بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Preparation and Characterization of a Cream containing *Citrullus colocynthis* extract

تحضير كريم من زيت الحنضل وصمغ *Azadirchta Indica* (النيم)

A Dissertation Submitted in partial fulfillment of the Requirements

Of M.Sc., degree in Chemistry

By:

Suha Mohammed Hassan mohammed (B.sc., Honours)

Supervisor: Prof. Mohammed Elmubark Osman

November 2016

بسم الله الرحمن الرحيم

(قالَ لَا تُؤَاخِذْنِي بِمَا نَسِيتُ وَلَا تُرْهِقْنِي مِنْ أَمْرِي عُسْرًا)

صدق الله العظيم

سوره الكهف الآية (73)

Dedication

This work is dedicated to

My family

Acknowledgment

First and foremost, praise to Allah, who give me the strength to do this work

I wish to express my gratitude and thanks to Prof. Mohammed El Mubark Osman for his suggestions, guidance, encouragement and support throughout the period of study.

Thanks are also due to Unamid pharmaceutical, Sudan for their cooperation and help.

Special thank to consolidated pharmaceutical industry for technical support.

Abstract

Citrullus colocynthis plant was gathered from soba area; south-east of Khartoum; the seeds were collected and dried. Using soxhlet apparatus *citrullus colocynthis* seeds oil was extracted and the yield was found to be (20.8%).

Physicochemical characterization results showed that the density of the oil was 0.9297(g/cm^3), sponification value was 215.9, acid value was 1.35 and peroxide value was 5.3.

GC-MS analysis of the oil indicates that it contains linoleic acid (66.86%), palmitic acid (7.01%) and stearic acid (6.0%) as major fatty acids, in addition to 4-(2,2-dimethyl-6- methylenecyclohexyl)butanal(3.80%) and sitostenone(16.33%).

Azadirchta indica gum was used to prepare an emulsion cream base, 90% of the cream base and 10% of *Citrullus colocynthis* oil were homogenized to formulate the muscle relaxant product.

The physical properties of the homogeneous product were determined: pH (6.06), viscosity (7888 cp), specific gravity (0.89140 g/cm) and conductivity (126 µs/cm).

المستخلص

تم جمع ثمار الحنضل من منطقه سوبا جنوب شرق الخرطوم، واستخراج البذور ومن ثم تجفيفها.

وباستخدام جهاز السوكسليت تم استخلاص زيت الحنضل بنسبه 20.8%.

الخواص الفيزوكيميائية لزيت الحنضل هي كثافة الزيت 0.9297g/cm ، رقم التصبن 215.9 ، رقم الحموضة 1.35 و رقم البيروكسيد 5.3.

الاحماض الدهنية الموجودة في الزيت هي حمض اللينوليك % 66.86 ، حمض البالمتيك 7.01% ، حمض الاستريك 6.0% بالاضافة الى الكيتون (سيتوستينون 16.33 %) و الألدهيد (4-(2,2 ثنائي ميثايل -6-ميثايلين سايكلو هيكسايل) بيوتانال (3.80%) .

و استخدم صمغ النيم في تحضير مستحلب كريم وتمت اضافة 90% من الكريم الى زيت الحنضل 10% وبدراسة الخواص الفيزيائية للكريم وجد ان:

الرقم الهيدروجيني (6.06) ، اللزوجة (7888 cp) ، الكثافة النوعية ($0.8194\ g/cm^3$) و التوصيلية (126 µs/cm) .

Table of contents	
Contents	Pages

List of Table	

Chapter One

Literature review

Literature review

1-1*Citrullus colocynthis*

Citrullus colocynthis, a member of the *Cucurbitaceae* family, is known as Bitter Apple in English, Handel in Arabic, and Abujahl watermelon in Persian. The extract of the fruit is called colocynthine due to its extreme bitter flavour (Hajar, et al, 2012).

Cucurbitaceae, also called cucurbits, are a plant family known for its high proteins and oil content. Seeds of cucurbits are rich sources of oils and protein. The oil content of the seeds was found to be in the range of 17 – 19% w/w (Schafferman *et a*l, 1998).

1-1-1 Botanical classification of *citrullus colocynthis*

Botanical Name: *Citrullus colocynthis*

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Cucurbitales*

Family: *Cucurbitaceae*

Genus: *Citrullus*

Species: *C. colocynthis* (Abbah,et al,2014).

1-1-2 Chemical contents

Chemical contents of *citrullus colocynthis* are shown in Table (1.1) and Table (1.2) show physical and chemical properties of *citrullus colocynthis* seed oil.

Table (1.1) Chemical content of *Citrullus colocynthis (*Borhade,et al,2013)

s.No	Part	Chemical content(reported/investigated)
1	*Seed*	1. Fatty acid like Stearic, Myristic, Palmitic, oleic, Linoleic, Linolenic acid. 2. Protein 8.25 % rich in lysine, leucine and methionine 3. Vitamin B1 , B2 and Niacin 4. Mineral like Ca, Mg, K, Mn, Fe, P and Zn
2	Aerial part and Fruit	Flavonoid glycoside quercetin, Flavone- 3- glucoside viz iso-vitexin, iso-orentine and isoorentine -3-methyl ether.
3	*Fruit*	1. Cucurbitane type triterpen glycoside viz colocynthoside A and B. 2. Cucurbitane type triterpen glycoside viz cucurbitacin E 2 O-beta-D-glcoside and its aglycone Cucurbitacin E. 3. 2-O-beta-D-glucopyranosyl-16alpha-20R-dihyroxy-cucurbita-1,5,23E,25(26)-teraen-3,11,22-trione. 4. 2-O-beta-D-glcopyranosyl-cucurbitacin B and 2, 25-di-o-beta-D glucopyranosylcucurbitacin L.

Table (1.2) physical and chemical properties of *citrullus colocynthis* seed oil:

Physic-chemical properties	**Ref (Mirjana,2005)**	**Ref (Hiba,2015)**
Specific gravity	0.914(20º C Kg/dm³)	0.886(g/cm³)
Refractive index	1.4733	1.4873(20º C)
Acid value(mgKOH/g)	1.00	3.91
Saponification value(mKOH/g)	188	196.66
Iodine value(g/100g)	119	119.53
Peroxide value(mmolO2/Kg)	7.9	6.97
Unsaponifiable matter(%)	1.02	1.44
Ester number	187	ND

1-1-3 Nutritional value

C.colocynthis (Egusi melon) is used as a common component of daily meals in West Africa. Its seed, which is edible but similarly bitter, nutty-flavored, and rich in fat and protein, is eaten whole or used as an oilseed. As an oilseed, of course, of natural origin, it is not out of place that different reseachers may have results of its oil composition that differ slightly. These differences may be due to various factors acting independently or in synergy and could include, but not limited to, the quality of the stock planted, the nature of the farmland, climatic conditions, processing techniques and analytical methods. Interestingly, one thing that has been reported in unison is that egusi melon oil, predominantly, contains unsaturatted fatty acids. According to Schafferman et al. (1998), the oil content of the seeds is 17-19% (w/w), consisting of 67-73% linoleic acid, 10-16% oleic acid, 5-8% stearic acid, and 9-12% palmitic acid. They also reported that its oil composition is similar to safflower oil, with a total of 80-85% unsaturated fatty

acids, and that its oil yield value is estimated at 250-400 L/hectare. Oluba *et al*. (2008) report the percentage composition by weight of the oil is: lauric, 0.21%; myristic, 0.78%; palmitic, 13.45%; stearic 13.71%; oleic, 14.50%; linoleic, 56.94% and linolenic, 0.46%. This composition amounts to about 72% by weight unsaturated fatty acids, with 57.4% of it being polyunsaturated fatty acids (PUFA). Polyunsaturated fatty acids (PUFAs) are essential fatty acids, that cannot be synthesized biologically and as such, they must be provided in the diet. In fact, essential fatty acid deficiency has been reported to result in dermatitis similar to that seen in zinc or biotin deficiency (Abbah, et al, 2014).

