Sudan University of Science and Technology

College of Graduate Studies

Preparation of Mosquitos Repelling Cream From *Gum Arabic*, Shea Butter and *Ocimum basilicum L. Oil* (Rehan)

تحضير كريم طارد للباعوض باستخدام الصمغ العربي وزبدة الشيا وزيت الريحان

A Dissertation Submitted in Partial Fulfillment of the Requirements of M.Sc Degree in Chemistry

By

Hajer Abdalla Mohammed Ahmed

(B.Sc. Honors ,Chemistry)

Supervisor: Prof. Mohammed Elmubark Osman

2018

الاستهلال

قال تعالى:

﴿وما توفيقي إلا بالله عليه
توكلت وإليه أنيب﴾

صدق الله العظيم **سوره هود الآية (88)**

Dedication

TO,

the soul of my father,

my mother,

brothers and sisters.

Acknowledgements

Praise to Allah, Almighty, who gave me strength and patience to complete this work.

I would like to express my deep thanks and gratitude to my supervisor Prof: MOHAMMED ELMUBARK OSMAN for his precious guidance and advice.

Special thanks are due to the staff department of Chemistry, College of Science, National Central Institute of Health (Stack Laboratory) Khartoum State for technical Support. .

Special thanks are also due to my friends Reem, Afaf and Khadija for moral support.

Abstract

The aim of this study is to extract volatile oil from leaves of *Ocimum basilicum L. Purple*, and determine the chemical components. It also aimed at preparing cream using Gum Arabic and basil oil, and shea butter and testing its efficiency as a mosquitoe repellent at10% concentration.

Ocimum basilicum L. purple gave a 1.5% yield of essential oil. The chemical composition of the oil(investigated by GC-MS) showed that it contained fifty sixconistuents.The main components were1.6-Octadien-3-ol,3,7-dimethyl(18.01%), methyleugenol(13.00%), geraniol (11.33%), cineole (8.33%), D- carvone (6.95%), benzoic acid,4-ethenyl-,methyl ester(4.21%), geranyle acetate (3.84%), tau cadinol (2.86%), D-limonene (2.45%), gamma-muurolene(2.15%),

Some of the physicochemical properties of the extracted essential oil were determined: pale yellow colour, refractive index (1.49), density (0.91g/cm^3), viscosity (3.37), saponification value (109), Iodine number (31.7), total acid number (2.8), peroxide value (0.0).

Acacia senegal var *senegal* gum and shea butter were used to prepare a stable emulsion(cream).

The cream showed 100%repellency at 10% concentration of Ocimum basilicum L purple.

المستخلص

هدفت هذه الدراسه الى استخلاص الزيت الطيار من احد انواع نبات الريحان وتحديد المكونات الكيميائيه له. تكمن اهميه هذه الدراسه في تحضير كريم طارد للباعوض من الصمغ العربي و زبدة الشيا وزيت الريحان بنسبه 10%. هذا الزيت الطيار تم استخلاصه من الاوراق الجافه لنبات الريحان خلال التقطير المائي.

نسبه الزيت في النوع ذو الازهار البنفسجيه هي1.5%.تم التعرف علي المكونات الكيميائيه لزيت الريحان بواسطه جهاز مطياف الكتله ,56 مركب قد حددت لهذا النوع من الزيت وكانت اعلى المركبات نسبه من ضمن هذه المركبات هي اوكتادابنول−3 (18.01%), ميثايل ايجينول (13.00 %),جيرينول(11.33%) سينول(8.33%), كارفون (6.95%),, جيرنايل استر(3.84%), , كادينول (2.86%) ليمونين (2.45%),.

ومن الخواص الفيزيائيه والكيميائيه لهذا الزيت هي: أن اللون اصفر باهت, ومعامل الانكسار (1.49),الكثافه (0.91جرام/سم^3),اللزوجه(3.37), رقم التصبن (109), رقم اليود(31.7), رقم الحموضه الكليه (2.8), رقم البيروكسيد(0.0).

تم تحضير مستحلب من 27جرام من الصمغ العربي الهشاب مذاب في 54مل من الماء المقطر و 9جرام من زبدة الشيا (كريم مرطب) و10مل من زيت الريحان في تحضير كريم طارد للباعوض و اوضحت النتائج ان نسبه الطرد لفعالية الكريم بلغت 100%باستخدام 90% من الكريم و10%من زيت الريحان.

.

Table of content

Content	Page No.
الإستهلال	I
Dedication	II
Acknowledgement	III
Abstract	IV
المستخلص	V
Table of Contents	VI
List of Tables	VIII
List of figures	IX
CHAPTER ONE: INTRODUCTION	
1-1Gum Arabic	1
1-1-1 Introduction	1
1-1-2 Definition of Gum Arabic	2
1-1-3 Botanical Description	3
1-1-4Botanical Classification of *Acacia Senegal(hashab*)	4
1-1-5 History and Origins of Gum Arabic	4
1-1-6 Structure of Gum Arabic	5
1-1-7 Application	6
1-1-8 Physicochemical properties of Gum Arabic	10
1-2 Basil Ocimum oil	11
1-2-1 Cultivation of Basil (Ocimum basillicum)	12

1-2-2 Botanical classification of Ocimum basilicum	12
1-2-3 Botanical Description of Basil	13
1-2-4 Chemical component of Ocimum basilicum	14
1-2-5 Uses of Ocimum Basil	15
1-3 Shea Butter	16
1-3-1 Botanical classification of Shea Butter	16
1-3-2 Origin of Shea butter	17
1-3-3 Chemical Composition of shea butter	17
1-3-4 Uses of shea butter	17
1-4 Mosquitoes Repellent	19
1-4-1 Preparation of Repellent compounds	19
1-4-2 Type of repellent	19
1-5 Emulsions	20
1-5-1Type of Emulsion	21
1-5-2 Stability of Emulsion	
1-5-3 Thermodynamic and Kinetic Sstability	23
1-5-4Instability of Emulsion	23
1-5-5 Chemical Instability	24
1-6 Objective	28
CHAPTER TWO :MATERRIALS AND METHODS	
2-1 Materials	29
2-1-1 Collection of plant Material	29
2-1-2 Preparation of Materials	29
2-2 Methods	30
2-2-1Photochemical Screening	30
2-2-2Physio-chemical of Essential Oil Extract	31

2-2-3Preparation of Cream Base (Aqueous base)	33
2-2-4 Preparation of Mosquitoes	34
2-2-5Repellency test	35
CHAPTER THREE: RESULTS AND DISCUSSION	
3-1 Physio-chemical of Essential Oil	37
3-2 Chemical composition by GC-MS	39
3-3 Repellent test results	43
3-4 Conclusion	44
3-5Recommendation	44
References	45

List of Tables

Table No	Topic	Page No
1-1	Physicochemical properties of Gum Arabic	10
3-1	Physical and chemical properties of Ocimum L. pruple	38
3-2	Chemical composition by GC-MS	39-42
3-3	Repellent test Result	43

List of Figures

Table No	Topic	Page No
1.1	Gum Arabic Nodules and Tears	3
1.2	Schematic representation structure of Gum Arabic	14
1.3	Type of Emulsion	22

CHAPTER ONE

INTRODUCTION

CHAPTER ONE

INTRODUCTION

1.1 Gum Arabic:

1.1.1 Introduction:

Plant gums are obtained as an exudation from fruit, trunk or branches of the trees or after mechanical injury of the plant by incision of the bark, after the removal of the branch, or after invasion by bacteria or fungi . The exudates become hard nodules or tears on dehydration they form a protective sheath against microorganisms. These plant products have been collected since about 3000 BC, during the Egyptian civilization from Acacia and gum Arabic trees, native to North – Africa and used as adhesive in hieroglyphic paints and in the embalming of Egyptian mummies. Nowadays, gums have been found to have application in the food industry where emulsifying and stabilizing properties are utilized. Gums are also used in the pharmaceutical and medical fields, in addition to other industries (cosmetic, mining, adhesive paints and inks).

The complex and heterogeneous nature of plant gums in terms of their chemical composition makes it very difficult to predict their properties. Yet, their industrial application are based on well characterized gum samples, i.e. samples whose quality and safety of application can be assured because their physicochemical properties are well-known.

The physicochemical properties of a compound are the measurable physical and chemical characteristics by which the compound may interact with other systems, and these characteristics collectively determine the quality, applicability or end-use of the compound. (paul,2015)

Gum Arabic readily dissolves in cold and hot water in concentrations up to 50%.

Because of the compact, branched structure and therefore small hydrodynamic volume, Arabic gum solutions are characterized by a low viscosity, allowing the use of high gum concentrations in various applications (Dziezak ,1999).

1.1.2 Definition of Gum Arabic:

Gums obtained from other acacia species, and occasionally from *Albizia* and *Combretum,* are also traded as "Gum Arabic". Current regulation surrounding Gum Arabic does not distinguish between gum obtained from *Acacia senegal* and *Acacia Senegal var Senegal*. Therefore, although gum from *Acacia senegal* is of inferior quality to *Acacia senegal var senegal*. *The* gum from *Acacia senegal var senegal,* is commonly traded as Gum Arabic.

