properties of the crude gum neem and fractions

Neem Gum	HPF	LPF	Crude gum
Moisture content (%)	7.56	7.54	7.5
Ash content (%)	8.41	8.31	3
Nitrogen content (%)	1.41	1.31	0.35
Protein content (%)	2.85	0.91	2.28
Intrinsic viscosity	17.6	6.6	10.3
Specific optical rotation (°)	+57.8	+55.8	-17.3o
pH value	4.5	4.3	4.9
Molecular weight	7.31×10^5	7.01×10^5	1.36×10^5
Equivalent Weigh	4.78×10^5	4.57×10^5	-

3.1 Moisture content

The moisture content values of Azadirachta Indica samples in table (3.1) were (7.56% and 7.54%) which showed similarity to moisture content value (7.54%) that studied by both (Abayomiet al, 2014) and (Omer, 2004).

3.2 Ash content

The ash content values of Azadirachta Indica samples in table (3.1) were (8.41% and 8.31%), the HPF value was more than that reported by (Muhammed et al, 2013) (8.31%), but LPF value was showed similarity to that reported by (Muhammed et al, 2013). Both HPF and LPF were more than that reported by (Omer, 2004).

3.3 Nitrogen content

The nitrogen content values of Azadirachta Indica samples in table (3.1) were (1.41% and 1.31%), the HPF value showed similarity to that studied by (Muhammed et al, 2013) (1.42%), but LPF value was less than that reported by (Muhammed et al, 2013). Both HPF and LPF were more than that reported by (Omer, 2004).

3.4 Protein

The protein content values of Azadirachta Indica samples in table (3.1) were (2.85% and 8.91%), the HPF value was very less comparable to that studied by (Muhammed et al, 2013)(8.93%),but LPF value showed similarity to that studied by (Muhammed et al, 2013). Both HPF and LPF were more than that reported by (Omer, 2004).

3.5 Viscosity

The viscosity values of Azadirachta Indica samples in table (3.1) were (17.6 and 6.6), the HPF value was very high comparable to that studied by (Nahla, 2000) and (Omer, 2004), but LPF value showed similarity to that studied by (Nahla, 2000).

3.6 Specific optical rotation

The specific optical rotation values of Azadirachta Indica samples in table (3.1) were (+57.8° and +55.8°) which showed similarity to that studied by (Nahla, 2000) and different from that studied by (Omer, 2004).

3.7 pH

The pH values of Azadirachta Indica samples in table (3.1) were (4.5 and 4.3) which were similar to that reported by (Muhammed et al, 2013) and less than that studied by (Omer, 2004).

3.8 Conclusion

Physicochemical properties of two fractions of Azadirachta Indica samples have been studied (moisture, ash, nitrogen, protein, viscosity, specific optical rotationand and pH). From all the data obtained it is clear that the two fractions of Azadirachta Indica gum investigated differ in their composition, structure and physicochemical properties. The results were good comparable to other studied.

In this experiment, the high protein fraction HPF (Hydrophobic fraction) has high protein content and high viscosity

References

Abayomi T. Ogunjimi and GbengaAlebiowu (2014). Material and tableting properties of AzadirachtaIndicagumwith reference to official Acacia Gum. ActaPoloniaePharmaceutica N Drug Research, Vol. 71 No. 1 pp. 107-118.

Anderson, D. M. W. and Hendrie, A. (1971) Carbohyd. Res., 20, 259.

Anderson, D.M.W. (1986). Nitrogen conversion factors of the proteinaceous content of gums permitted as food additives and contaminants.No 3,p 231–234.

Andreotti A, Bonaduce I, Colombini MP, Modugno F, Ribechini E(2008).
Characterization of natural organic materials in paintings by GC/MSanalytical procedures. In New Trends in Analytical, Environmental andCultural Heritage Chemistry. Edited by Colombini MP, Tassi L. Kerala:Transworld Research Network; pp 491.