1-1-4 Uses

Citrulus colocynthis seed oil used for frying and cooking in some African and Middle Eastern American countries owing to its unique flavor. (Mirjana,et al,2005).Conventionally, *Citrullus colocynthis* fruits are used in antidiabetic treatment in Mediterranean countries. The seed extract induces insulin secretion and that this could at least partially account for the antidiabetic activity of these fruits. Earlier, the antioxidant effects and the effect of the aqueous extract of the pulp on kidney and liver functions were reported. The leaves of *Citrullus colocynthis* show significant antimicrobial, anti-diabetic, anti-oxidant, local anesthetic, and anti-inflammatory activity reported (S. Gurudeeban,et al, 2011).Table (1-3) show medicinal uses of different part of citrullus colocynthis.

Table (1.3) Medicinal uses of different part of citrullus colocynthis (Mahesh,et al, 2014)

Plant part	How to use	Use
Seed	Seed oil	Bowel complaints
		Epilepsy
		Cooking purpose
		Useful in hair growth and maintaining its Black luster
	Seed oil apply on hair	Blackens grey hair
	Pulp of seeds	Treatment of Malaria
	Seed powder	Soup thickener and flavouring agent
Fruits	Small fruits are collected during rainy season, stuffed with salt and ajwain	Acute stomach ache
	Extract of pulp	Anti-bacterial
	Juice of fruit mixed with sugar	Dropsy
	Juice of the fresh fruit is made. Cotton dipped in juice is placed over the mouth of uterus	Timely and easy delivery
	Decoction is drunk	Treatment of Hepatitis
	Fruits in low doses	Treatment of Urticaria, constipation and toxemia
	Dried Fruit pulp and powdered and taken Orally	Cause abortion
Root	A poultice of root	Inflammation of the breasts
	Powder of roots mixed with ginger And jiggery	Inflammation of joints.
	Equal parts of the root with long pepper are given as pill.	Rheumatism
	A paste of root applied to the enlarged abdomen of children.	Enlarged abdomen

	Root paste	Ascites, jaundice, urinary disease and rheumatism.
	Root base mixed with cow milk is applied on hypogastrium	Easy delivery
Fruit and root	Fruit and root paste with water.	Boils and pimples
	Fruit and root-antidote	Snake poison
Fruit and seed	Jam prepared from pulp of fruits and seeds	Effective in curing biliousness in animals
Whole plant	Glycosidic extract (50 mg/kg)	Lowering glucose level

1-2Plant gum

Plant gums such as gum arabic (*Acacia senegal)*, gum talha (*Acacia seyal*) and grewia gum (*Grewia mollis*) are among important agroforestry resources in Africa gum belt. Gums are harvested from the stems and branches of the resource gum trees as dry exudates. A gums, in general, are water-soluble or water-swellable polysaccharide that is extractable from marine and land forst, or from microorganisms that possess the ability to contribute viscosity or gelling ability to their dispersions. (Abu Baker, *et al*, 2007)

Plant gums are biopolymeric materials composed of complex heteropolysaccharides and proteinaceous material, in addition to some mineral elements(Williams and Phillips, 2000), Also The gum has a high molecular weight (approx. 350-850 kDa) and has galactose (44%), rhamnose (13%), glucuronic acid (16%) and arabinose (27%) residues, but also minerals like potassium, magnesium and calcium(Ali, et al,2013).

1-2-1Properties of *Acacia* gum

Gum Arabic readily dissolves in cold and hot water in concentration up to 50%. Because of the compact, branched structure and therefore small hydrodynamic volume, gum Arabic solutions are characterized by low viscosity,

allowing the use of high gum concentration in various applications (Dziezak, 1991). solution exhibit Newtonian behavior at concentration up to 40% and become pseudo plastic at higher concentration. The pH of the solution is, normally, acidic around 4.5-5.5, but maximal viscosity is found at pH 6.0.The other major functional characteristic of gum Arabic is its ability to act as an emulsifier for essential oils and flavours, gum Arabic has excellent emulsifying properties due to its high molecular weight arabino galactan protein (AGP) fraction. The hydrophobic polypeptide backbone of the AGP strongly adsorb at oil-water interface, while attached carbohydrate units stabilize the emulsion by steric and electrostatic repulsion.

Fractionation studies show that, emulsifying properties, generally, improve with increasing molecular weight and protein content, the best result are obtained with mixtures of different fractions (Ray,et al,1995). The hetero genous nature of the gum makes it an excellent emulsifier.

Buffo et al (2008) reported that the stability of beverage emulsions is influenced by a number of processing factors, such as pasteurization and demineralization, pH of the emulsion.

Emulsions can remain stable for long periods of time(several months); prolonged heating of gum Arabic solutions causes the protein to precipitate out of solution thus affecting the gums emulsification properties.

1-2-2 Chemical constituent of gum Arabic

Crude gum Arabic is a complex copolymer of polysaccharide with a high molecular mass and a complex structure (Connolly,et al,1987;1988). It is a branched molecule (Snowden,et al,1987)with a protein content of about 2.0-2.5%.
The gum is present a mixed calcium, magnesium and potassium salts of polysaccharidic acid (Standford and Barid,1983). It is composed of six carbohydrate moieties; galactopyranose, arabinopyranose, arabinofuranose,

ramnopyranose, glucuropyranosyln uronic acid and4-O-methylglucuropyronosyl uronic acid (Sharma, 1981; Glickman, 1979; Aspinal, et al, 1956; Ekhadem, 1956) polysaccharides that contain arabinose and galactose as their major constituent are called arabinogalactan(AG) (Fincher,et al,1983).

So, all *Acacia* gums are chemically arabino galactan proteins (AGP) And it was described as"hetero poly molecular",i.e,having either a variation in monomer composition and/or avariation in the mode of linking and branching of the monomer units, in addition to adistribution in molecular weight (Lewis,1957; Jermyn,1962; Anderson and Stoddart,1966).

1-2-3 Uses of gums

Due to its desirable properties such as being extremely highly soluble in water, low viscosity and it's abilities to reduce the surface tension(Osman,1993), gum Arabic witnessed aremarkable development in the area uses, especially to that concern to human health and safety such as application in food, pharmaceutical, medicinal and cosmetics…etc, some of these applications are described below;

1-2-3-1Food uses

Gum Arabic is used mainly as food additives, where it act as:

- Emulsifying agent:

  In confectionery gum Arabic function in keeping fat uniformly distributed, and prevents fat from migrating to the surface. This property makes gum useful in caramel and Toffee confections(Glicksman and sand,1973),and retard sugar crystallization.