For Sudanese exports, the distinction is quite clear: gum from *Acacia senegal* is sold under the name "hashab gum" while gum from *Acacia senegal* is sold as "talha gum". In Zimbabwe, the gum traded locally as Gum Arabic comes from *Acacia karoo* However, synthetic substitutes, namely modified starches" such as xanthan and gellane, are rapidly replacing Gum Arabic as dietary hydro-colloids. Gum Arabic is traditionally defined as a substance, which exudes from *Acacia Senegal* or related species'. This definition encompasses a variety of species, which

from a taxonomy point of view, are not related. To date, though, only the gum from *A. senegal var senegal* has been effectively demonstrated as an innocuous food demonstrated as an innocuous food additive. As a result of growing global pressure demanding trade specifications and stricter labeling regulation regarding the identity and quality of products, a publication of revised specifications was created3. The report describes Gum Arabic as derived from *A. senegal*, or closely

linked species, comprising of an optical rotation range of -26o and -34o and a Kjedahl index indicating nitrogen content between 0.27% and 0.30%. Gum Arabic is tear-shaped, round, with an orange-brown colour and a surface with a matte texture. After it is crushed or shattered, the pieces are paler in colour and have a vitreous appearance.fig (1.1)

Contrary to other vegetable gums, Gum Arabic dissolves very well in water (up to50%). The viscosity of *Acacia senegal var senegal* gum, though, is weak (16ml/g on average). The resulting solution is colourless, tasteless and does not interact easily with other chemical compounds. (Salif, 2008)

Figure (1.1): Gum Arabic Nodules and Tears

1.1.3 Botanical Description:

Variable species. Deciduous tree or shrub up to 15 m tall but usually less; umbrella-shaped crown; bark variable. Short, black prickles, normally in threes, the central curved downwards. Leaves small, 1-10 cm long, 3-8 pairs of pinnae,

each with 7-25 pairs of leaflets. Flowers white and fragrant, in 3-12 cm long spikes, 2-3 together in the leaf axils.(Joker, Dorthe ,2000)

1.1.4 Botanical classification of *Acacia Senegal(hashab)*:

Family: *Leguminosae.*

Subfamily*: Mimosoideae.*

Genus*: Acacia.*

Subgenus: *Aculeiferum*.

Species: *Senegal*.

Series: *Vulgares* .(Hamza , 1990)

1.1.5 History and origins of Gum Arabic:

Gum Arabic is, certainly, the most ancient and the most well known of all gum types. The term ‘Gum Arabic’ was coined by European merchants who imported it from Arabian ports such as Jeddah and Alexandria. Egyptians referred to it as kami’ and allegedly used it from the third dynasty onwards (around 2650 BC) to secure bandages around mummies. The gum was, supposedly, also used to fix pigments into hieroglyphic paintings. According as the best food available to man is, in fact, a direct reference to Gum Arabic. The word ‘mana’ seemingly also refers to Gum Arabic in the Torah where it is described as an essential food and designated by Moses to the Israelis as God-given bread. In the 15th century, European navigators discovered Gum Arabic on the coasts of modern-day Senegal and Mauritania. In the 18th century, following a bloody and determined ‘gum war’ France acquired the monopoly of gum trade along the West African coast1 At the beginning of the 20th century England opened up access to the other primary source of Gum Arabic by building a railroad between Elobeid, in the heart of the

Kordofan region, and the Sudanese port. England and France, aboard their merchant fleet, transport the gum from the trading post back to Europe to be processed. Consequently, Gum Arabic became a prized commodity given its popularity with these two colonial powers2. There are close to 900 acacia species capable of producing gum. These are primarily located in tropical climates, with about 130 of them specifically located on the African continent. Africa, therefore, quickly became the major site of the production of gum; this is the reason why it is also referred to as 'Senegal Gum'. Gum is essentially the secretion of several *Acacia* (leguminous) trees. *Acacia* Gum species, of which there are up to seventeen, produce acacia gum of varying quality and quantity. Interestingly, close to 80% of Gum Arabic is produced by the *Acacia senegal* (in Sudan). The remainder is produced *Acacia*, with each species contributing 40% to the total supply of gum. The gum produced by the *Acacia senegal var senegal* is commonly referred to as "hard gum" and the gum from *Acacia senegal*, as "flaky gum". Gum exudes naturally from slits incisions in tree barks; or by creating additional man-made slits, which yields larger quantities. The amount produced varies, however, from20g to 2000g depending on the tree; on average, a tree yields 250g. (Salif toure, 2008)

1.1.6 Structure of Gum Arabic:

Arabic gum is abranched, slightly acidic, complex polysaccharide obtained as a mixed calcium, magnesium, and potassium salts of uronic acid . The backbone consists of 1,3-linked B-d-galactopyranosyl units fig(1.2). 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-l-arabinofuranosyl, a-l-rhamnopyranosyl, B-dglucuronopyranosyl, and 4-O-methyl-b-d-glucuronopyranosyl.and the latter two mostly as end –units Asmaller fraction

is ahigher molecular weight (Ghada et al , 2015).The smallest fraction having the highest protein content is a glycoprotein which differs in its amino acids composition. Reported GA to be comprised of 39-42% galactose, 24-27% arabinose, 12-16% rhamnose, 15-16% glucuronic acid, 1.5-2.6% protein, 0.22–0.39% nitrogen, and 12.5– 16.0% moisture. The chemical composition of GA can vary with its source, the age of the trees from which it was obtained, climatic condition and soil environment. (Azzaoui, 2015)

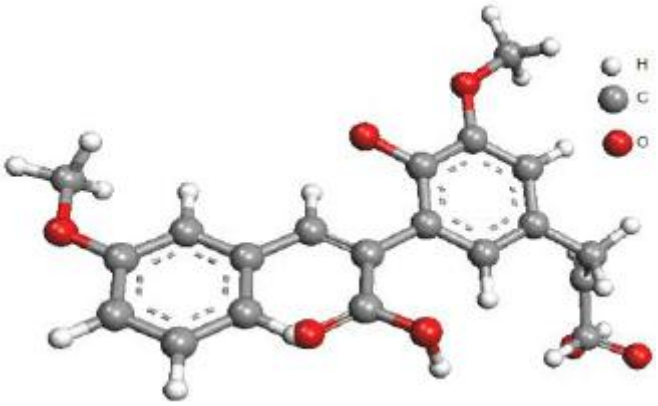

Figure (1.2): Schematic representation structure of Gum Arabic

1.1.7 Application:

Gum Arabic is used in many industrial applications including the food industry. However, due to stringent regulations imposed on all food additives, Gum Arabic is subject to a quality and toxicological evaluation by countries, organizations and users. To comply with these requirements and to enter the market for Gum Arabic trade must meet certain chemical specifications.

* Emulsions and Beverages:

Gum Arabic is recognized as a key ingredient in the industrial production of beverages. Its emulsifying, stabilizing and suspending ability provide key advantages for the production of emulsions in beverages. It is used for encapsulation of flavor essential oils, vitamins, aromatic compositions, plant essences etc. The low calorie and highfibre content of Gum Arabic makes it the first choice for functional beverages, diet products and other drinks with nutritional claims. In oil in water or water-in-oil systems, Gum Arabic is coating the fat or oil droplets, providing excellent flavor retention, stability and, homogeneous texture and a pleasant mouthfeel.

* Nutraceutical products:

Gum Arabic is used in a wide range of food and nutritional applications to provide dietary fiber and additional benefits such as moisture management and extended shelf life. Guaranteed to contain a minimum of 90% soluble dietary fiber, Gum Arabic is used as a binder in tablets, nutrition bars, beverages, juices, baked goods, and most any application where additional fiber is required.

* Dietetic and Diabetic products:

Gum Arabic is often used as a source of soluble fiber in low calorie and dietetic beverages. Gum Arabic is used for dietetic and diabetic products such as reduced sugar and sugarless candies and other confectionery. It is replacing very well the bulk and texture of sugars in combination with other sweeteners and it is providing binding and fiber in low calorie products. It seems to be the perfect solution for reduced-fat ice cream and dairy desserts: it is replacing the fat content but preserving the creaminess of the products.

* Pharmaceuticals:

In pharmaceutical industry Gum Arabic as a natural ingredient is used for emulsification, stabilization and binding as well as other functions like: suspending agent, demulcent action, film forming, encapsulation. In medicated cough drops it is used as a main ingredient, preventing sugar crystallization and giving good texture to the drops. Used in syrups as a suspending agent and demulcent, it contributes to the stability of the end-product. Its adhesive properties make it a good choice for compressed tablets and coating of pills. Gum Arabic is also used for encapsulation of oil soluble vitamin sin powder form, giving an increased resistance to oxidation.

* Cosmetics:

The cosmetic industry is using gum Arabic for a large range of functionalities: stabilizer, viscosity agent, film former, emulsifier, binding agent . Gum Arabic is used in creams, lotions, mascaras and cake cosmetics as a protective colloid.

* Industrial applications:

1- Lithography:

Gum Arabic is used as a sensitizer for lithographic plates as an element in the light sensitive composition and as an ingredient of the fountain solution.

2- Inks, Water Paints and Pastels:

Gum Arabic is an important constituent of many special purpose inks. Water color and quick drying inks utilize the suspending and binding properties of Gum Arabic.

