CHAPTER ONE

INTRODUCTION

1.1 Volatile oils:

Essential oils are defined as any volatile oils that have strong aromatic components and that give “distinctive” scent to the plant. Essential oils are complex mixtures of organic compounds, composed of terpenoid hydrocarbons and oxygenated compounds derived from these hydrocarbons. They originate from the plant of secondary metabolism and are responsible for their aromatic characteristics (Ladan, 2011).

Essential oils are volatile liquids at room temperature. The amount of essential oil found in most plants is 1 to 2%, but can contain amounts ranging from 0.01 to 10% (Vincent, 2013). They are located in different parts of the plant. They can be found in the root such as Vetiver grass (*Vetiveria zizanioides*), in stems like that of Peteribi wood (*Cordia trichotoma*), in leaves like Eucalyptus trees (*Eucalyptus citriodora)*, Citronella (*Cymbopogonnardus*), Chinchilla (*Tagetes minuta*), Basil (*Ocimum basilicum)* and Lemon grass (*Cymbopogon citratus*), in flowers like Lavenders (*Lavandula officinalis*), in fruit like Lemon, Orange (*Citrus spp.*) and even in seeds as in the case of Anise (*Pimpinella Anisum), C*oriander(*Coriandrum,sativum*) and Pepper (*Pipernigrum*).

Essentials oils accumulate in cells, secretory cavities or glandular hairs of plants (Ester, 2012). The fragrance and chemical composition of essential oils can vary according to the geo-climatic location and growing conditions (Abdelouaheb, 2012).

1.2 Methods of Extracting Essential Oils:

Essential oils can be extracted using a variety of methods, although some are not, commonly, used today. Thus the chemical composition of the oil, both quantitative and qualitative, differs according to the extraction technique, for example over fourty two different plants materials used are hydro-distilled or, steam and steam/water-distilled (Temel Özek *et al*, 2010). Hydro-distillation and steam distillation methods yield oils rich in terpene hydrocarbons. In contrast, the super-critical extracted oils contain a higher percentage of oxygenated compounds. Currently, the most popular method for extraction is steam distillation (Özcan, 2002).

1.2.1 Principles of Distillation:

The choice of a particular process for the extraction of essentialoil is, generally, dictated by the following considerations:

- Sensitivity of the essential oil to the action of heat and water.
- Volatility of the essential oil.
- Water solubility of the essential oil (Sukhdev *et al,* 2008).

There are three types of hydro-distillation for isolating essential oils from plant materials.

1.2.1.1. Water Distillation:

In this method, the aromatic plant material is, completely, immersed in water which is boiled by direct heating, closed steam jacket, closed steam coil or open steam coil. The main characteristic of this process is that there is direct contact between boiling water and plant material.The plant material in the apparatus must be agitated as the water boils. The laboratory apparatus recommended for trial distillations is the Clevenger system. During water distillation, all parts of the plant must be kept in motion by boiling water; this is possible when the distillation material remains loose in boiling water. For this reason only, water distillation possesses one distinct advantage, i.e. it permits processing of, finely, powdered material or plant parts that are in contact with steam, would otherewise form lumps through which the steam can not penetrate. Other practical advantages of water distillation are that the apparatuse are inexpensive, easy to construct and suitable for field operation.

Disadvantages of Water Distillation:

- Oil components like esters are sensitive to hydrolysis while others like acyclic monoterpene hydrocarbons and aldehydes are susceptible to polymerization (since the pH of water is often reduced during distillation, hydrolytic reactions are facilitated.
- Oxygenated components such as phenols have a tendency to dissolve in the still water; hence their complete removal by distillation is not possible.

- As water distillation tends to be a small operation (operated by one or two persons), it takes a long time to accumulate much oil, and so good quality oil is often mixed with bad quality oil.
- The distillation process is treated as an art by local distillers, who, rarely try to optimize (both oil yield and quality).
- Water distillation is a slower process than steam distillation (Sukhdev *et al*, 2008).

1.2.1.2. Water and Steam Distillation:

In water and steam distillation, the steam can be generated either in a satellite boiler or within the still, and separated from the plant material. Also, the equipment used is, generally, similar to that used in water distillation, but the plant material is supported above the boiling water on a perforated grid (Murray, 2001).