Anyaehie, U.S.B. (2009). Changes in Some Physiological Parameters of HIV/AIDS Patients on Antiretroviral and Neem (AzadirachtaIndica) Extract in Enugu, Nigeria. Ph.D. Thesis, Abia State University, Uturu.

Biswas, K., Chattopadhyay, I., Banerjee, R.K. and Bandyopadhyay, U. (2002). Biological Activities and Medicinal Properties of Neem (AzadirachtaIndica). Curr. Sci. 82 (11): 1336 – 1345.

Bhattacharyya, K.G. and Sharma, A. (2004). AzadirachtaIndicaLeaf Powder as an Effective Biosorbent for Dyes: A Case Study with Aqueous Congo RedSolutions. Journal of Environmental Management. 71 (3): 217-22.

Bleton J, Mejanelle P, Sansoulet J, Goursaud S, Tchapla A (1996). Characterizationof neutral sugars and uronic acids after methanolysis andtrimethylsilylation for recognition of plant gums. J Chromatogr A,720:27–49.

Churms, S.C, Merrifield, E.H. & Stephen, A.M.(1983). Some new aspects of the molecular structure of Acacia senegal gum (gum arabic). Carbohydrate.Res.123, p 267-264.

Colombini MP, Ceccarini A, Carmignani A (2002). Ion chromatographycharacterization of polysaccharides in ancient wall paintings.J Chromatogr A, 968:79–88.

David, P.H., Hommel, M., Miller, L. H. Udeinya, I. J. and Oligino, L. D. (1983). Parasite Sequestration in Plasmodium Falciparum Malaria: Spleen and Antibody Modulation of Cytoadherence of Infected Erythrocytes. ProcNaH. Acad. Sci. 80 (16): 5075-5079.

Defaye, J& Wang, E. (1986), Carbohydr,Res , 150, p 221-231. Dermyn, M.A.(1962). Chromatography of Acidic Polysaccharide on DEAE. Cellulose. Australian Journal of Biological Science, Vol.5, p 787-791.

Ekanem, O. J. (1971). Has AzadirachtaIndica (Dogonyaro) any Antimalarial Activity? Niger. Med. J.8: 8-11.

Elkhatim, K.S. (2001). Factors affecting the emulsifying properties of Acacia gums, MSc Thesis .U. of Khartoum, Sudan.

Elnour, A.H. (2007). Fractionation; Physicochemical and Functional properties of Acacia Polyacantha gum. M.Sc. Thesis, University of Khartoum, Sudan.

Ganguli, S. (June 2002). Neem: A Therapeutic For all Seasons. Current Science.82. (11): 1304.

Gandhi, M., et al. (1988). Acute Toxicity Study of the Oil from Azadirachta Indica Seed (neem oil).

Journal of Ethnopharmacology.23(1):39-51.

Glieksman, A.M and Saud, R.E. (1973). In whistler, R. L. ed "Industrial Gums" 2nd ed. Academic Press, New York. Girish K., Shankara B. S. (2008). Neem –A Green Treasure. Electronic Journal of BiologyVol. 4(3):102-111. Harvey DJ (2011). Derivatization of carbohydrates for analysis bychromatography; electrophoresis and mass spectrometry. J Chromatogr B, 879:1196–1225.

Johnson S., Morgan E. D. and Peiris C. N. (1996). Development of the Major Triterpenoids and Oil in the Fruit and Seeds of Neem (Azadirachta Indica). Journal of Annals Botany. 78: 383-388.

Kharbade BV, Joshi GP (1995). Thin-layer chromatographic and hydrolysis methods for the identification of plant gums in art objects. Stud Conserv, 40:93–102.

Lewis, B.A.,Smith, F. (1957). Journal of the American Society. 79, p 3929.

Masoumeh, M.M., et al. (2002). Sterility Effects of Neem (Azadirachta Indica) Extract on Male Rat. Journal of Reproduction and Infertility.3 (2): 10.