3- Ceramics and Porcelain:

Gum Arabic is used as a glaze thickener.

4- Firework and Explosives:

Gum Arabic is used as a binding, adhesive agent.

5- Pesticide and Insecticide:

Gum Arabic is used as a suspending agent. (Azzaoui ,2015)

Stamps and Cigarette papers: its adhesive capability is used to form a transparent adhesive film.

6- Photography:

The historical photography process of gum bichromate photography uses Gum Arabic mixed with ammonium or potassium dichromate and pigment to create a colored photographic emulsion that becomes relatively insoluble in water upon exposure to ultraviolet light. In the final print, the acacia gum permanently binds the pigments onto the paper.

7- Shoe polish:

Today, shoe polish is usually made from a mix of natural and synthetic materials, including Gum Arabic as natural ingredient.

8- Detergent:

Gum Arabic is used as surface-active additive for removing oil.

9-Textiles:

Gum Arabic gives body in finishing silk and rayon fabric without loss of transparency. It is also used as a sizing and finishing agent in printing formulations for imparting designs or decorations.(Azzaoui ,2015)

1-1-8 Physicochemical Properties of Gum Arabic:

The physical parameters examined included colour, odour, pH, solubility in water and taste. From the results obtained, it is evident that the colour is yellowish brown to pure yellow (Table1.1). The gum was also found to be odourless. From the measured pH of the gum, it was found that it is acidic,. The acidity of the plant gum is not unexpected; it is known to contain salts (K, Na, Ca, Mg, and Fe) of acidic polysaccharides, the acidity of which is due to uronic acids in their structures. (paul et , al , 2015)

Table (1.1): Physicochemical Properties of Gum Arabic:

Colour	yellow
Odour	Odourless
Taste	Bland
pH	4.8
Solubility % w/v in	50
Intrinsic viscosity (η),	g/cm^3 0.53
Protein	(%) 3.498

1.2 Basil Ocimum Oil:

Plants are of the important sources of medicinal and a large numbers of drugs in use today are derived from plants. *O. basilicum* L. commonly known as Sweet basil (*Lamiaceae*) is used in both Ayurvedic and Unani system of medicine Sweet basil is indigenous to lower hills of Punjab in India, Persia and Sindh but also grown in several Mediterranean countries including Turkey. *O. basilicum* is being known by different names in different languages around the world In Hindi and Bengali, it is known as Babui Tulsi. In English, it is known as Basil, Common Basil or Sweet Basil. In Arabic the plant is known as Badrooj, Hebak or Rihan; as Nasabo or Sabje in Gujrati and as Jangli Tulsi in Urdu. Tohrakhurasani and Okimon are the names of the plant in Persian and Unani languages respectively. Besides fresh leaves have been normally used *in natura*, one can extract a valuable essential oil from basil, used in the manufacture of perfumes and flavors for food and beverages. Essential oil has insecticide and insect repellent properties reported the potential uses of basil essential oil in order to replace molecules obtained from endangeredsspecies.(Mueen,2015)

Basil (*Ocimum* L.) species have been used for centuries as medicinal plants. Basil essential oils are responsible for characteristic aroma and biological activity .The chemical composition of sweet basil essential oil, similarly to other oil plants, depends on genetic, ontogenetic, and environmental factors .Hence, the morphological and chemical variability of the basil creates great possibilities for growing different cultivars of this valuable herbal plant. Numerous basil cultivars and forms, differing in the plant size, habitus, color, and shape and size of the leaves and flowers, content and chemical composition of the essential oil ,as well as other biologically active substances are, currently ,cultivated in Europe and in the world. classified four major chemotypes of basil based on essential oil

composition:

methyl chavicol (estragole)-rich; linalool-rich; methyl eugenol-rich; methyl cinnamate-rich, and also numerous subtypes. According to the geographical origin of basil varieties and on the basis of their major constituents, they are classified in four chemotypes :(1) European chemotype, the oil of which is characterized by high amounts of linalool (35–50%) and estragole (15–25%); (2) reunion chemotype (estragole basil) whose main essential oil component is estragole (80% or more); (3) tropical chemotype (cinnamon basil), the oil of which is dominated by methyl cinnamate, and (4) eugenol chemotype whose major oil component is eugenol.

The qualitative and quantitative profiles of particular basil varieties can differ significantly depending on their geographical origin as it can be seen, for example, for the 'Purple Opaal' variety or the 'Red Rubin' variety.In addition, the components that occurred in larger amounts were geraniol (12.6%), 1.8-cineole (4.1%), and epi-α-cadinol (3.8%). (Andrea et al, 2017).

1.2.1 Cultivation of Ocimum basilicum:

It is grown in cold areas the season of it's planting is mid spring in an inner place and the seeds are shed directly in the soil at warm places away from the snow ,or frost .The plant needs moderate temperature and regular irrigation . Basil shrubs are planted as a fence, with a distance between each tree of one meter and 3.5 meter between each raw . It is, rarely , infected with bests and can bear sun rays.(Mario, 2012)

1.2.2Botanical classification of Ocimum basilicum:

Belongs to the genus Ocimum; the word basil derived from the Greek oza which means strong odours. Basileus is still considered the "king of herbs" by many

cookery authors, recognized more than 150 species of *lamiaceae* ; however ,paton proposed that Ocimum has only 65 species and other attributions should considered as synonyms. Among the species of the genus , *Ocimum basilicum L.* (basil) is the major essential oil crop worldwide these species are highly aromatic and economically important , the five species of *Ocimum L. vis O. tenuiflorum L. syn. O. sanctum L. O. americanum L. Syn . O. canum Sims, O. basilicum L. O. gratissimum L. and O.Xcitriodorum vis*. Were identified using their seed characteristics, difference in shapes, size , colour , texture.

The aromatic oil of this species possesses a characteristic pleasant aroma with an appreciable note of cloves and sometimes licorice. The type is used in Italian food is called sweet basil, as opposed to "Thai basil" Obasilicum var. thyrsiflora.

Ocimum basilicum Lamiaceae is commonly known as mint. The species of Ocimum basilicum is the most cultivation in Algeria is well known as "hbek" .(Mario, 2012)

1.2.3 Botanical Description of Basil:

Sweet basil is an autogamous, aromatic and herbaceous plant that is annual and perennial,grows 1-2 feet in height . Basil produce large green leaves around 2 inches in length, throughout the summer . Basil flowers are commonly removed to increase yield of leaves. Calyx is five mm long, enlarging in fruit and very shortly pedicels. Its lower lip with the two central teeth is longer than the rounded upper lip The bracts are stalked (shorter than the calyx), ovate and acute. Corolla is 8-13 mm long and. white, pink or purplish in color. Nut lets are about two mm long, ellipsoid, black and pitted [6]. Sepals of flower are five and remain fused into a2-lipped calyx. Ovary is superior and is a 2- carpellary, 4-locular and a 4-partite fruit of four achiness.(Mueen, 2015).

Figure (1.3): Schematic representation of Ocimum Basilicum

1.2.4 Chemical Component of Ocimum Basilicum:

compounds in basil oil reported were linalool, methyl chavicol, eugenol, bergamotene, and methyl cinnamate, or methylchavicol, camphor, citral, limonene, methylcinnamate, caryophyllen-β, anethol, terpinen-4-ol, myrcene, thymol, ocimene, and cinnamaldehyde .Chalchat and Özcan showed different concentrations of linalool and other important components in the basil essential oil extracted from the different parts of the herb, such as flowers, leaves, and stems. Among the main constituents of flower oil were estragole (58.26%), fenchone (10.1%), limonene + β-phellandrene (19.41%), and α-phellandrene (4.37%), while estragole (52.60%), limonene (13.64%), exo-fenchyl acetate (10.99%), fenchone (5.70%), α-phellandrene (4.15%), and endo-fenchyl acetate (1.30%) were found as the main components of basil leaf oil. Recently, the effect of elicitation with jasmonic acid (JA) on the plant yield, the production and composition of essential oils of 'Lettuce leaf' basil was evaluated . The continual increase of the production of basil essential oil reflects in demands on the quantity of basil vegetable matter useful for the extraction. Therefore, great effort is devoted not only to the evaluation of new potent basil varieties, but also to the maximization of periodical harvests (cycles) of the basil plants with acceptable content of essential oil. However, only very few works have been published on the study of seasonal variation of basil oil yield during the year. Valuable

Europeanbasil varieties such as ‘Ohˇre’, ‘Lettuce Leaf’, ‘Purple Opaal’, ‘Dark Green’, ‘Mammolo Genovese’, ‘Red Rubin. **(**Mohamed, 2015**)**

1.2.5 Uses of Ocimum Basilicum:

Biological activity of essential oils may be due to one of the compounds or due to the entire mixture. The compounds presented in essential oils have high economic value due to their toxic effects, such as phenyl propanoids or terpenoids , linalool enantiomers as the main active agent responsible for antimalarial and antimicrobial activity against human pathogenic bacteria and fungi. Basil oil contains some compounds like benzene derivatives are generally more toxic and insects repellent. Physiological or homological basis for these bitrophic interactions between plants and insects mediated by methyl eugenole, furthermore methyl eugenole was most effective in repelling and killing toxicity effects for 24 and 48 hours. Estragole and safrole have shown mutagenic and carcinogenic effects in rats and cats. **(**Hadj et al , 2012**)**

1-Anti – inflammatory: the fixed oil and linolenic acid were tested in vivo using rats and animals models with experimentally. Activity: mechanism seems to involve linolenic acid ability to block cyclooxygenase and lipoxygenase inhibiting pathways of arachidonate metabolism and histamine antagonistic effects**.**

2-Antiulcer: Aqueous extract of aerial parts in vivo; rat. Activity: is comparable to Ranitidine (stander drug); prevented ulcer genesis; administrated intragastrically inhibited secretion of peptic -acid induced by aspirin; potently neutralized secretion of the acid in stomach.