A. Advantages of Water and Steam Distillation are:

- High oil yield.
- Components of the volatile oil are less susceptible to hydrolysis and polymerization. The control of wetness on the bottom of the still affects hydrolysis, whereas the thermal conductivity of the still walls affects polymerization.
- If refluxing is controlled, then the loss of polar compounds is minimized.
- Oil quality produced by steam and water distillation is more reproducible.
- Steam water distillation is faster than water distillation, so it is more efficient.

B. Disadvantages of Water and Steam Distillation:

- Due to low pressure of rising steam, oils of high-boiling range require a greater quantity of steam for vaporization, hence longer hours of distillation.
- The plant material becomes wet, which slows down distillation as the steam has to vaporize the water to allow it to condense further up the still.To avoid that the lower plant material resting on the grid becomes waterlogged, a baffle is used to prevent water from boiling too, vigorously coming in direct contact with the plant material (Sukhdev *et al*, 2008).

1.2. 1.3. Steam Distillation:

In this process, plants are placed on a perforated base or sieve at a certain distance over a distilling tank (Figure 1.1). The tank contains water at a level less than the depth of the sieve. Heating is via saturated steam when the water is heated using a built in heater. Steam flows at low pressure and penetrates the vegetable matter. The component parts volatilise and are then condensed in a refrigeration tube and collected in a florentine flask where water and oil are separated because of difference in density.

Essential oils contain substances with boiling points up to 200°C. In the presence of steam or boiling water, however, these substances are volatilized at a temperature close to 100°C under atmospheric pressure. A number of factors determine the final quality of an essential oil. Aside from the plant material itself, most important factors are time, temperature, pressure, and the quality of distillation equipment (Murray, 2001).

A. Advantages of Direct Steam Distillation:

- Amount of steam can be readily controlled.
- No thermal decomposition of oil constituents.
- Most widely accepted process for large-scale oil production, superior to the other two processes.

Steam distillation is a relatively cheap process, and the properties of oils produced by this method are well known. Steam distillation is one of most preferable extraction methods to obtain essential oils. It is still reliable a longside modern techniques to produce 93% of oil extract while the remaining 7% is extracted by other techniques. Although conventional steam distillation system is characterized by a simple process, cleaner production and low operational costs but losses of some volatile compounds, solvent residue and volatile compounds were significantly diluted with water this mostly affect colours of essential oil and results in undesirable off-flavor compound (Zakiah *et al,* 2013).

B.Disadvantage of Direct Steam Distillation:

- Much higher capital expenditure needed to establish this activity than for the other two processes. Extraction ofl oil depends on the temperature. The temperature will influence the final product of the extraction process.

- Sensitive materials like oils, resins, hydrocarbons, which are insoluble in water and may decompose at their boiling point. The yield and quality of essential oil from steam distillation is affected by:
- mode of distillation or technique, proper design of equipment, material of fabrication of equipment, condition of raw material, time for distillation, loading of raw material and steam distribution, operating parameters, condition of tank and equipment, Purification of crude essential oils continuous steam distillation (Sukhdev *et al*, 2008).

Figure (1.1 a) Steam Distiller

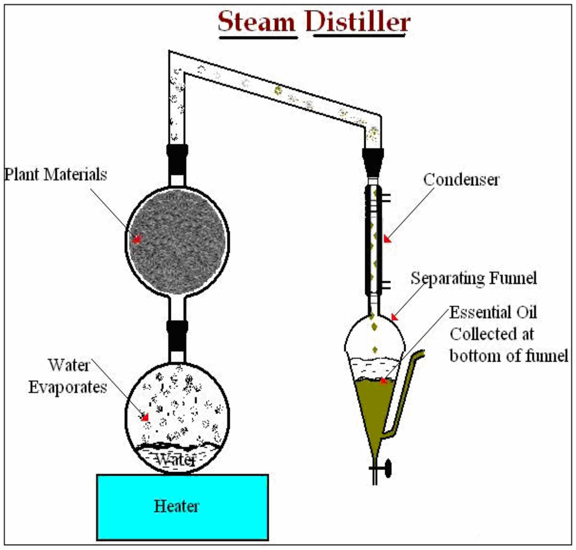


Figure (1.1 b) Steam Distiller

Other methods include:

- Cold pressing extraction.
- High pressure extraction.
- Turbo distillation extraction.
- Microwave–assisted hydro-distillation.
- Solvent extraction.
- Enfleurage distillation extraction.