- Flavour and Fragrance encapsulation:

  In spray dried flavours and fragrance production. Gum Arabic is used, extensively, to stabilize flavour and fragrance emulsions and when subsequently spray-dried, the gum prevents evaporation and oxidative deterioration of flavour, fragrance oil or absorption water from air(Revie and

Thomas,1962).many flavour emulsions such as orange juices, lemon, cherry and cola are prepared using gum Arabic(Johnstone,1939).

- Glazing and adhesive properties:

  In baking industry gum Arabic is widely used for its low water absorption properties and its impressive adhesive properties for use in glazing and toppings (Werbin,1953).

- Foaming and clarifying agent:

  Gum has ability to stabilize foam in the manufacturing of drinks. The gum is also used clarify wine and fix tanning (Williams,2000).

Uses of gum Arabic in food industry are summarized in table (1-4)

Table (1.4) Application of gum Arabic in food industry (Sharma,1981)

Function	Food application
Crystallization inhabitor	Ice cream sugar syrups
Coating agent	Confectionery
Emulsifier	Salad dressing,soft drinks
Encapsulating agent	Powdered“fixed”flavours
Film-forming	Sausage casing, protective coating
Flocculating agent	Wine
Foam stabilizer	Whipped topping, beer
Gelling agent	Pudding,desserts,mousses
Protective colloid	Flavour emulsions
Stabilizer	Beer,mayonnaise
Suspending agent	Chocolate milk
Thickening agent	Jams,sauces,gravies

1-2-3-2 Pharmaceutical and cosmetics uses

Gum Arabic is described as a suspending agent, it has been used to suspend soluble drugs and to prevent the precipitation of metals through the formation of colloidal suspensions (Osborn and Dekay, 1941).

Also it has been used as demulcent in preparations designed to treat diarrhea, dysentery, coughs etc.(U.S. pharmacopoeia,1955). In addition gum Arabic act as emulsifying, binding, and film-forming agents in different types of drugs. Among its many uses gum Arabic functions as stabilizing, emulsifying and suspending agents in lotions and creams. Where it provide a protective coatings and smooth feel. The most important characteristic which encouraged the use of gum in cosmetics formulation is that it is non toxic and free from dermatological and allergic reactions (Feinberg and schoekerman,1940).

1-2-3-3 Medicinal uses

In folk medicine the demulcent, emollient effects of gum are used to treat inflammation of intestinal mucosa, and externally to cover inflamed surfaces, burns, some nipples and nodular lesproy. also,is said to be used for antitussive, astringent, catarrh, colds, coughs and dysentery(Duke and Wain,1981).

Most dietary attempts to treat chronic renal failure and to decrease uraemia use a protein restricted regime (Fouque,et al,2006;Chaturvedi and Jones,2007). It has been also reported that’s supplements to the diets of patients suffering from chronic renal failure with gum Arabic fibers result in a significant reduction in urinary nitrogen excretion and in an increase of faecal nitrogen excertion(Bliss, et al,1996).

GA has also been claimed to act as antioxidant (Al-majed,et al,2002). This finding has not been confirmed by others(Ali, et al,2003). It was reported that gum arabic has lipid per oxidation lowering effect(Trommer and Neubert,2005), and it was suggested by Ali et al(2008) that GA ingestion can reduced plasma cholesterol

concentration; as well as providing protection against hepatic and cardiac toxicities in rats.

Wadood et al (1989) reported that *Acacia* arabica seeds powder, significantly, reduced blood glucose concentration of normal, but not diabetic rabbits.

1-2-3-4 Other industrial uses

In textile industry gum Arabic is used for fabric sizing, water proof emulsions, binding, thickening agents etc (Meer,1980). In lithography gum Arabic functions as a sensitizer for plates, as ingredients in fountain solutions, plate washings and protective coating (Wood,1952). In paints industry, uses of gum Arabic are based on its emulsifying properties in controlling the chemotropic properties of paints (Gamble and Gardy,1938). Gum Arabic acts as suspending and binding agent in Ink formulation (Neil,et al,1947

1-3Emulsions

An emulsion is a dispersed system that consists of two immiscible liquids (usually oil and water), with one of the liquids dispersed as small droplets in the other called continuous phase. (McClements, 1999) Oil droplets dispersed in water are known as “direct emulsions”, whereas water droplets dispersed in oil are called “inverse emulsions” (Mason, et al,2006).

Emulsions are thermodynamically unstable systems that have a tendency to break over time. The breakdown of an emulsion manifests itself through different physicochemical mechanisms such as gravitational separation, coalescence, flocculation, Ostwald ripening and phase inversion.(Sabah El – Kheir, et al,2008) Emulsions take place, partially or completely, in the structures of many natural and processed foods or some foods are already emulsified in certain stages of production . Foods like milk, cream, butter, margarine, juice, soup, cake, pastry,

mayonnaise, cream liqueur, coffee creamer, sauce and ice cream. (Serdaroğlu, et al, 2015)

1-3-1 Types of emulsion

Oil in water emulsion: If the oil droplets are dispersed throughout the aqueous phase, the emulsion is termed oil-in-water (O/W) as shown in Figure1.1

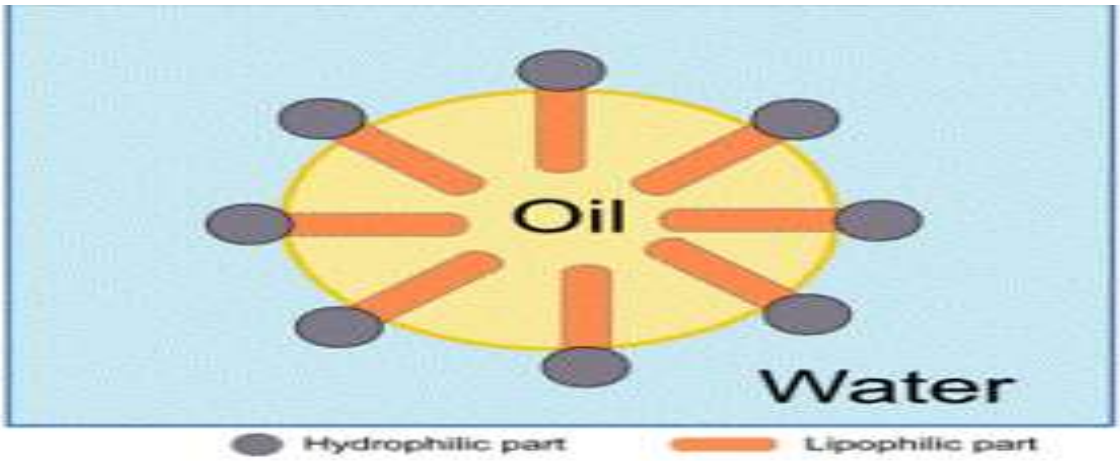

Figure (1.1) O/W emulsion

Water in oil emulsion: A system in which the water is dispersed as globules in the oil continuous phase is termed water-in-oil emulsion (W/O) as shown in Figure 1.2