3-Ear infection treatment: Essential oil and active constituents (thymol, carvacrol, and salicylaldehyde) applied to ear canal, in vivo: rat experimentally – induced acute otitis media caused by pneumococci or Homophiles influenza. Activity:

healed 58% - 81% of animals infected with H. Influenza and 6% - 75% of those with pneumococci (by comparison; only 5.6%-6% of placebo group were cured); recommended as effective treatment.

4- Glutathione S-transferase inhibition: essential oil; dosage 30mg/to animal. In vivo mouse. Activity: Effective in inhibiting enzyme transfer in the small intestine and liver, but not in the stomach**.(**Hadj et al , 2012**)**

1.3 Shea Butter:

The shea tree, also known as Butyropermum parkii or vitellaria paradoxa, is primarily found in the semi-arid savannah belt, extending across sub-saharan Africa and ranging from Mali to Sudan and Uganda. The shea tree is most valued for its "butter" extracted from the fruit kernels, which commonly is used as askin care emollient, but also as a replacement or addition to cocoa butter in the food industry. Shea butter, also known as karate butter, is highly appreciated in the cosmetics industry for its sensory and skin care properties. Its high unsaponifiable lipid content makes it a valuable source of bioactive triterpene esters, offering a naturally derived and functional this ingredient for cosmetic formulations the present article profiles. (Ann et al, 2015)

1.3.1Botanical classification of Shea butter:

Botnical name: *Vitellaria paradoxa*.

Family: *Sapotaceae.*

Genus: *Vitellaria.*

Subspecies: *SSP, Paradoxa –SSP,Nilotica.*

Commonly known *:Nkuto,karate.*(Kobomje et al,2013)

1.3.2 Origin:

Shea-butter tree is found in areas with 400-1800 mm rainfall per year. The species is of African origin. Its distribution area spreads from Senegal to Uganda (West-East Africa) and up to the Adamaoua Province in Cameroon (North-South Africa). The locations from which Shea germplasm was introduced to other countries in Africa is not well known due to vegetation changes. The species has probably spread out from refugia. Secondly, there have been aspects of human management that suggest semi-domestication is ongoing. (Ann et al, 2015)

1.3.4 Chemical Composition of Shea Butter:

As a plant fat, shea butter consists of approximately 90% or more of triglycerides and a minor unsaponifiable fraction. Triglycerides are responsible for shea butter‟s emollient properties, while the unsaponifiable fraction contains the bioactive substances that include hydrocarbons, tocopherols, sterols, and alcohols and thus responsible for shea butter‟s medicinal properties. (Esuoso et al, 2000)

1.3.5 Uses of Shea Butter:

Shea butter has many useful properties and has been traditionally used as a decongestant, an antiinflammatory for sprains and arthritis, a healing salve for babies' umbilical cords, a lotion for hair and skin care, as cooking oil, and for lamp fuel. However, the protective and emollient properties of shea butter are most valued for skin care. In Africa, shea butter is applied to the skin and hair as a moisturizer and is also a main ingredient in traditional black soaps. Vitamin A in Shea Butter is important for improving a number of skins conditions, including blemishes, wrinkles, eczema, and dermatitis. Additionally, Premium Shea Butter cream has properties that treat skin allergies, insect bites, sunburns, frostbites, and a number of other conditions of the skin. Shea Butter’s unparalleled

moisturizing property is due to several natural moisturizers present in the cream. The moisturizers in Shea Butter are the same moisturizers produced by the sebaceous glands in the skin.

If the skin sebaceous glands produce moisturizers for the skin, then it's no wonder that Shea Butter is such a superior moisturizer. The positive biochemical and physiological effect Shea Butter has on skin injuries makes this cream ideal for wound healing. Many users of Shea Butter have reported that Shea Butter promotes and accelerates wound healing. The exact benefit of the Vitamin E in Shea Butter is less clear. Vitamin E is a vitamin whose exact function in human being is not entirely clear, although it has been described as effective in a number of conditions or circumstances. These benefits include being anti-aging, an anti-free radical agent, and exerting a positive effect on increasing the micro-circulation.

If the vitamin E in Shea Butter is helpful for the skin, such benefits could be accomplished by at least two methods: (1) by increasing the micro-circulation to the skin, which results in increased blood supply to and from the skin; (2) vitamin E may serve by as an anti-free radical agent, thereby aiding in preventing the deleterious effects of sun and environmental exposure. (Esuoso et , al, 2000)

1.4 Mosquito's Repellents:

The Eggs from mosquitoes were dried and stored in an incubator until needed. Eggs were placed in deoxygenated water and two to three drops of a ground TetraMinTM fish food solution were added to the water to feed the larvae. Pupae were removed fkom the larval pans as they appeared and were placed into mesh covered paper cups. Following emergence, adult females were tested over a six-

day period. Mosquitoes were continually allowed to feed on a cotton ball soaked with 0.3 M sucrose solution. At 1-2 hours before testing.(Gretchen schmaltz, et.al, 2006)

1.4.1 Preparation of Repellent Compounds:

Besides being used in their natural state or "straight" repellents have been, commonly, embodied in lotions, creams, pastes or other preparations, either to facilitate their applications, for a material to be valuable as a mosquitoes repellent it must effectively, discourage insect attack on the treated area of the skin for many hours. It must work in different environmental conductions, and must be environmental friendly when applied to human or animal skin, it must also be, cosmetically, acceptable, having a pleasant odour, and feel, it should also be harmless to clothing, and have a relatively low cost, effective against insects.

1.4.2 Type of Repellents:

Lotions: Mixtures containing the repellent dissolved in or diluted with alcohol or other thin fluid, or thickened other oils.

- **Creams (ointment type):** Mixtures of the repellent with some solid greasy base such as hard and soft paraffin, petroleum jelly, cetyl alcohol, lanolin, magnesium stearate with or without modifying materials. Early repellent creams were mostly of this type.
- **Creams (vanishing cream type):** Essential oil dissolve in water emulsions which disappear on application to be absorbed by the skin. The chief requirements are an oily or greasy base, an emulsifier such as triethanolamine, triton X,and water ect.
- **Creams (waxy base type):** And mixtures of the repellent with wax and such solvent (which may be the repellent itself) as is necessary to give a correct

consistence. (Koech et al, 2013)

1.5 Emulsions:

The term emulsion is derived from the word emulgeo meaning “to milk”. Milk is example of a natural emulsion. An emulsion is a biphasic liquid preparation having two immiscible liquids, which cannot be dispersed for a long period. An emulsifying agent is added, which form film around the globules, so that a stable emulsion is produced. The size of globules is 0.25 to 25 Um diameters. Micro-emulsions are the globules of smaller diameter as small as 10 nm, which in appearance is milky and transparent. Transparent emulsion is formed when two phases having same refractive index. Emulsions are thermodynamically unstable and revert back to separate oil and water phase.[1] when to immiscible liquids are mechanically agitated, both phases initially tend to form droplets. When the agitation is stopped, the droplets quickly coalesce, and the two liquid separate. The lifetime of the droplets is materially increased if an emulsifier is added to the two immiscible liquids. Usually only one phase persists in droplet form for a prolonged period of time. This phase is called the internal phase and it is surrounded by the external phase. An assembly of close packed mono dispersed spherical droplets as the internal phase can occupy not more than approximately 74 % of the total volume of an emulsion. It is evident that the internal phase can exceed 74 % if the spherical particles are not mono dispersed. A further increase in the ratio of internal and external phase can result if the internal phase is assumed to consist of polyhedral rather than spheres. It is almost universally accepted that the term emulsion should be limited in liquid systems.[2] Emulsions are normally formed by “mixing “two immiscible liquids. I necessary the two phases are heated to ensure that they are liquids during emulsification. The most common types of pharmaceutical or cosmetics emulsions include water as one of

the phases and an oil and lipid as the other. Since approximately 1978, two additional types of emulsion classified as multiple emulsions with the characteristics of oil in water in oil (O/W/O) or of water in oil in water (W/O/W) emulsions. Such emulsions.

1.5.1 Type of Emulsion:

Emulsions are classified in two group:

1.5.1.1 Oil/water Emulsion:

In oil in water type emulsion, oil is dispersed phase and water is continuous phase. These are mostly used internally. Emulsifying bases used in this type is gum acacia, tragacanth, methyl cellulose, saponins, synthetic substances, etc. When the oil phase is dispersed as globules throughout an aqueous continuous phase, the system is referred to an O/W emulsion. An O/W emulsion is formed if the aqueous phase constitutes more than 45 % of total weight and if a hydrophilic emulsifier is used. It employs the following emulsifiers: sodium laurel sulfate, sodium oleate and glyceryl monostearate.