Modern Methods of Extraction:

- Headspace trapping techniques (static headspace technique, vacuum headspace technique, dynamic headspace technique).
- Solid phase micro-extraction (SPME).
- Supercritical fluid extraction (SFE).
- Phytosol (phytol) extraction.
- Protoplast technique.
- Simultaneous distillation extraction (SDE).
- Microwave distillation.
- Controlled instantaneous decomposition (CID).
- Thermomicro distillation.
- Microdistillation.
- Molecular spinning band distillation.
- Membrane extraction (Sukhdev *et al*, 2008).

1.3. Analysis of Essential oils:

Gas Chromatography-Mass Spectrometry (GC-MS):

One of the weaknesses in gas chromatography is the separation of compounds will often show overlapping peaks, which make compounds difficult to identify. The gas chromatograph alone will not distinguish the difference between impurities and other compounds. Through the coupling of a mass spectrometer at the end of a capillary column of a gas chromatograph, the detector can capture and ionize (bombard with electorns) then each molecule is identified according to relative charge of broken molecule fragments (ions). Through combining retention time and ionization detection, most molecules can be, accurately, identified. Compounds are identified through comparing spectrum to a library database of compound spectrum in a connected computer.The GC-MS quantifies molecules in the mixture through comparing their relative concentrations among the atomic masses in the generated spectrum.The advent of the gas chromatograph mass spectrometer (GC-MS) has revolutionize analysis and identification of compounds in essential oils (Francisco *et al*, 2008). Gas chromatography (GC) has a great impact on essentialoil industry. Before chromatography, it took many days to undertake an analysis. Compound structures and identities could only, superficially be identified; using fractional distillation where pure compounds could not be isolated and thus were contaminated with other constituents in the fraction. Gas chromatography-Mass spectrometry (GC-MS) added further improvements to essential oil analysis where broad categories of monoterpenes and varying isomers closely resembling each other in terms of chemical structure could be, individually, identified. The GC-MS allows recognition of overlapping peaks on a GC and, separately, identify constituents (Murray, 2001).

Gas chromatography and gas chromatography-Mass spectrometry (GC and GC-MS) analyses were carried out using an Agilent 6890N gas chromatograph equipped with a flame ionization detector (FID) and coupled to a quadrupole Agilent 5973 network mass selective detector working in electron impact mode at 70 eV (scanning over 35–350 amu range). The gas chromatograph was equipped with two HP-1 fused silica capillary columns (PDMS, 50 m x 0.2 mm i.d., film thickness: 0.33 μm). The analytical parameters were the following: the carrier gas was helium at a flow rate of 1 mL/min. The oven temperature was programmed from 60 to 250°C at 2°C/min and held isothermal for 40°C for 1 min. The injector (split mode, ratio 1/100) temperature was 250°C. The FID temperature was set at 250°C, and in the GC-MS analyses, the temperatures of the ion source and transfer line were 170 and 280°C, respectively. To remove some co-elutions, another series of analysis were performed with the same analytical parameters as above, but with a HP-5 capillary column. In both cases, retention indices (RI) were determined from the retention times of a series of n-alkanes with linear interpolation. The constituents of the essential oil were identified by comparison of their mass spectral pattern and RI with those of pure compounds registered in commercial libraries and literature data and with a laboratory-made database built from authentic compounds. Quantitative data were obtained by internal standardization using Nonane as an internal standard. For a given compound, its relative response factor (RRF) was predicted according to its chemical class (Akono Ntonga *et al,* 2014).