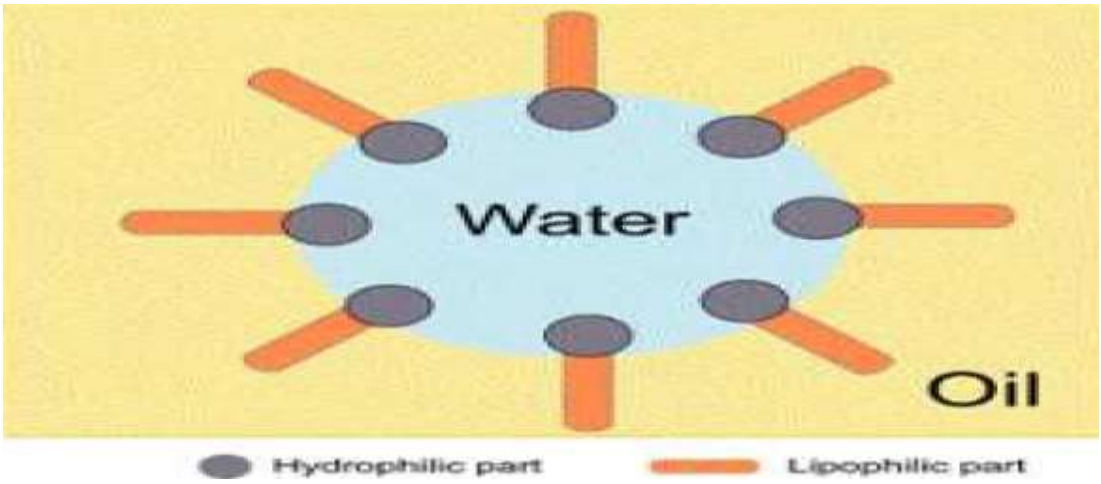

Figure (1.2) W/O emulsion

Multiple emulsions: are complex systems, It is a complex type of emulsion system in which the oil-in-water or water-in-oil emulsions are dispersed in another liquid medium. In this way an oil-in-water-in-oil (O/W/O) emulsion consists of very

small droplets of oil dispersed in the water globules of a water-in-oil emulsion and a water-in-oil-in-water (W/O/W) emulsion consists of droplets of water dispersed in the oil phase of an oil-inwater emulsion .(Khan,et al,2011)

1-3-2 Emulsifying Agents

Emulsifying agents (EAs) are very important ingredients having an essential role to ensure emulsification and stability in the formation of emulsions in aqueous solutions. EAs are amphiphilic molecules including both hydrophilic and lipophilic parts. EAs are surface-active compounds being absorbed on the surface of droplets and ensuring kinetic stability in a certain period by being added into emulsion before or after homogenization Components like thickening agents and stabilizing agents used in the formation of emulsions are different and shouldn't be confused with the term of emulsifying agent. Thickening agents typically increase the viscosity in the continuous phase of emulsion and limit the movements of droplets and develop emulsion stability in this way. Stabilizing agents are used to increase the stability provided by both emulsifying agents and thickening agents. (Serdaroğlu, et al, 2015)

1-4 *Azadrichta indica* (neem) tree

Azadrichta indica is used for thousands of centuries by many cultures for their medicinal values. Herbal treatment is very popular because of its available and non toxicity. *Azadirachta indica* (neem) is a plant, widely, distributed in Asia and Africa subcontinents. Neem has two closely related species: *A. indica (*A. Juss) and *Azedarach melia*(Linn.), the former is popularly known as Indian neem (margosa tree) or Indian lilac, and the other as the Persian lilac (Asif,2013).

It has been used in ayurvedic medicine for more than 4000 years due to its medicinal properties, Most of the plant parts such as fruits, seeds, leaves, bark and roots contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory, antiulcer and antifungal uses(Ogbuewu,et al,2011).

Neem is called '*aristha*' in Sanskrit a word that means 'perfect, complete and imperishable'. The people of India have long revered the neem tree; for centuries, millions have cleaned their teeth with neem twigs, smeared skin disorders with neem leaf juice, taken neem tea as a tonic and placed neem leaves in their beds, books, grain bins, cupboard and closets to keep away troublesome bugs (Pandey,et al,2014).

1-4-1 Botanical classification of Neem

Order*: Rutales*

Suborder: *Rutinae*

Family: *Meliaceae*

Subfamily: *Melioideae*

Trib: *Melieae*

Genus: *Azadrichta*

Specie*: Indica (Pankaj,et al,2011).*

1-4-2 Description of Neem tree

Neem is fast –growing tree, generally 15-20 m tall (sometimes up to 40 m tall), with a crown diameter up to 20 m. Neem is evergreen but can shed most of its leaves under dry conditions. The compound (pinnate) leaves are alternate, 20-40 cm long, with 20-30 dark green, serrated leaflets, each about 3-8 cm long. The terminal leaflet is often absent. Young leaves are reddish to purplish in colour.

Petioles are 70-90 mm long. The bark is deeply fissured. Flowers are cream coloured, perfumed and arranged in axillay clusters (each cluster is called an inflorescence).

Each inflorescence is 15-25 cm long and comprises 150-250 individual flowers. Each flower is about 1 cm in diameter with five petals.

Neem has a strong root system with a deep tap root and extensive lateral roots. Suckers can be produced following damage to roots (Basak,et al,1968).

1-4-3 physicochemical properties of Neem gum (Eman awad and Mohamed Elmubark Osman, 2016) table (1.5)

Table (1.5) Physiochemical properties of Neem gum

Moisture	10.6
Ash	3.7
Optical rotation %(-)	65.2
Viscosity g/cm^3	10.7
Acid equivalent weight %	26.8*10^3
Uronic acid	0.71
Tannin content %	0.152
Nitrogen %	4.02
Protein %	25.17
PH	6.775
Molecular weight x10^6	2.45
Arabinose	15.413
Glactose	20.205

1-4-3Medicinal uses

Neem contained various compounds which showed various biological activities such as anti-inflammatory; Antiarthritic, Antipyretic, Hypoglycaemic, Antigastric ulcer; Spermicidal; Antifungal, Antibacterial, Diuretic, Antimalarial, Antitumour, Immunomodulatory. Table (1.6) show Pharmacological activity of various parts of Neem(Asif,2013).

Table (1.6) Pharmacological activity of various parts of Neem(Asif,2013)

Part used	Medicinal properties
Leaves	Leprosy, eye problem, epistaxis, intestinal worms, anorexia, biliousness, skin ulcer
Barks	Analgesic, alternative and curative fever
Flowers	Bile suppression, elimination of intestinal worms and phlegm
Fruits	Piles, intestinal worms, urinary disorder, epistaxis, phlegm, eye problem, diabetes,wounds and leprosy
Twings	Cough, asthma, piles, phantom tumor, intestinal worms, spermatorrhoea, obstinate urinary disorder, diabetes
Gum	Scabies, wounds, ulcers, skin diseases
Seeds	Leprosy and intestinal worms
Oil	Leprosy and intestinal worms

1-5 Objectives

To formulate a muscle relaxant cream from *Citrullus colocynthis* seed oil and gum of *Azadrichta indica* (neem).

Chapter Two

Materials and Methods

2-1Materials

2-1-1 Sample collection

Representative samples of *citrullus colocynthis* were collected from Khartoum, suba. Figure (2.1)

Figure (2.1) Plant of citrullus colocynthis (Abbah,et al,2014)

Sample of azadrichta indica gum was collected from Khartoum, Sudan University of Science and Technology, Figure (2.2) and Figure (2.3)

Figure (2.2) Gum on Neem tree

Figure (2.3)*Azadrichta indica* gum

2-1-2 Preparation of the sample

The seeds were isolated from *citrullus colocynthis* fruits and then it was dried in shade, 300g from *citrullus colocynthis* seeds were weighted and crushed by moulinex grinder in to powder figure (2.4).