Mbah, A.U., Udeinya, I.J., Shu, E.N., Chijioke, C.P., Nubila, T., Udeinya, F., Muobuike, A., Mmuobieri, A. and Obioma, M.S. (2007). Fractionated Neem Leaf Extract is Safe and Increases CD4+ Cell Levels in HIV/AIDS Patients. AM. J. Ther.14(4): 369-374.

Mejanelle P, Bleton J, Tchapla A, Goursaud S (2002). Gas chromatography–mass

spectrometric analysis of monosaccharides after methanolysis and trimethylsilylation. Potential for the characterization of substances ofvegetal origin: application to the study of museum objects. J ChromatogrLibr, 66:845–902.

Mills J, White R (1999). Organic Chemistry of Museum Objects. 2ndedition. London: Butterworth-Heinemann.

Mohashine B.M.D., et al. Antibacterial Effects of Crude A Indica (Neem) Bark Extract on Streptococcus Sobrinus. Dept of Pedia Dentistry, Okayama.

Molna´r-Perl I (2000). Role of chromatography in the analysis of sugars, carboxylic acids and amino acids in food. J Chromatogr A, 891:1–32.

Mongkholkhajornsilp D., Douglas S., Douglas P.L., Elkamel A., Teppaitoon, W. and Pongamphai, S. (2004). Supercritical CO2 Extraction of Nimbin from Neem Seeds-A Modeling Study. Journal of Food Engineering71: 331-340.

Muhammed Kashif and ShafqatUllah (2013). Chemical Composition and Minerals Analysis of Hippophaerhamnoides, Azadirachta indica, Punicagranatu and Ocimum sanctum Leaves. World Journal of Dairy & Food Sciences 8 (1): 67-73. Narsimhan, G. (2000). Foam Fractionation. Purdue University, West Lafayette, IN, USA.

Natarajan, V., et al. (2003). Effect ofAzadirachtaIndica(Neem) on the Growth Pattern of Dermatophytes. Indian Journal of Medical Microbiology. 21 (2):98-101.

Nwafor, S.V., Akah, P.A., Okoli, C.O., Onyirhioha, K.C. and Nwosu, C.S. (2003). Interaction between Chloroquine Sulphate and Aqueous Extract of Azadirachta IndicaA. Juss (Meliaceae) in Rabbits. Acta Pharm.53:305-311.

Ofusori, D.A., et al. (2008). Regenerative Potential of Aqueous Extract of Neem Azadirachta Indica on the Stomach and Ileum following Ethanol- Induced Mucosa Lesion in Adult Wistar Rats. The Internet Journal of Nutrition and Wellness.5 (2). Omer, E.A. (2004). Characterization and Analytical Studies of A.polyacontha Gum, Ph.D. Thesis, Sudan, University of Science and Technology, Khartoum, Sudan.

Pitthard V, Griesser M, Stanek S (2006). Methodology and application of GC-MSto study altered organic binding media from objects of the Kunsthistorisches Museum, Vienna. Ann Chim Rome, Italy,96:561–573.

Pitthard V, Griesser M, Stanek S, Bayerova T (2006). Study of complex organicbinding media systems on artworks applying GC-MS analysis: selected examples from the Kunsthistorisches Museum, Vienna. Macromol Symp, 238:37–45.

Pitthard V, Finch P (2001). GC-MS analysis of monosaccharide mixtures as the irdiethyldithioacetal derivatives: application to plant gums used in artworks. Chromatographia, 53:S317–S321.

Puri, H.S. (1999). Neem-The Divine Tree. Harwood Academic Publishers, Amsterdam.

Ragasa, C.Y., Nacpil, Z.D., Natividad, G.M., Tada, M., Coll, J.C. and Rideout, J.A. (1997). Tetranortriterpenoids from Azadirachta Indica. Journal of Phytochemistry.46: 555-558. Raveendra, M.P., et al. (2004). Evaluation of Antiplaque Activity of Azadirachta Indica Leaf Extract Gel- a 6week Clinical Study. Journal of Ethnopharmacology.90 (1): 99-103. Ruiz-Matute AI, Hernandez-Hernandez O, Rodriguez-Sanchez S, Sanz ML, Martinez-Castro I (2011).