1.5.1.2 Water/Oil emulsion:

In water in oil type emulsion, water is dispersed phase and oil is continuous phase. Emulsifying bases used in this type is wool fat, resins, beeswax and soaps. These mostly used externally as creams and lotions. It employs the following emulsifiers: spans, cholesterols and wool fats. When an aqueous phase is dispersed as globules and the oil phase serve as the continuous phase, the emulsion is termed as W/O emulsion.(vania,et al.2014)

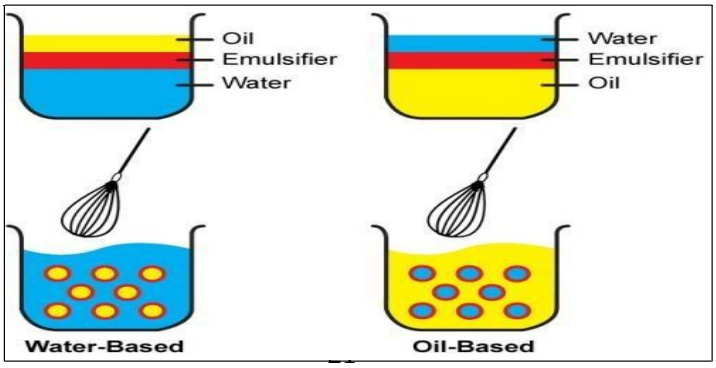

Figure (1.4): Type of Emulsion

1-5-2Emulsion Sstability:

The term “emulsion stability” refers to the ability of an emulsion to keep its properties unchanged over a certain period of time. However, as emulsions are thermodynamically unstable, changes of emulsion properties will occur; the more slowly the properties change, the more stable the emulsion is. There are many phenomena that can alter emulsion properties: coalescence, flocculation, creaming, Ostwald ripening, etc. Two or more of these instability phenomena may occur at the same time. It is then important to understand the cause(s) of instability to select suitable components to form stable emulsions. (Roman, 2010)

1-5-3Thermodynamic and Kinetic Sstability:

Thermodynamics gives information about processes taking place during emulsification or at quiescent conditions (after homogenisation). Kinetics gives information about the rate at which these processes occur. Mixing pure oil and pure water results in the formation of opaque emulsions. After a certain time, distinct layers of oil and water are visible. Phenomena (coalescence of oil or water droplets) taking place in this example are due to thermodynamic instability. The time taken by the droplets to merge is related to kinetics. In order to understand emulsion stabilisation mechanisms, it is important to distinguish thermodynamic stability and kinetic stability. It was shown that emulsions, and particularly food emulsions, are thermodynamically unstable systems23,44. This was demonstrated by considering the free energy of the oilwater system before and after emulsification. During emulsification, the overall free energy is positive, due to the increase of interfacial area, i.e. the food emulsion formation is thermodynamically unfavourable. Since emulsions are thermodynamically

unstable, kinetic stability is of great importance in many fields, including food; as emulsions are almost certain to break down, a crucial issue is to know how long emulsion properties remain the same. Despite the fact that emulsions exist in a thermodynamically unstable state, some remain kinetically stable for months or years. This metastable state (thermodynamically unstable and kinetically stable), is due to the fact that phenomena responsible for thermodynamically instability take place over a long period a time. The changes in emulsion properties occur then very slowly**.** (Roman, 2010)

1.5.4 Instability of Emulsion:

Physical Instability of Emulsion:

Probably the most important consideration with respect to pharmaceutical and cosmetic emulsions is the stability of the finished product. The stability of a pharmaceutical emulsion is characterized by the absence of coalescence of the internal phase, absence of creaming and maintenance of elegance with respect appearance, odor, color and other physical properties. Some workers define instability of an emulsion only in terms of agglomeration of the internal phase and its separation from the product. Creaming, resulting from flocculation and concentration of the globules of the internal phase, some times is not considered as a mark of emulsion.[2]

Creaming:

Creaming may be defined as the upward movement of dispersed globules to form a thick layer at the surface of the emulsion. Creaming is a temporary phase because it can be re-distributed by mild shaking or stirring to get again a homogenous emulsion; as far as possible creaming of an emulsion should be avoided because it may lead to cracking with complete separation of two phases. Under the influence of gravity suspended particles or globules tend to upward

movement, known as creaming while downward movement of particles or droplets is called sedimentation. Creaming or sedimentation depends on the differences in specific gravity between the phases. The rate at which a spherical droplets or particle sediments in a liquid is governed by stroke's law. Other equations have been developed for bulk system, but stroke's equation it still useful because it points out the factors that influence the rate of sedimentation. These are diameters of the suspended droplets, the viscosity of the suspending medium and the difference in densities between the dispersed phase and dispersion medium.[2,5] According to stroke's law, the rate of creaming depends on the number of factors which can be explained by the flowing equation

$$v = 2r^2 \frac{(\Delta p)g}{9\eta}$$

γ = Rate of creaming, r = Radius of globules, g = Gravitational constant,

Δp= Density difference between the two phases,

η = Viscosity of the continuous phase.

1.5.5 Chemical instability:

Chemical instability of an emulsion causes coalescence of particles of emulsion. It is necessary to insure that any emulgent system use is not only physically but also chemically compatible with the active agent and with the other emulsion ingredients. Anionic and cationic emulgents are thus mutually incompatible. It has already been demonstrated that the presence of electrolyte can influence the stability of an emulsion either by

- Reducing the energy of interaction between adjacent globules.
- A salting out effect, by which high concentration of electrolyte can strip emulsifying agents of their hydrated layers and so cause their precipitation.

Change in pH may also lead to the breaking of emulsion. Soap stabilized emulsions are therefore usually formulated at an alkaline pH. Environmental conditions, such as the presence of light, air, contaminating microbes adversely affect the stability of an emulsion. For light sensitive emulsion, light resistance container is used. For emulsion susceptible to oxidative decomposition, anti oxidants may be included in the formulation and adequate label warning provided to ensure that the container is tightly closed after each use. Many molds, yeasts, and bacteria can decompose the emulsifying agent, disrupting the system. Even if the emulsifier is not affected by the microbes, the product can be rendered unsightly by their presence and growth and will not off course not be efficacious from a pharmaceutical or therapeutic point. Because fungi (molds and yeasts) are more likely to contaminate emulsion than are bacteria, fungi static preservatives, commonly combinations of methylparaben and propylparaben are generally included in the aqueous phase of an o/w emulsion. Alcohol in the amount of 12 % to 15 % based on the external phase volume is frequently added to oral o/w emulsion for preservation.[7] Chemical instability is of three types namely:

- Oxidation
- Microbial contamination
- Adverse storage condition.

1.5.5.1 Oxidation:

Many of the oils and fats used in emulsion formulation are of animal or vegetable origin and can be susceptible to oxidation by atmospheric oxygen or by the action of micro organisms. Oxidation of microbiological origin is controlled by the use of anti microbial preservatives and atmospheric oxidation by the use of reducing agents or more usually, anti oxidants like butylatedhdroxyanisole (BHA) is widely used in the protection of fixed oil sand fats at concentration of up to 0.02

% and for some essential oils up to 0.1 %. The efficiency of an anti oxidant in a product will depend on many factors, including: (1) Its compatibility with other ingredients (2) its O/W partition coefficient (3) the extent of its solubilization within micelles of the emulgent (4) its sorption on to the containers and its closure.

1.5.5.2 Microbial Contamination:

Microbial contamination of emulsion occurs by micro organisms can adversely effect the physicochemical properties of the product, causing such problem as gas production, color and odor changes, hydrolysis of fat sand oils, pH changes in the aqueous phase and breaking of the emulsion. An emulsion can contain many bacteria and if these include pathogens may constitute a serious health hazard and most fungi and many bacteria will multiply readily in the aqueous phase of an emulsion at room temperature. Many moulds will also tolerate a wide pH range. Species of the genus *Pseudomonas* can utilize polysorbates, aliphatic hydro carbons and compounds. Some fixed oils such as arachiz oil, can be used by sum *aspergillus* and *rhizopus* species, and liquid paraffin by some species of penicillium.[6,7] Water in oil product have less chances of microbial spoilage as compared to oil in water emulsion as in the latter case the continuous oil phase act as a barrier to the spread of micro organisms throughout the product, and less water there is present the less growth there is likely to be. It is necessary to include an anti microbial agent to prevent the growth of any micro organisms that might contaminate the product. Example, because fungi are more likely to contaminate emulsion then are bacteria, fungi static preservatives, commonly contamination of methyl paraben and propyl paraben are generally included in the aqueous phase of an oil in water emulsion.[8]

1.5.5.3 Adverse Storage Condition

Adverse storage conditions may also cause emulsion instability by increase in

temperature that cause increasing rate of creaming, owing to a fall in apparent viscosity of the continuous phase. Increase in temperature cause an increased kinetic motion, both of the dispersed droplet and of the emulsifying agent at the oil in water interface. Increased motion of the emulgent will result in a more expended monolayer and so coalescence of more likely. Certain macro molecules emulsifying agents may also be coagulated by an increase in temperature. Freezing of the aqueous phase will produce ice crystals that may exert a pressure on the disposed globules and their absorbed layer of emulgent. In addition, dissolved electrolyte may concentrate in the unfrozen water, thus affecting the charge density on the globules. Certain emulgents may also precipitate at low temperature. The growth of micro organisms within the emulsion can cause deterioration and it is therefore essential that these products are protected as far as possible from the ingress of micro organisms during manufacture, storage and use. (vania,et al.2014)

1.6 Objectives:

In this study, demonstrated the important of natural products like Gum arabic, ocimum basil and shea butter.