Figure (2.4) Seeds of *citrullus colocynthis* (Abbah,et al,2014)

2-2 Methods

2-2-1 Extraction of the oil

300g of the crushed seeds were placed in a Soxhlet apparatus figure(2.5), using 600 mL of hexane (40-60)˚C, then heated at 40˚C for 5 to 6 hours. The

extract solution was evaporated. Hexane was removed, and the oil % yield was calculated as follows:

$$\%yield = weight\ of\ oil/weight\ of\ seeds\ x100$$

2-2-2 Determination of physiochemical properties of the oil

2-2-2-1 Determination of saponification value

2.5 g of the oil was weighed into a 250 ml conical flask. 25 mL of alcoholic potassium hydroxide solution (0.7N) was pipette into the flask. The flask was connected with air condenser and boiled until fat was completely saponified-as indicated by absence of any oily matter and appearance of clear solution. The flask contents was cooled and titrated with 0.45N hydrochloric acid using phenolphthalein indicator. Blank determination was conducted along with that on sample. The saponification value was calculated using the following equation:

$SV = 56.1 \times (B-S) \times N/Wt\ of\ sample$

Where B= volume in mL of 0.45N HCl required by the blank, S = volume in mL of 0.45N HCl required by the sample and N is normality of HCl solution.

2-2-2-2 Determination of acid value

2.35 g of oil were weighed into a 250 mL conical flask and 16.7 mL of ethanol alcohol (previously neutralized by adding 2 ml phenolphthalein solution and enough 0.1N NaOH to produce faint permanent pink) was added to the flask. The conical flask contents were titrated against 0.01 NaOH with vigorous shaking until permanent faint pink appears and persists > 1 min. The acid value was calculated using the following equation:

$Acid\ value\ (AV) = (V \times N \times 40)/\ Wt\ of\ sample$

Where: V is volume in mL of NaOH required by sample, N is normality of NaOH

2-2-2-3 Determination of peroxide value

2.5 g of the sample were weighed into a 250 mL conical flask and 15 mL glacial acetic acid and chloroform mixture (3:2) were added and stirred. 0.3 mL of saturated potassium iodide solution was added to the flask and shaked for 1 min.

15 mL of distilled water and 0.5 mL of 1% starch solution were added to the flask contents and titrated against 0.001N sodium thiosulphate solution with vigorous shaking until blue color just disappears. Blank determination was conducted also the peroxide value was calculated using the following equation:

Peroxide value (milliequiv peroxide /Kg sample) = V× N×1000/ Wt of sample

Where V is mL of sodium thiosulphate required by the sample, N is normality of sodium thiosulphate solution.

2-2-3 Cream preparation

20g of glusseren and 50g Vaseline were weighed and placed in water bath at 100˚C. Then 30g of wax was added with well shaking at the same temperature, the product was taken and added 200ml of gum solution (5%) until obtained the cream. 90g of the base cream product was taken and added 10g of *citrullus colocynthis* oil.

2-2-4 pH measurement

The instrument (pH meter METTLER TOLEDO 8603 Schwerzenbach, Switzerland) was calibrated carried out buffer solution before taking pH measurement. The electrode and the temperature probe was rinsed with alcohol then with distilled water and dried very well (to avoid impurities) and dipped them into the sample to be tested, allow for the electrode to stabilized.

2-2-5 Specific gravity measurement

An empty clean, graduated, cylinder was placed on the digital scale and zero it, the graduated cylinder was filled up with the cream, wait until air bubbles disappeared from the surface and was refilled to the maximum mark. The reading in the grams was taked, the weight of the grams divided by the volume of it as indicated to specific gravity of the cream.

2-2-6 Viscosity measurement

Before measurement, the instrument was calibrated (DV-E Viscometer BROOKFIELD model: LVDVE). A cream sample of 600 ml was prepared. The power was switched to the ON position by peressing motor ON/OFF button until

display shows on screen: spindle was replace press any key, then the following parameters were entered:

- Time of spindle rotation: 2 minutes /for CTR: 1minute
- Temperature :25˚C
- spindle number : LV64 / for CTR :(T-F)
- Rotational speed : 60 RPM/ For CTR:10 RPM
- viscosity in cP (centipoises)

The spindle (LV64) has been attached to the viscometer by screwing it onto lower shaft and it introduced into the prepared sample in the middle of container to avoid the presence of air bubble as much as possible. Auto range has been pressed. The screen has showed the value of viscosity with temperature.

2-2-7 GC-MS analysis

Citrullus colocynthis seeds oil analyzed by gas chromatography–mass spectroscopy. A shimadzu GC-MS-MS (TQ8040) ultra instrument with a RTX-5MS coloumn (5% diphenyl, 95% dimethyl polysiloxane),(30m,length; 0.25mm diameter; 0.25µm thickness) was used. Helium was used as carrier gas.
Chromatographic conditions are given in table (2.1)

Table (2.1) Chromatographic conditions

Column oven Temp.	80º C
Injection Temp.	250 C
Injection mode	Split
Flow control mode	Pressure
Pressure	122 KPa
Total flow	50 ml/min
Column flow	1.80ml/min
Linear velocity	49.4cm/sec
Purge flow	6 ml/min
Split ratio	-1.0

Chapter Three

Results and Discussion

Result and discussion

Table (3.1) shows the yield of extracted oil from *citrullus colocynthis* (20.8%) which exist within the range in good agreement with the literature (Zead,et al,2016).

Table (3.2) show the physiochemical properties of oil the density was 0.9297(g/cm³), saponification value was 215.9, acid value was 1.35, peroxide value was 5.3 there were good comparable to other studies. Saponification value of the oil which indicate a high content of fatty acid, it can use in soap industry.

Emulsion was analyzed to assure the formulation of desired properties by physical analysis, pH determination, electrical conductivity, viscosity determination, and specific gravity.

Table (3.2) shows the physical properties of the cream. The pH value for prepared cream is (6.06) within the Sudanese Standars and Metrology Organization (SSMO) standard of (5-9).The specific gravity was 0.8914 g/cm³, the conductivity was 126 µs/cm. The viscosity of the emulsion was too high 7888cp due to use Vaseline, wax in the oily phase and *azadirchta indica* gum in water phase. No change in the color of the formulation at the end of the observation period was noticed.

Table (3.1) percentage and density of oil extracted:

Percent yield	20.8%
Density (25º C)	0.9297(g/cm³)

Table (3.2) physiochemical properties of *citrullus colocynthis* seeds oil:

Physiochemical properties	Ref (Mirjana,2005)	Ref (Hiba,2015)	Results this study
Specific gravity	0.914(20ºC Kh/dm³)	0.886(g/cm³)	0.9297(g/cm³)
Acid value(mgKOH/g)	1.00	3.91	1.35
Saponification value(mKOH/g)	188	196.66	215.9
Peroxide value(mmolO2/Kg)	7.9	6.97	5.3

Table (3.3) physical properties of cream:

Physical properties	Result
Viscosity	7888 cp
Specific gravity	0.8914 g/cm³
PH	6.06 at (25º C)
Conductivity	126.0 µs/cm at (25º C)
Color	yellowish White

GC-MS analysis of *citrullus colocynthis* seed oil was conducted and identification of the constituent by comparison with the MS library (NIST)

The GC-MS spectrum of the oil revealed the presence of 5 component table (3.4), the typical total ion chromatograms (TLC) is depicted in Fig (3.1).