1.4. Chemical Properties of Essential Oils:

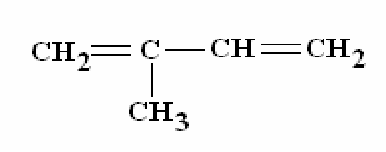
Pure essential oils are mixtures of terpenes or phenylpropanic derivatives, in which the chemical structural differences between compounds are minimal. They can be, essentially, classified into two groups:

1.4.1. Volatile fraction:

Essential oil constituting of 90–95% of the oil in weight, containing the terpenes hydrocarbons, as well as their oxygenated derivatives and other sulpher i.e. (4-mercapto-4-methyl-2-pentanone, allyl sulfide) and nitrogene-compounds i.e.,(Indole,Methyl anthranilate).

1.4.1.1. Hydrocarbon:

Essential oils consist of chemical compounds that have hydrogen and carbon as their building blocks. Basic Hydrocarbon found in plants is isoprene unit (Figure 1.2).



$CH_2{=}C(CH_3){-}CH{=}CH_2$

Figure 1.2: Structure of Isoprene Unit

1.4.1.1.1Terpenoids:

Terpenes have names, generally, ending in “ene” terpenes they are analgesic, bactericidal, expectorant, and stimulant, cleaning solvents, paint stripper and botanical insecticide,and anti-inflammatory, antiseptic, antiviral, and anti-allergic, used as an intermediate. Terpenes can be further categorized in monoterpenes, sesquiterpenes and diterpenes. Referring back to isoprene units under the hydrocarbon heading, when of one these isoprene units join head to tail the result is a terpene (Figure 1.3). Isoprene unit quickly react to air and heat and consequently lack stability and long shelf life as they are quickly oxidized (Abdelouaheb, 2012).

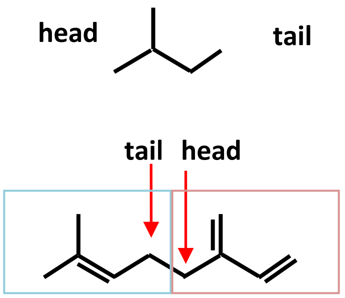


Figure 1.3: Terpene

1.4.1.1. A. Monoterpenes [C_{10} H_{16}]

The branched-chain C_{10} hydrocarbons are composed of two isoprene units connected to form either linear derivatives (Geraniol, Citronellol) or cyclic molecules i.e., menthol (monocyclic); camphor (bicyclic); Pinenes (α, β) in black pepper, limonene. Monoterpenes are formed through the mevanolate pathway by the conversion of isopentenyl pyrophosphate (IPP) with dimethylallyl pyrophosphate and combine to form geranyl pyrophosphate (Figure1.4), (a precursor of monoterpenes). For example, monoterpene hydrocarbon in some citrus oils, are synthesized from geranyl pyrophosphate through the terpenoid pathway in the plant (Figure.1.5).