The main objective of this study was to:

-To extract the essential oils of ocimum basilicum L purple.

-To identify components Basil oil by GC-MS.

-To study physicochemical characteristic of essential oil of ocimum basilicum L purple.

-To prepare moisturizing cream with basil oil extract and shea butter, using gum Arabic as emulsifier agent.

-To study the effectiveness of basil oil cream as mosquitoes repellency under laboratory conditions.

CHAPTER TWO

MATERIALS AND METHODS

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials:

Basil oil, gum Arabic, Shea batter, distal water, Instrument: Water bath, balance, steam distillation, Gc mass, PH meter, Conductivity meter, type of apparatus.

2.2 Methods:

2.1.1 Collection of Plant Material:

Essential oils were extracted from leaves of Ocimum basilicum . The aerial parts of cultivated O.basilicum leaves (grown without pesticides and chemical fertiliziers), the leaves were collected from Omdurman ,Khartoum state. All plant matirales were taxonomically , authenticated at the herbarium of medicinal and aromatic plants at Traditional Medicinal Research Institute (National Center For Research).

2.1.2 Preparation of Materials:

Fresh leaves for basil were picked from the plant, washed with clean tap water before being dried under shade to constant weight. Drying was done slowly to avoid the loss in biological activity. The leaves were dried at room temperature for three days. The dried leaves powdered marital of type Ocimum basilicum Purple (50g) with (500ml) distilled water were subjected to hydro distillation for 5 hours using Clevenger-type apparatus (National center for Research). The steam and vaporized oil were condensed into liquid by a vertical condenser and collected in measuring tube. Being immiscible and lighter than water, the volatile oil separated out as an upper layer. The oil was then separated from water and volume of the

obtained essential oil was calculated as a percentage volume per weight. The oils were collected in glass with air- tight cover and were dried over anhydrous Na_2SO_4 and stored in a dark glass bottle in light resistant at 4-6ºC until analyzed by GC/MS to determine oil constituents.(Hussain et als ,2008).

2.2 Methods:

2.2.1 Photochemical Screening:

Phytochemical screening for the major constituents was carried out using standard methods described by gas chromatography-mass spectrometry (GC-MS). Physicochemical characterization of essential oils was performed.

Identification of components Basil oil by GC-MS:

Essential oil chemical composition was analyzed using a gas chromatograph (GC) fitted to a mass spectrophotometer(MS) (GC-MS, Shimadzu QP-5000, Kyoto, Japan) operating in electron-impact (70 eV, *m/z* 40 – 450) mode; the fused-silica capillary column used was an OV–5 with diameter of 30 m long., 0.25 mm i.d., 0.25 □m film thickness (Ohio Valley Special Chemical Inc., USA). The chromatographic conditions were as follows: sample preparation 1µL in 1 mL of hexane; injection volume 1 µL; split ratio 1:55; helium flow rate 1.0 mL/minutes temperature programme ramp from 60 to 240 °C with a gradient of 3 °C/minutes (holding the initial and final temperature for 10 minutes); injector temperature 240 °C; and detector temperature 230 °C The identification of the essential oil components was performed by retention indexes and by comparing their mass spectra with a data bank (System GC-MS, Nist. 62 lib) and the literature (adams, 1995; mclafferty; staufer, 1989). Retention indexes were obtained by co-injection with a hydrocarbons (C9-C24) standard mixture using the van den Doll equation (van den et al, 1963).

2.2.2 Physicohemical Charcteristics of Oil Extract:

2.2.2.1 Color Determination:

Color of respective oils was determined by physical observation in day light and under ultraviolet radiation of 254 and 366 nm using ultraviolet chamber.

2.2.2.2 Determination of Percentage Oil Yield:

The percentage oil yield was calculated by using the following relationship: Essential oil (%) = amount of essential oil recovered (g) /amount of crop biomass distilled (g)*100 (Gabi Baba, 2012).

2.2.2.3 Determination of Refractive Index:

The refractive index of the oil samples was determined using an Abbe refractometer model A 80251 (BS). Two drops of respective oil were placed on the prism with the help of syringe and the prism was firmly closed by tightening the screw head. The apparatus was allowed to stand for 5 min, after that reading was recorded from display screen (Barkatullah et al, 2012).

2.2.2.4 Determination of Viscosity:

Viscosity is the resistance to the flow and it was determined by using a viscometer with a selection of spindle number four which was properly fixed to the holder. The container having the oil was, carefully, placed below the rotor holding the spindle. The spindle was allowed to immerse in to the oil inside the container. The meter was turned on and adjusted to speed of 6m/s. Then the spindle was allowed to rotate in the oil for a period of 30 min until stable reading displayed on the meters display screen. The viscosity value of the oil was measured in centipoises.

2.2.2.5 Determination of Total Acid Number (TAN):

1.25g of oil were placed in a flask 50ml of solvent were added to the flask, well shake and titrated against o.1N KOH solution, using 1ml phenolphthalein as indicator. Alkali was added till a pink color was established for (20-30s) were noted that number of milliliters of standard alkali required and calculated the acid value of the fat. 0.1M KOH contain 5.6g/l or 5.6mg/l. acid value was calculated using the following formula: V*N*56.1/W.

Whereas:

N= normality of potassium hydroxide.

W= weight in g of the sample.

2.2.2.6 Determination of Iodine Value:

2g of respective oil was weighed and transferred into conical flask. 25ml of iodine monochloride 1ml were added and the solution was kept in the dark for one hour at room temperature. 10 ml of potassium iodide solution and 100 ml of distilled water were added to the flask. The resulting solution was titrated against sodium thiosulphate (0.1M) using starch as indicator tell the end point where the blue black coloration becomes colorless. A blank titration was carried out at same time starting with 10ml carbon tetrachloride. The difference between the blank and test reaging (B-T) gives the number of milliliter of 0.1M thiosulphate needed to react with the equivalent volume of iodine. The amount of fat taken is 0.2g and 1Lof mol/L iodine contained 12.7g of iodine, iodine value was then calculated by t5he following formula:

Iodine value = (B-T) *12.7/1000*100/2 = (B-T)* 6.35.

Where as B=0.1N Na_2SO_3 required by sample.

2.2.2.7 Determination of the Saponification Value:

1g of oil sample was weighted and dissolved in 3ml of ethanol 95% and ether. The contents were transferred to a clean dried conical flask (250ml) and 25ml of alcoholic potassium hydroxide 0.5M was added. A reflux condenser was attached to the flask and heated for one hour with periodic shaking. The appearance of clear solution indicated the completion of saponification. Then 1ml of 1% phenolphthalein indicator was added and the hot excess alkali was titrated with 0.5M hydrochloric acid until it reached the end point where it turned colorless. A blank titrated was carried out at the same time and under the same condition. The difference between the blank and test reading gives the number of milliters of 0.5M KOH needed to saponify 1g of fat. The molecular weight of KOH is 56 and, since three molecules of fatty acid are released from a triglyceride then:

Saponification value (S) = 3*56*1000/Average mol.wt of sample.

Average Mol .Wt of sample (fat) =3*56*1000/S (plummed, 1978)

2.2.2.8 Determination of Peroxide Value:

1.29g of the oil were weighted and added to 15ml of acetic acid chloroform solution then the mixture was shaked slowly in a flask of 250ml capacity until the contents were completely dissolved. Then 0.2ml of potassium iodine solution and shaked for one for one minute, then 1ml of starch solution was added as an indicator and titrated slowly with sodium thiosulphate (0.1N).

Peroxide value = (T –B)*10/W

T=Titration volume (ml)

B= Titration Blank (ml)

W=Weight of sample

2.2.3 Preparation of Cream Base (Aqueous base):

27g of Gum Arabic were dissolved in 54 ml of distilled water, 9g of shea butter were weighted, at same time all these component heated in water bath at 60ºC to desired concentration and form primary emulsion by ratio (27:54:9), added solution of Gum Arabic in shea butter oil and vigorously shaking . Keep at room temperature over night to observe stability of emulsion. 10g of basil oil extracted was added to emulsion and vigorously stirred until product become a soft cream of high viscosity and very smoothes to the skin.

2.2.4 Preparation of Mosquitoes:

Mosquitoes, used in this study, were laboratory reared anopheles females (malaria vector), according to the standard protocol of the medinical Insecticide Section, National Central Institute of Health (STACK Laboratory) Khartoum State. The eggs were placed on a plastic tray containing tap water to hatch and yeast pellets served as food for the emerging larvae. The eggs batched, collected daily, kept wet for 24 hours and then placed in distilled water in the laboratory at 24-26ºC and natural condition for hatching. The newly emerging larvae were then isolated in 150 groups of ten specimens in 100ml tubes with 50ml of mineral water and a small amount of cat food (Gabi Baba, 2012).