Table (3.4) Constituents of *citrullus colocynthis* seeds oil

Peak#	Constituent	R.Time	Area	Area%
1	Ethyl palmitate	12.675	10139039	7.01
2	9,12-octadecadienoic acid(Z,Z)-ethyl ester	14.354	96643508	66.86
3	Ethyl stearate	14.616	8667887	6.00
4	4-(2,2-dimethyl-6-methylenecyclohexyl)butanal	16.486	5486876	3.80
5	Sitostenone	18.835	23610185	16.33
			144547495	100.00

Table (3.4) show that *citrullus colocynthis* oil is composed of 80% esters

9,12-octadecadienoic acid(Z,Z)-ethyl ester, ethyl palmitate and ethyl stearrate.

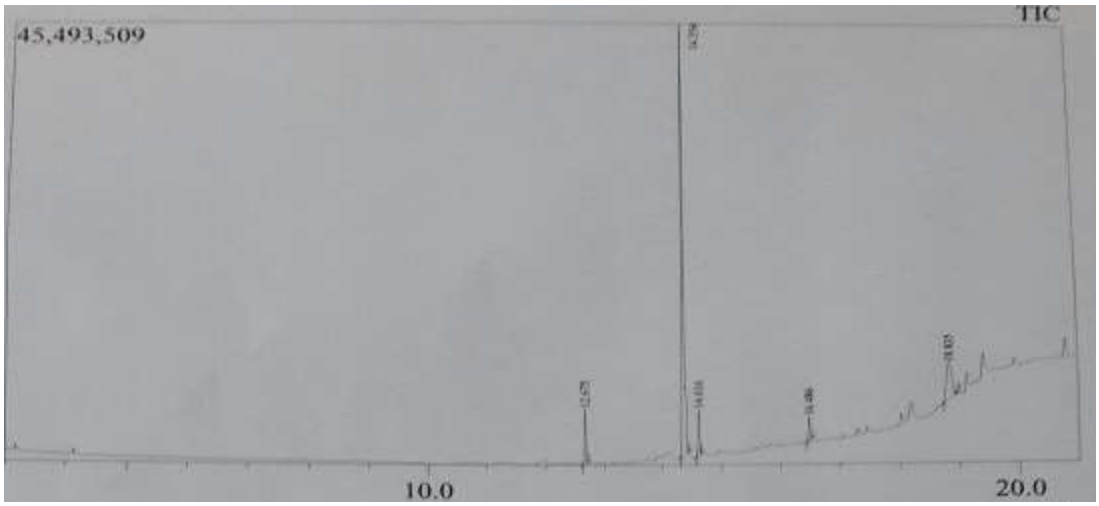

Fig (3.1) Chromatograms of *citrullus colocynthis* seeds oil

The following major constituents were detected:

- 9,12-octadecadienoic acid(Z,Z)-ethyl estar

- Sitostenone

-Ethyl palmitate

-Ethyl stearate

-4-(2,2-dimethyl-6-methylenecyclohexyl)butanal

9,12-octadecadienoic acid(Z,Z)-ethyl ester (66.86%)

The mass spectrum of 9,12- octadecadienoic acid ethyl ester is shown in fig (3.2). The peak at m/z 308, which appeared at R.T 14.354 in total ion chromatogram, corresponds to $C_{20}H_{36}O_2$. So the peak at m/z 279 corresponds to loss of an ethyl function.

Sitostenone (16.33%)

The mass spectrum of Sitostenone is shown in fig (3.4), the peak at m/z 412, which appeared at R.T 18.835 in total ion chromatogram, corresponds to $C_{29}H_{48}O$.

Ethyl palmitate (7.01%)

The mass spectrum of ethyl palmitate is shown in fig (3.3). The peak at m/z 284, which appeared at R.T 12.675 in total chromatogram, corresponds to $C_{18}H_{36}O_2$. So the peak at m/z 255 corresponds to loss of an ethyl function.

Ethyl stearate (6.0%)

The mass spectrum of ethyl stearate is shown in fig (3.5). The peak at m/z 312, which appeared at R.T 14.616 in total ion chromatogram, corresponds to $C_{20}H_{40}O_2$. So the peak at m/z 283 corresponds to loss of an ethyl function.

4-(2, 2-dimethyl-6-methylenecyclohexyl) butanal (3.80%)

The mass spectrum of 4-(2, 2-dimethyl-6-methylenecyclohexyl) butanal is shown in fig (3.6). The peak at m/z 194, which appeared at R.T 16.486 in total ion chromatogram, corresponds to $C_{13}H_{22}O$.

The fatty acid in the *citrullus colocynthis* oil were linoleic acid (66.86%) followed by palmitic (7.01%) acid and stearic acid (6.0%). This result agree with other studie(Nehdi Ia, et al,2013).