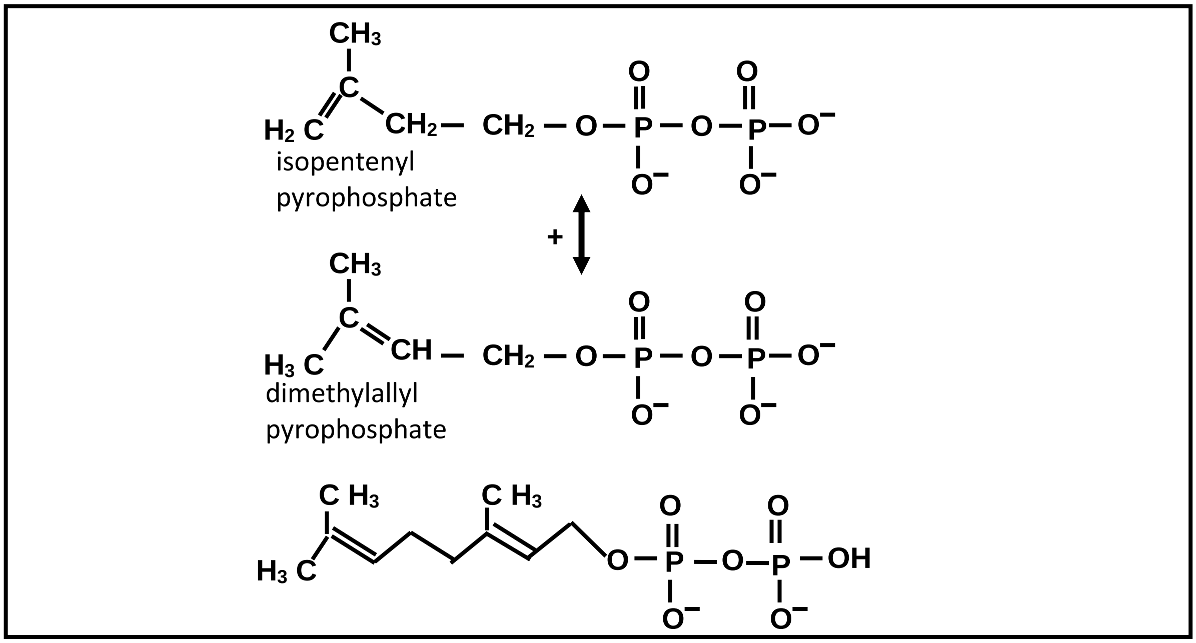


Figure1.4: Formation of geranyl pyrophosphate.