Larvae were transferred to wells containing 1ml of water and 2g of food in a 24 hours well polystyrene plate. The larvae were reared continuously for several generations and kept under 25-30ºC, 60-70% relative humidity and photoperiod of 13:11 hours (light / dark). Larvae were fed on ground biscuit. At larvae stage, they were surface. Water, in rearing trays, was refreshed every two days in order to avoid scum formation that might kill the larvae. Trays were washed in clean tap water and the larvae sieved out of the trays and cleaned thoroughly with tap water

before being returned to the fresh water in rearing trays. On pupation, the pupae were collected using a Pasteur pipette and transferred in a container, three quarters full of water that was then inserted in a wooden cage. The adults were reared in humidified cages and supplied with 10% sugar solution as an energy source and 10% multivitamin syrup supplied in plates. The prepared solution was placed in feeding tube, Whatman No.1 filter paper inserted and placed in the cage. The solution was changed every three days to avoid fermentation and growth of moulds. Female mosquitoes were periodically blood –fed on restrained rabbits to obtain protein used principally for egg production under these conditions, the full development from egg to adult lasted for three weeks. Batches of 3-5day –old healthy female mosquitoes were used in the repellency bioactive. And stored under conditions during the test followed a standard diel cycle, with air temperature 25 ±2º, 60 ±10% relative humidity and 12 h : 12 h(light :dark).

2-2-5 Repellency test:

Test one :for repelling activity (Hand in cage method).The mosquitoes repellency of the basil oil cream was evaluated by using hand in cage test (WHO,1996) with little modification.

Procedure hand in cage method for step one and step two: The repellency of the basil oil cream was evaluated using the human – bait technique. The number of 50 female mosquitoes 3-5 day –old (without water and sugar for 18-24 hours) was aspirated randomly using a mouth aspirator introduced in to (40*40*40cm) net cage. Evaluations were carried out by placing the arms of volunteers inside cages. The temperature was maintained at 25-30ºC and the relative humidity at 55-60%. Four human volunteers were employed for one repellency tests. The one step to measurement the ability of mosquitoes Both arms of volunteers, which would later be placed one in each cage, were carefully covered with thick paper, except for

area of 3*10cm exposing the arm to mosquito bites. Step two this area of (3*10cm) was treated with anything for control (one arm) approximately 0.1cm^3 of cream base was applied to the one arm and 0.1cm^3 of basil oil cream (10%) was applied to the other arm. Each arm of a volunteer was placed inside the cage containing 50 female mosquitoes for 5 min. After that the arms of the test person were cleaned with ethanol, all this step at the same condition and exposure the number of mosquitoes landing on each 3*10cm test area was observed. Mosquitoes that land and bite are clearly distinguished by the visible blood in their bodies. The number of these mosquitoes taken throughout the test period 6h was used as the number of bites calculated per hour. Each mosquito that has bitten once was removed from cage by the visible blood in their bodies and replaced by a new one. These test repeated at the same conditions. (Nour et al, 2009).

Data analysis: The median protection time was used as a standard measure of the repellency of the volatile oil against mosquito in the laboratory. Percent protection from mosquito landing/biting or repellency was computed as compared to control by the following equation.

Percentage repellency %= Ta-Tb/Ta*100

Whereas:

Ta= is the number of mosquitoes that fed on the untreated skin.

Tb= is the number of mosquitoes that fed on the treated skin (pin yang, 2005).

CHAPTER THREE

RESULTS AND DISCUSSION

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Physio-chemical properties of essential oil:

The appearance of essential oil was pale yellow, transparent and clear with strong odour, with characteristic insoluble in water and soluble in vegetable oils and alcohol. This result is similar to result obtained by (Mawada, 2005).

The essential oil yield obtained was (1.5±0.1%) for Ocimum basilicum L. purple (purple flower). The result similar to (Hussain et al, 2008) the oil content varied from (0.1- 1.98%) depend on the seasonal factor and locality. Table(3-1) show the yield obtained and physiochemical properties of Ocimum basilicum L.purple oil showed that the percentage of oil yield (1.5±0.1%) observed when to comparison with study (Hussain et al,2008),difference appeared ,might be due ecological factor , and influences of storage.

3.1.1 Refractive index: the range is 1.51±0.4. It is a ratio found within the range of essential oils.

3.1.2 Density ranged: The percentage of O.basilicum L. purple is 0.91g.cm^3.

3.1.3 Viscosity value: O.basilicum L. purple is 3.37.All essential oil characterized by low viscosity value.

3.1.4 Saponification value: Saponification value for extracted oil was 109 for O.basilicum L. purple. The saponification values showed richness in long fatty acid chain.

3.1.5 Iodine value: Iodine value was ranged from 31.73 .The iodine value of the most oils are were ranged from 118±0.11 to 157± 0.65 this value indicate on number of unsataureted bonds.

3.1.6 The acid value: Acid value is 2.this value indicate the molecular decomposition for oil during distillation.

3.1.7 Peroxide value: It was found equal zero that indicate to the extract oil doesn't contain any peroxide substance.

Table.3.1: physical and chemical properties of Ocimum basilicum .L.purple:

Properties	Rustles of Ocimum basilicum purple
Yield oil%	1.5%
Colour	Yellow
Refractive index	1.49± 0.6
Density	0.91g/cm^3
Viscosity value	3.37
Saponification value	109
Iodine value	31.73
Acid value	2.8
Peroxide value	0.0

3.2 Chemical Compositions by GC-MS Spectrometry:

The Chemical compositions of essential oil of Ocimum basilicim .L. purple are shown in (Table 3.2).In the order of the retention times of the constituents 56 constituents were identified in O.basilicum L. purple, representing (100% of the total oil).

D-limonene (2.45%), 1.6-Octadien-3-ol,3,7-dimethyl(18.01%), Cineole (8.33%), 1.6-Octadien-3-ol,3,7-dimethyl(18.01%), alpha-Terpineol (1.13%), Geraniol(11.33%), ,Cinnamaldehyde, E(1.38%), Geranyle acetate (3.84%),Benzoic acid,4-ethenyl-,methyl ester(4.21%), Methyleugenol(13.00%), D- carvone (6.95%),Gamma-Muurolene(2.15%),Tau-Cadinol(2.86%),Methylcinnamate(0.74%),Linalool(0.13%).Was found as the major compunds for Ocimum basilicum Lpurple.

Table 3.2: Show Chemical Composition by GC-MS of Ocimum basilicum L. purple:

NO.	Compound Name	Retention Time	Area %	Area
1	Alpha- pinene	4.702	0.70	3057130
2	Camphene	4.975	0.09	376127
3	Beta-phellandrene	5.403	0.62	2723034
4	Bicycle[3.1.1]heptane,6,6-dimethyl-2-methyle	5.479	1.32	5753705
5	Beta myrcene	5.685	0.32	1392426
6	D-Limonene	6.449	2.45*	10690557
7	Cineole	6.517	8.33*	36330022
8	Beta –Ocimene	6.793	0.28	1200121
9	Bicycle[3.1.0]hexan-2-ol,2-methyl-5-(1-	7.244	0.53	2304586

	methyl)			
10	Alpha-methyl –alpha –[4-methyl-3penten]	7.324	0.27	1165752
11	L-Fenchone	7.667	1.07	4687947
12	1,6- Octadine -3-ol,3,7 dimethyl	7.908	18.01*	78550767
13	Bicycle[2.2.1]heptan-2-ol,1,3,3-trimethyl	8.223	0.22	940959
14	Oxirane ,2-(hexyn-1-yl)-3-methoxymethylene	8.700	0.09	372604
15	(+)-2-Bornanone	8.837	0.93	4044611
16	L –alpha-terpineol	9.294	0.41	1795339
17	Terpinen-4-ol	9.494	0.12	544883
18	Alpha –terpineol	9.774	1.13*	4925544
19	Cyclohexanol, 2-methyl-5-(1-methylethenyle	9.852	0.28	1201708
20	Fenchyl acetate	10.306	0.35	1529716
21	2-Cyclohexen-1-ol,2-methyl-5-(1-methylet)	10.349	0.13	577088
22	2,6-Octadien -1-ol,3,7-dimethyl,(Z)	10.482	0.54	2377440
23	2,6-Octadienal,3,7,-dimethyl-(Z)	10.734	0.35	1548362
24	D-Carvone	10.846	6.95*	30318845
25	Geraniol	11.015	11.33*	49429791
26	2,6-Octadienal, 3,7-dimethyl(E)	11.309	0.94	4108832
27	Cinnamaldehyde ,(E)	11.414	1.38*	6023882
28	Bornyl acetate	11.626	0.10	445414
29	Linalool	11.882	0.13*	552017
30	Methyl Cinnamate	12.038	0.74*	3243168