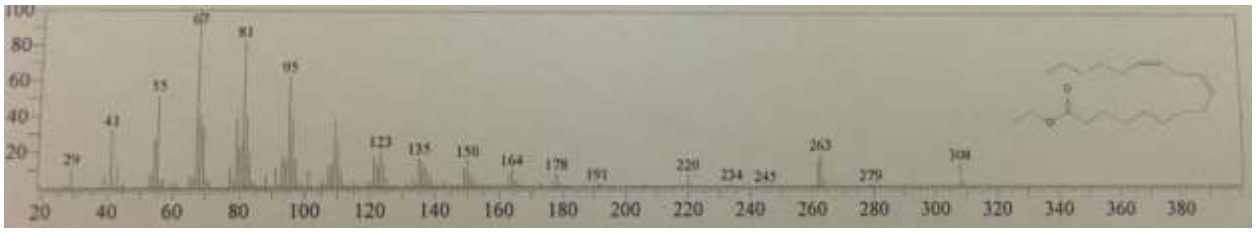

Fig (3.2) Mass spectrum of 9,12-octadecaienoic acid ethyl ester

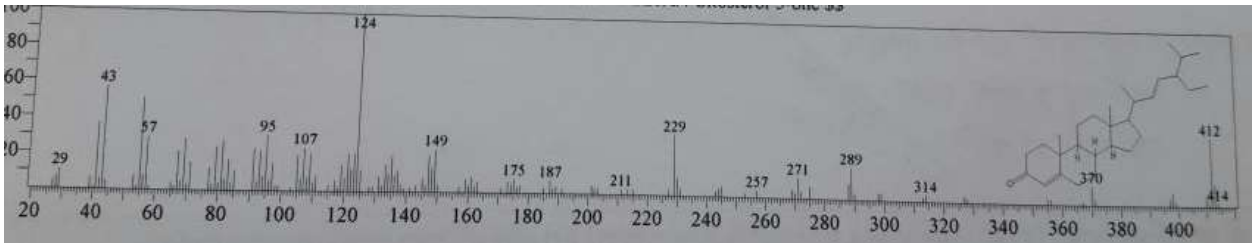

Fig (3.3) Mass spectrum of Sitostenone

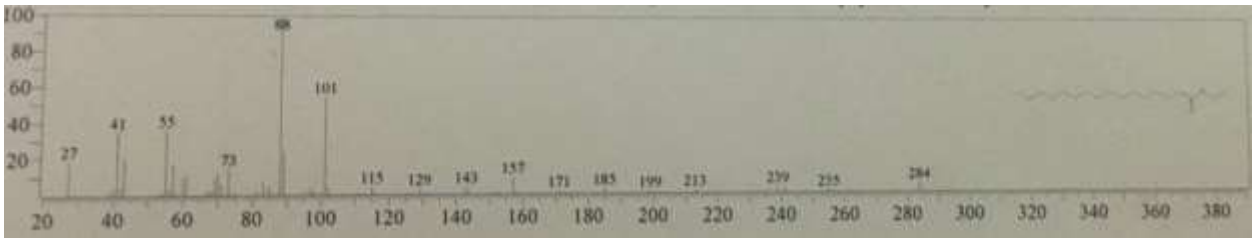

Fig (3.4) Mass spectrum of ethyl palmitate

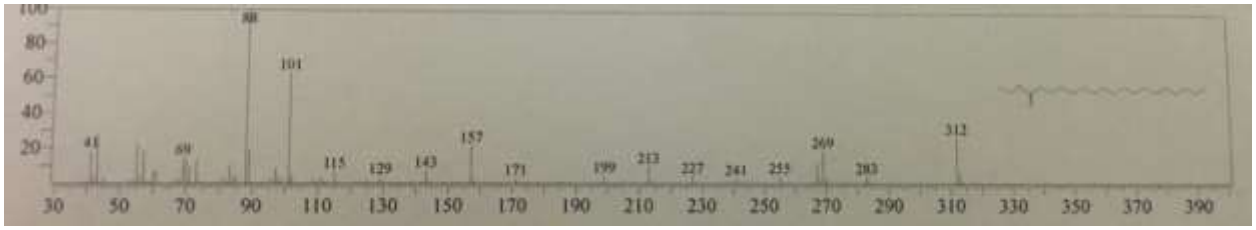

Fig (3.5) Mass spectrum of ethyl stearate

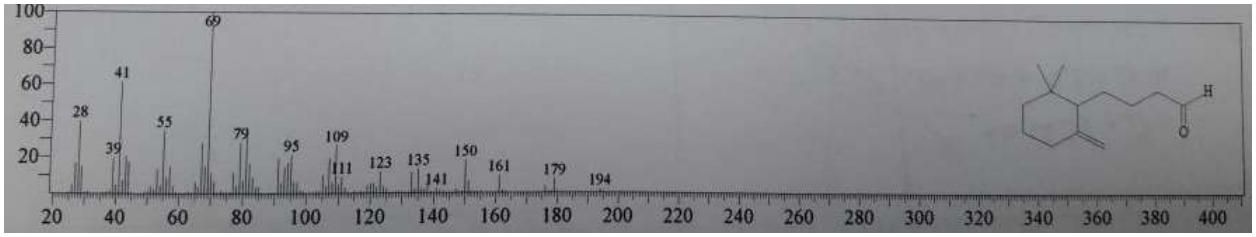

Fig (3.6) Mass spectrum of 4-(2, 2-dimethyl-6-methylenecyclohexyl) butanal

Conclusions

It can be concluded that a stable W/O formulation using extract of *citrullus colocynthis* seeds to lessen muscles and joint pain without causing any skin irritation.

The pH of the emulsion within the pH of the human skin, the viscosity was too high (Vaseline and glycerin which have emollient properties that relive dryness).

The color and the shape of the cream was acceptable, no liquefaction of the cream at 25˚C but observed slight liquefaction in the cream at more than 25˚C.

The main fatty acid in the *citrullus colocynthis* oil were linoleic acid (66.86%) followed by palmitic (7.01%) acid and stearic acid (6.0%).

Recommendation

- Separate the active ingredient in the oil, study the effectiveness of the active ingredient over a period of time.
- Prepare the cream as spray body with increase the oil concentration and study the effectiveness with through stability and safety studies.
- Study the shelf life of the cream.
- Feasibility studies for the product.

References

1. Abbah, O. C., Sanni, M. and Ejembi, D. O. (2014).Nutritional aspect of egusi melon-Citrullus colocynthis l, *Asian Journal of Science and Technology*,**5**,**3**,176-180.
2. Abu Baker, A.; Tahir, A. and Sabah Elkheir; M.K.(2007). Effect of Tree and Nodule Age on some Physicochemical Properties of Gum from Acacia senegal (L.) Willd., *Sudan Research Journal of Agriculture and Biological Sciences*,**3**(6),866-870.
3. Ali,B.H.,AL-Qarawi A.A.,Haroun E.M.and H.M.Mouse,(2003).The effect of treatment with G.A.On gentamicin nephrotexicity in rats:apreliminary study,*Ren.fail*.**25**,15-20
4. AliA.A.;AliK.E.;FadlallaA.and Khalid,K.H.(2008).The effects of G.A.oral treatment on the metabolic profile of chronic renal failure patients under regular haemodialysis in central sudan,*Nat.Prod.Res*.**22**,12-21.
5. AL-Majed,A.M.Mostafe A.C.AL-Rikabi and O.A.AL-Shabanah(2002),Protective effects of oral Arabic gum administration on gentamicin-induced nephrotoxicity in rats,*Phamacol.Res*.**46**,445-451.
6. Anderson,D.M.W.,Hirst,S.E.and Stoddart,J.F.,(1966).*J.Chem.Soc*1959.
7. Aspinal,G.O.,Hirst,E.L.and Matheson,N.K.,(1956).*J.Chem.Soc.pt*.1,989.
8. B. H. ALI; S. BEEGA;, I. AL-LAWATI,;M. I. WALY; M. AL ZA'ABI; A.NEMMAR.(2013). Comparative Efficacy of Three Brands of Gum Acacia on Adenine- Induced Chronic Renal Failure in Rats, *Physiological research*,**62**,47.
9. Barkat Ali Khan; Naveed Akhtar; Haji Muhammad Shoaib Khan; Khalid Waseem; Tariq Mahmood; Akhtar Rasul; Muhammad Iqbal and Haroon Khan.(2011). Basics of pharmaceutical emulsions: A review, *African Journal of Pharmacy and Pharmacology*,**5**(25),2715-2725.
10. Basak; S.P.; andChakraborty D.P.(1968). Chemical investigation of *Azadirachta indica* leaf (*Melina azadirachta*), *Journal of the Indian chemical society*, 466-467.
11. Bliss,O.Z., stein,T.P.,Charles,R.,Schleifer,G.R.and settle,R.G.(1996) supplementation with gum Arabic fiber increases fecal nitrogen excretion and lowers serum urea nitrogen concentration in chronic renal failure patients consuming alow.protein diet. *Am Journal of clinical nutrition*, **63**,392-398.