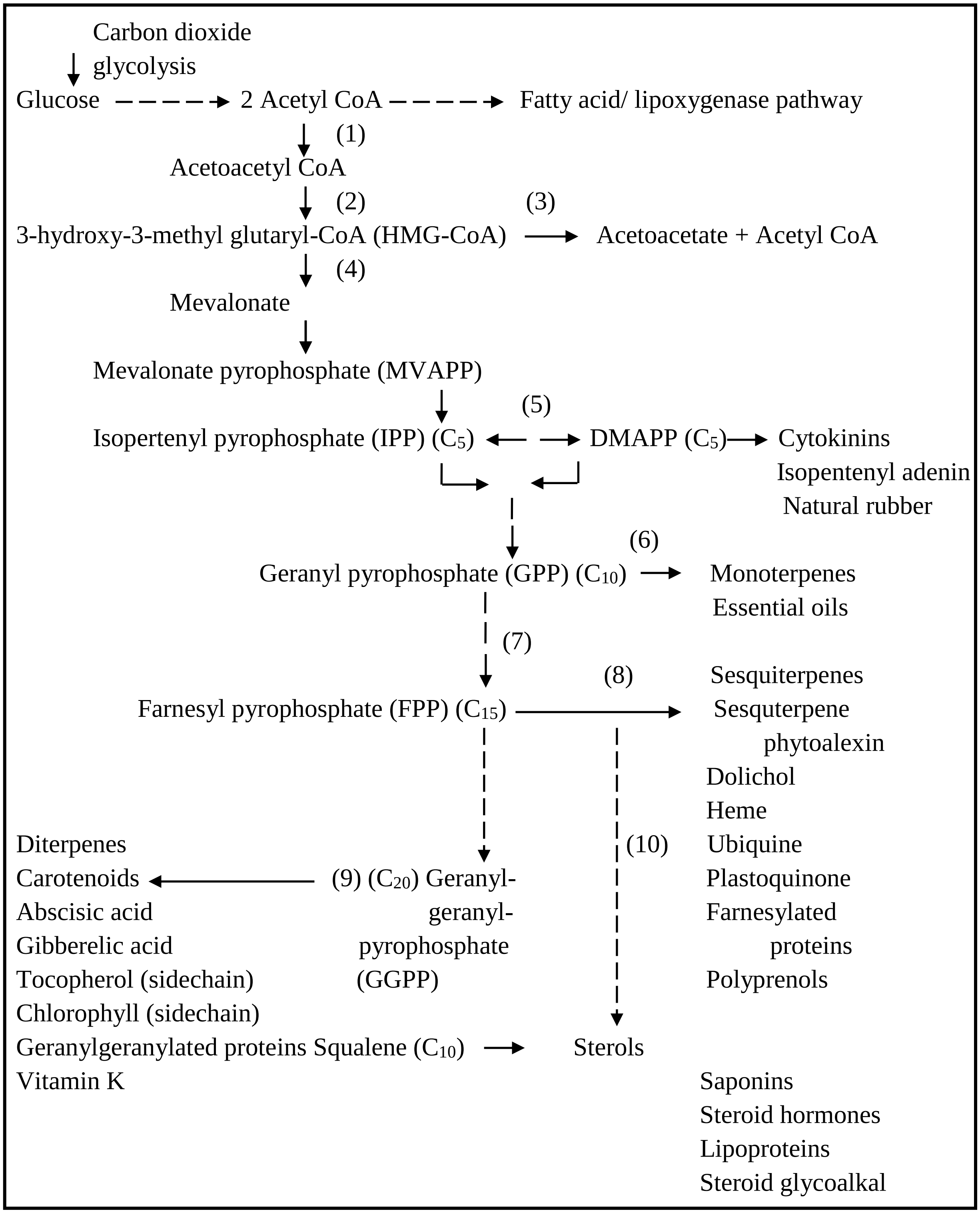


Figure.1.5: Mevalonate pathways in plant.

1.4.1.1. B. Sesquiterpenes:

Sesquiterpenes consist of C_{15} carbon atoms or three isoprene units linked to each other, head to tail. Sesquiterpenes are synthesised from farnesyl pyrophosphate which is condensed from geranylpyrophosphate, derived from isoprenyl diphosphate (IPP) and dimethylallyl diphosphate $(CH_3)_2C=CHCH_2OPP$-(DMAPP), along the mevalonate pathway. For example: Caryophyllene, Camphene

1.4.1.2. Oxygenated Compounds:

Through further oxidative transformations, a number of other terpene compounds are formed. Oxygenated compounds contain oxygen molecules within their structures. These include alcohols, aldehydes, amides, carboxylic acids, esters, ketones, nitro-compounds and oxides. Their Properties include: anti-septic, anti-viral, bactericidal and germicidal.

-Alcohols (Menthol, bisabolol, Terpineol, Terpinen-4-ol, Cineole, Eucalyptol, Citronellol, Linalool)

-Phenols (Timol, Thymol, Eugenol, Safrole, Para-Cresyl, methyl Ether, carvacrol).

-Aldehydes (geranial, citral) and a cetones (camphor, thuyone).

-Esthers (bornile acetate, linalile acetate, methyl salicilate),

-Ethers (1.8 cineol) and peroxides (ascaridol) (Murray, 2001).

1.4.2. Nonvolatile residue: that comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids (Ester *et al*, 2012).

1.5. Physicochemical Characteristics of Essential oils:

Physicochemical properties of oil like colour, odour, density, refractive index, optical rotation, viscosity, specific gravity, acid value, iodine value, saponification value, etc, directly, influence the quality of essential oil. The commercial importance of oils mostly depends on these physio-chemical properties, which provide baseline data to determine its suitability for consumption. Physicochemical characteristics provide a baseline for suitability of oils.

Colour: Volatile oils differ in degree of colour after distillation.

Odour: Most of volatile oils have fragrant smell, due to that they contain some compounds of small molecular weights, and are volatile in normal temperature and atmosphere (oxygenated compounds).

Density: Its density is lesser than water, density of volatile oils range from (0.8 – 1.1gcm^{-3}), which is due to terpeniod and aliphatic compounds composition. They are soluble in organic solvents, such as ether, chloroform, Petroleum ether alcohol. They are, also, lipo-soluble and not very soluble in water, but can be dragged using steam

Optical rotation: It reveals the type of extracted oil, naturally or artificialy by determining the compounds of oil.

The refractive index: is the degree of the deflection of a beam of light that occurs when it passes from one transparent medium to the other. It increases with the length of chains and with the number of carbon atoms present. Therefore, the refractive index determines evidences that the sample might be unsaturated long carbon chain. The range of refractive index for volatile oils is (1.45 – 1.69)

Viscosity: Is a measure of resistance of a fluid to deform under shear stress. It is commonly perceived as thickness, or resistance to pouring.

Acid value: The acid value is the number of milligrammes of KOH required to neutralize the free fatty acid present in 1g of fat.

Acid value is an important physicochemical property index of oil which is used to determine the quality, age, edibility and suitability of oil for industrial use such as paint. This value is used to measure the extent of glycerides in the oil, which have been decomposed by lipase and other physical factors such as light and heat. Number of acidity differ according to aromatic oil produced from different parts of plant it is ranged between (0.3 – 3). If acid value has increased, it would be due to molecular analysis process for oil components during water distillation.