31	(-)- 8-p-Menthen-2-yl, acetate ,trans	12.413	0.47	2036467
32	Cyclohexane ,1-ethenyl-1-methyl-2-(1-methenyl)	12.609	0.18	766163
33	2-Oxabicyclo[2.2.2]octan -6-ol,1,3,3-trimethyl acetate	12.689	0.18	805979
34	Alpha –Cubebene	12.841	0.10	420404
35	2,6-Octadien -1-ol,3,7-methyl-acetate (Z)	13.054	0.44	1902893
36	Geranyl acetate	13.413	3.84*	16768359
37	Benzoic acid , 4, ethenyl-1-methyl ester	13.536	4.21*	18378731
38	Cyclohexane 1-ethenyl –methyl -2,4-bis(1-methylethenyl)	13.657	1.58	6900526
39	Methyleugenol	13.908	13.00*	56692368
40	Caryophyllene	14.217	0.96	4176255
41	Bicyclo[3.1.1] hepta-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)	14.437	4.93	21500362
42	Alpha –Guaiene	14.516	0.35	1522110
43	Humulene	14.842	0.43	1862021
44	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene	14.999	0.23	982041
45	Benzene,1-methyl-4-(1,2,2—trimethycyclopentyl)	15.272	0.13	576484
46	Beta .ylangene	15.329	1.11	4832669
47	1,5-Cyclodecadiene,1,5dimethyl-8-(1-methylethylidene)	15.603	0.46	2001197
48	Azulene,1,2,3,5,6,7,8,8a-octahydro-1,4-	15.737	0.87	3804365

	dimethyl			
49	Gamma –Muurolene	15.894	2.15*	9387865
50	Cyclohexene,3-(1,5-dimethyl-4hexenyl)-6-methylene	16.001	0.20	867167
51	Trans-calamenene	16.039	0.21	911264
52	Cubedol	16.194	0.12	516707
53	Alpha Muurolene	16.292	0.07	313548
54	Spathulenol	17.042	0.55	2408536
55	Cubenol	17.642	0.46	2017551
56	Tau .Cadinol	18.061	2.68*	11679341

Among the oxygenated compounds such as linalool, Cineole, Methyl eugenol, Geraniol, Cinnamaldhyde, Geranyl acetate, Methyl cinnamate.In sample was found to be high .that me induce to activation of cream in repellency of mosquitos.

Repellent Test Results:

Table 3.3: shows repellency test result and the numbers of mosquitoes biting on the control and treated areas of the arm by basil oil cream.

The skin repellent test at 10% concentration of basil oil cream offered the complete and total percent of protection as 100%.

3.3 Repellency test Results:

Table (3.3) Repellency test Results:

NO.Cage	Ability of mosquitoes interval 3mint		Control interval 5mint		Treatment 10% interval 5 mint	
	landing	Biting	landing	Biting	landing	Biting
1	8	6	8	8	-	-
2	5	4	-	-	0	0
3	4	4	-	-	0	0
4	7	5	-	-	0	0

Percentage of repellency = Ta –Tb/ Ta *100

Percentage of repellency = 8 -0 /8 * 100 = 100%

	Treatment of basil oil cream 10%
Percentage of repellency	100%

In this study, the repellency activity of basil oil cream against Anopheles mosquitoes was used. These results, are in good agreement with earlier studies in repellency activity of basil essential oil against mosquitoes, by (Swssan, 2016), but differ with some materials (light liquid paraffin, white petroleum jelly, paraffin wax).The results this work indicated that the repellency potential of the oil is concentration and activity of components in basil oil depend.

Basil oil cream based repellent are safe for humans. It did not cause any adverse effect in terms of discomfort or skin irritations on the human volunteers during and after the study period. And also shea butter it beneficial in cosmetic

cream industrially. Cream was stability found to be over 60 days, no reports are available about any possible side-effects on external usage of basil oil.

3.4 Conclusion:

In conclusion, this study appear the potential of cream preparation with volatile oil derived from O.basilicum, repellent against mosquitoes. O.basilicum purple has strong odour like clove odour and safety. And shea butter choses to form cream of repellent due to beneficial in cosmetic industrial. The cream has the advantage of being prepared from Natural product in contrast to the petrochemical product used by swsan (2016).

3.5 Recommendation:

Due to the high beneficial of basil oil cream as repellent against mosquitoes, and importance of shea butter as a skin moisturizer the researcher recommends the following:

1- Preparation the basil oil cream industrially and registering it as a pharmaceutical product for public use.

2- This study demonstrated importance of natural products, and how useful without high cost.

3-Conduct research to determine the source of activation of O.basil oil in repellency test, possible replaces by chemicals components in some industrial cream repellents.

4-Conduct research using basil oil and shea butter in the treatment of wounds and cosmetic products (soap, hand lotion, hear cream.), and anti bacterial activity.

Reference:

Andrea Murarikova ,(2017). Characterization of Essential oil Composition in Different Basil species and pot cultures by GC-MS method ,*J American Journal of applied sciences*,2-3

Ann. Charlotte Ander soon and Jari Alander ,(2015) , Bio active skin Care, Shea Butter Extract Cosmetics &Toiletries .45-47. *J pharmacology and Tixicology*

Azhari H .Nour, salah A. Elhussein, Nour. A.Osman and Abduelrahman H .Nour (2009) Repellent activities of essential oils of four Sudanese types of essential oils of basil (ocimum basilicum) aginst anopheles mosquitoes .J *Applied Sciences*,2645-2648.

Barkatullah, Muhammed Ibrar, Abdur Rauf and Inyat. Ur- Rahman , (2012). Physicochemical characterization of essential and fixed oils of skimmia. Laureola and zanthoxylum armatum. *J Medicinal plants Research1*(3),51-58.

Dziezak,(1999) Gum Arabic,*J Environmental and Biological Sciences*.6-8.

EL. Amin, H.M.(1990).Trees and shrubs of the sudan. Ithaca press Exeter pp. 160-163.

Esuoso K.O.;Lutz H;Bayer E.Kutubuddin ,(2000).Unsopnifiable lipid constituents of som underutilized Tropical seed oils . *J.Agric food chemistry*. 48-52

Gabi Baba , A.O.Lawal , Hauwa B.shariff (2012). Mosquito repellent activity and phytochemical characterization of essential oil from striga hermonthica.hyptis spicigera and Ocimum basilicum leaf extracts.*J pharmacology and Tixicology* (3).43-48.

Gen ,Joker,Dorth, (2000) . Botanical,Acacia Senegal –Seed leafet , *J university of copenna*.3-4

Gretchen S chultz, Chris Peterson and Joel Coats, (2006).Natural Insect repellents activity against Mosquitoes and Cockroaches,147-148 ,*American chemical society.*

Hadj Khelifal – M-Brada, F.Brahmi , D.Achour, M.L.Fauconnier G.lognay (2012) . Chemical composition and antioxidant activity of essential oil of ocimum basilicum leaves ,*J Herbal Medicin.*25-30.

Hussain , Abdulah Ijaz, farouq Anwar, Syed Tufail Hussain sherazi, Roman przybylski (2008). Chemical composition, antioxidant and antimicrobial activities of basil (ocimum basilicum) essential oils depends on seasoned variations.*J food chemistry*.986-995.

K-Azzaoui ,B-Hammouyi, A-Lamhamdi ,E-Mejdoubi, M-Berrbah,(2014). Gum Arabic in the Southern region of morocco, *J food chemistry* .102-103.

Koech peter kiplang, Richard W. Mwangi, (2013), Repellent Activities of Ocimum basilicum ,Azadirachta indica and Eucalyptus citriodora extracts on Rabbit skin against Aedes a egypti.*JEntomology and zoology studies*,84-91.

Mario Vargas (2012). Botanical categories on plant essential oils with effects on mosquitoes *J De malariologia Y Salud Ambiental*. 143.

MarioVargas (2012).Planting botanical Categories on plant essential oils with effects on mosquitoes Ysalud Ambiental., *JDe Malariologes* Vol.l.143.

Mohamed H.M.AbdEl.A, Ahmed. AM.Abdelgawad,(2015). Chemical composition and antimicrobial activity of essential oil of Egyptian Ocimum Basilicum. *j International journalof chemical sciences*.837-842.

Mr.SalifToure (2008) , Market news service. Gum Arabic – http://WWW.intracen.org/mns.4-5

Mueen Ahmad , Syda Batool, Asifa sharif, (2015). Biological and pharmacological properties of the sweet basil, *J Interntional research of pharmacy*. 13-16

O.S.Kobompje,A.O.Mohammed and P.F.Omojasola,(2013).The production of citric from shea nut shell using Aspergillus niger,*Jpelagia Researchlibrary* . 25.

Paulo.A. Ameh, Umar M.Sani, Elias E.Nwoye , (2015).Studies on some physiochemical and rheological properties of Albizia furriguinea.*J Environmental and Biological Sciences.* 10-11

Roman pIchot, (2010) .Stability and characterization of Emulsion in the presence of colloidal particales and surfactants. university of Birmingham (2), 17-18,

Sonali Naik, (2014). Natural Gums and its pharmacist application,113-119.

Vanden Dool H .; KRATZ, D.J.A. (1963).A generalization of the retention index system including line Temperture programed gas liquid partition chromatography.

Vania Madaan , Arsh chanana, Mahesh Kumar, ajay bilandi ,(2014), emulsion technonlogy and recent trendsin emulsion application – *Interntional research J of pharmacy*.

WHO(1996).Report of the informal consultation on the evaluation and testing of Insecticides CTD/WHOPES/IC 96.1 Control of Tropical Diseases Division .World Health organization Geneva.