12. Borhade Pravin; Deshmukh Tushar; Patil Vijay and Khandelwal Kishanchnad.(2013). Review on *Citrullus colocynthis*, *International Journal of research pharmacy and chemistry*, **3**(1), 46-53.
13. Buffo.R.O.Reineccius.G.A,Oehlert,(2001).Factor affecting the emulsifing and rheological properties of gum acacia in beverage emulsions. *Food hydrocolloids*, **15,** 53-66.
14. Chaturvedi.S.and C.Jones.(2007).Protein restriction for children with chronic renal failure, cochrance Database syst.Rev. 17,p.CD00686
15. Connolly,S.,Fenyo,J.,C.and Vandevelde,M.C.,(1988).*Carbohydrpolym*.,**8**,23-32.
16. Connolly,S.,Fenyo,J.C.and Vandevelde,M.C.,(1987).*Food hydrocolloids*,**1**,477-480.
17. Duke,J.A.and WAIN,K.K.(1981).Medicinal plants of the world.computer indexwith more 85,000 entries,3vols.
18. Dziezak,J.D.(1991)Afoucs on gums.*Food Technol*,**45**,116-132.
19. Eiman Awad,(2016). Elneelaean University
20. Ekhadem,E.and Megadad,M.M.,(1956).*J.ChemSoc*.,3.3953.
21. Feinberg,M, and schoenkerman,B.B.(1940)*Wisconsin Med.J*.,**39**:734.
22. Fincher,G.R.,Stones,B.A.and Clarke,A.E.,(1983).*Ann.Rev.Plant physiol*.,34-47.
23. Gamble,L.D.and Grady,D.L.,(1938).*chem.Abstr*.**2**,135-936.
24. Garima pandey; KK verma; Munna singh.(2014).Evaluation of phytochemical, anti bacterial and free radical scavenging properties of *azadirachta indica*(neem),*International journal of pharmacy and pharmaceutical sciences*,**6**(2),444-447.
25. Glicksman,M.,(1979).Gelling Hydrocolloids in Product Application in Polysaccharides in Food.CRS.Inc.Florida.
26. Glicksman,R.L.(ed),Industrail Gums, second Ed.Academic press,New York,p197.
27. Hajar Shafaei; Abolghasem Esmaeili; Jafar Soleimani Rad; Abbas Delazar and Mohaddeseh Behjati.(2012). *Citrullus colocynthis* as a medicinal or poisonous plant: A revised fact, *Journal of Medicinal Plants Research*,**6**(35),4922-4927.
28. Hiba Riaz; Shahzad Ali Shahid Chatha; Abdullah Ijaz Hussain; Shazia Anwer Bukhari; Syed Makhdoom Hussain and Kashif Zafar.(2015). Physico-chemical characterization of bitter apple (*Citrullus colosynthis*) seed oil and seed residue, *International Journal of bioscience*,**6**(1),283-292.

29. Imededdine Arabi Nehdi; Hassan Sbihi; Saud Ibrahim Al-Resayes, (2013). Evaluation and characterization of *citrullus colocynthis* (L.) scharad seed oil:comparision with helianthus annuus (sun flower)seed oil,*food chem*.,**136**(2),348-353.
30. I.O.ogbuewu; V.U.odoemenam; H.O.obikaonu; M.N.opara; O.O.emenalom; M.C.vchegbu; I.C.okoli; B.O.esonu and M.U.iloeje.(2011). The growing importance of neem(*azadirachta indicia*.juss)in adriculture, industry, medicine and environment:*Areview*,**5**(3),230-245.
31. Jermyn,M.A.,(1962).*Australian Journal of Biological Science*,**5**,787-791.
32. Johnstone,C.(1939)*Mfg.confect*.,19,14.
33. Lewis,B.A.,Smith,F.,(1957).*J.Am.Chem.Sec*.,79,3929.
34. Mahesh Chand Meena; Rishi Kesh Meena and Vidya Patni.(2014). Ethnobotanical studies of *Citrullus colocynthis* (Linn.) Schrad. An important threatened medicinal herb, *Journal of Medicinal Plants Studies*,**2**(2),15-22.
35. McClements, D.J., 1999. Food Emulsions: Principles,practices and Techniques. CRC Press, Florida, pp: 378.
36. Meer,W.H.,(1980)"Hand book of water soluble gums and resin"In Davidson,R.L.(ed)Mcgraw-Hill,Newyork.
37. Mirjana Milovanovic and Ksenija Picuric-Jovanovi.(2005).Characteristic and composition of melon seed, *Journal of Agriculture Sciences*,**50**(1),41-47.
38. Mohammad asif.(2013).Areview on spermicidal activities of *azadirachta indica*, *Journal of pharmacognosy and phytochemistry*,**1**(5),61-79.
39. Murwan K. Sabah El – Kheir; Abu El Gasim A. Yagoub and Asma A. Abu Baker.(2008). Emulsion-Stabilizing Effect of Gum from Acacia senegal (L) Willd. The Role of Quality and Grade of Gum, Oil Type, Temperature, Stirring Time and Concentration, *Pakistan Journal of Nutrition*,**7**(3),395-399.
40. Neils,R.,Baily,C.F.and Casey,R.S.,(1947).*J.chem.E.d*.,24-42.
41. Osborn,G.E.,and Dekay,H.G.(1941)prac.pharm.Ed.2:42.
42. Osman,M.E.,(1993).ph.D. thesis,department of chemistry and applied chemistry of salford . faculty of science, health and medical studies,North East wales institute,Deeside,Clwyd.
43. Ray,A.K.,Bird,P.B.,Lacobucci,G.A.,&clark,C.B.(1995).Functionality of gum Arabic.Fraction,characterization and evaluation of gum fractions in citrus oil emulsions and model beverage.*Food hydrocolloids*,**9**,123-131.
44. Revie,C.N., and Thomas,B.J.R.(1962)*Mfg*.,37-40.

45. S. Gurudeeban, T.; Ramanathan and K. Satyavani.(2011). Characterization of Volatile Compounds from Bitter Apple (*Citrullus colocynthis*) Using GC-MS, *International Journal of Chemical and Analytical Science*,**2**(8),108-110.
46. Sharma pankaj; Tomar lokeshwar; Bachwani mukesh; Bansal Vishnu.(2011).Review on neem (*azadirchta indica*):Thousand proplem one solution,*International research journal of pharmacy*,**2**(12),97-102.
47. Sharma,S.C.,(1981).*Food Technology*,59.
48. Sharma,S.C.,(1981).Gum and hydrocolloids in oil water emulsions,*Food technology*,59-67.
49. Snowden,M.J.,Phillips,G.O.,andWilliams,P.A.,(1987).*Food hydrocolloids*,**1**,291-300.
50. Standford,P.A.and Baird,J.,(1983). In Aspinall,G.O.(ed),"The polysaccharides 2",Academic.
51. T.G.Mason; S.M.Graves; J.N.Wilking and M.Y.Li.(2006). Extreme emulsification:formation and structure of nanoemulsions, *Condensed Matter Physics*,**9**,193-199.
52. U.S.pharmmacopoeia,(1955)15th E.d.Mackmillan publishing co.,Easton pensylvania.
53. Wadood.,A.,N.Wadood and S.A.shah,(1989),Effect of acacia Arabica and caralluma edulis on blood glucose rabbits,*J.pak.Med.Assoc*.**39**,208-212.
54. Werbin,S.J.(1953)Backers digest 27:7
55. Williams, P.A. and Phillips, G.O. (2000). Gum arabic. In: G.O. Phillips and P.A. Williams, Editors, Handbook of Hydrocolloids, Vol.9, C.R.C. Press, New York, pp 155-168.
56. Williams,P.A. and G.O.phillips.(2000)."Gum Arabic"In: handbook of hydrocolloids,G.O Phillips and P.A. Williams(Eds.)combridge(UK): wood head publishing.155-168.
57. Wood,W.H.(1952).*chem.Abstr*.,46,6024.