Derivatization of carbohydrates for GC and GC–MS analyses. J Chromatogr B, 879:1226–1240.

Salve U.S. (2014). Neem, Azadirachta IndicaJuss. A New Host Plant of Insect Aeolesthes Holosericea Fabricius from Maharashtra, India. Online International Interdisciplinary Research Journal, {Bi-Monthly}, ISSN2249-9598, Volume-IV, Issue-I, Jan-Feb 2014.

Schmutterer, H., Ascher, K.R.S. and Isman, M.B. (1995). The Neem Tree: Azadirachta Indica A. Juss and other Meliaceous Plants. Weinheim.1-3.

Schneider U, Kenndler E (2001). Identification of plant and animal glues inmuseum objects by GC-MS after cataliytic hydrolysis of the proteins bythe use of cation exchanger, with simultaneous separation fromcarbohydrates. Fresenius J Anal Chem, 371:81–87.

Singh V, Sethi R, Tewari A, Srivastava V, Sanghi R (2003). Hydrolysis of plant seedgums by microwave irradiation. CarbohydrPolym, 54:523–525.

Singh V, Tiwari A, Kumari P, Tiwari S (2006). Microwave-promoted hydrolysis ofplant seed gums on alumina support. Carbohydr Res, 341:2270–2274.

Stephen, A.M.; Nunn, J.R. and Charles, A.J.(1955). J. Chem. Soc., p 1428.

Stephen AM, Churms SC, Vogt DC (1990). Exudate Gums. Methods Plant Biochem, 2:483–522.

Stix, G. (1992). Village Pharmacy: The Neem Tree Yields Products from Pesticides to Soap. Sci. Am.266:132. Stoddart, J.F.; Andersn, D.M.W. (1966b). Studies on Uronic

Acid Materials: Part XV. The Use of Molecular Sieve Chromatography in Studies on Acacia senegal Gum. Carbohydr. Res. 2: pp. 104-111.

Street, C. A.; and Anderson, D. M. W.(1983). Talanta, 30, p 887-893.

Uday, B., et al. (2002). Clinical Studies on the Effect of Neem (Azadirachta Indica) Bark Extract on Gastric Secretion and Gastroduodenal Ulcer. Life Sciences.75 (24): 2867-2878.

Udeinya, I.J. (1993). Antimalarial Activity of Nigerian Neem Leaves. Trans. Royal Soc. Trop. Med. Hyg.87:471.

Udeinya, I.J., Brown, N., Shu, E.N., Udeinya, F.I. and Quakeyie, F. (2006). Fractions of an Antimalarial Neem Leaf Extract have Activities Superior to Chloroquine and are Gametocytocida. Ann. Trop. Med. Parasitol.100(1): 17-22.

Udeinya, I.J., Mbah, A.U., Chijioke, C.P. and Shu, E.N. (2004). Antimalarial Extract from Neem Leaves is Antiretroviral. Trans. Royal Soc. Trop. Med. Hyg.98: 453-437.

Voget, K. (1995). Common trees and shrubs of dry land. Sudan, London.

Williams, P.A, Phillips, G.O. and Randal, R.C.(1989). Food Hydrocolloids, 3: p 65-75.

Willför S, Pranovich A, Tamminen T, Puls J, Laine C, Suurnäkki A, Saake B, Uotila K, Simolin H, Hemming J, Holmbo B (2009). Carbohydrate analysis of plant materials with uronic acid-containing polysaccharides-a comparisonbetween different hydrolysis and subsequent chromatographic analytical techniques. Ind Crop Prod, 29:571–580.

Yinusa, R., et al. (2004). Effects of Azadirachta Indica Extract on Gastric Ulceration and Acid Secretion in Rats. Journal of Ethnopharmacology. 90 (1): 167-170.