The iodine value: The iodine number is the number of grammes of iodine taken up by 100g of fat (plummed, 1978). Iodine value is a useful tool in predicting the drying properties of oils. High iodine value of oils indicate high degree of unsaturation bond and suggests that the oils may be used as drying agent for the manufacturing of oil paints, varnishes, cosmetics and also as cooking oil manufacturing index. The iodine value is also an index of assessing the ability of oil to go rancid. It is also used for determining the level of oxidation.

Saponification value: Saponification value is the number of milligrammes of KOH required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of fat. The saponification value gives an indication of the narture of the fatty acid in the oil since the longer the carbon chain the less acid is liberated per gramme of fat hydrolysed. Saponification value is an index of average molecular mass of various fatty acids in oil samples. The lower value of saponification means molecular weight of fatty acids is low and has lower limit of use in industry. The saponification value suggests the use of oil in production of liquid soap, shampoos and lather shaving creams. Physicochemical characteristics provide a base line for suitability of oils. Volatile oils are charaterisd by low saponification value (Plummed, 1978).

1.6 Ues of Essential Oils:

Essential oils are used in pharmaceutical and food industries and perfumery, etc. They are used in flavours and fragrance preparations (Ladan, 2011). Essential oils are used in veterinary products, deodorants industry (Abdelouaheb, 2012). Traditionally employed in the manufacture of foodstuffs, cosmetics, cleaning products, and have been used for therapeutic properties, according to the composition of the oil (Zakiah et al, 2013).

1.6.1. Pharmaceutical Industry:

Essential oil constituents are used as feed-stock or precursors utilized in the production of modern prescription drugs. Several of these oils have been used in traditional medicine since ancient times as digestives, diuretics, expectorants, sedatives, neurotoxic, decongestant, antitumoral (Amir *et al,* 2011) anti-rheumatic, cholagogue, and antiepileptic, antioxidant, antiviral, antibacterial, stimulators of central nervous system (Koech *et al*, 2013). It is now broadly accepted that certain class of plant-based compounds such as dietary fiber, phenolic acids, flavonoids, vitamins, and neuro pharmacological agents play preventive role against the incidence of some common diseases like cardiovascular (Wang, 2008). Essential oils are used in cure of coughs, wounds Anti-emetic Effect (Barbara, 2010), and other skin ailment, commonly, used as anti-inflammatory anti-malarial (Montasir *et al*, 2013)

1.6.2 Cosmetics Industry:

This industry uses essential oils to make cosmetics, the use of essential oils in hair care perfumes and oral care. Skin care products help protect the skin from the damage that UV causes (anti-aging) and help protect the environmental effects (barrier products) (Murray, 2001).

1.6.3 Biocides and Insecticides:

Many plant essential oils show a broad spectrum of activity against pest insects, antifeedant, repellent, oviposition deterrent, growth regulatory and antivector activities (Opender *et al*, 2008). Essential oil works in aromatherapy (Maria, 2006). Essential oils are use as intermediates in the production of other compounds (Murray, 2001).

1.7 Methods for Using Essential oils:

An essential oil is inhaled directly or absorbed by the olfactory system to the limbic system of the brain. Thus the brain responds to the particular scent affecting our emotions and chemical balance.

Essential oils are also absorbed by the skin and carried throughout the body via the circulatory system to reach all internal organs. The action of essential oils begins by entering the human body via three possible different ways: (inhalation, topical application, and ingestion). Once essential oil molecules enter the body, they interrelate with physiological functions by three distinct modes of action:

- Biochemical (pharmacological).
- Physiological: by acting on specific physiological function for example (Phytohormone).
- Psychological: By inhalation (Abdelouaheb, 2012).

1.8 Toxicity of Essential Oils:

The toxicity of essential oils can also be, entirely, different to that of herbs, because of their ability to pass across membranes, very efficiently, due to their lipophilicity. And possible dangers of using essential oils internally and externally in large doses, any biotransformation in the body is affected by, individual, enzymes, but the activity of enzymes in the skin is much lower than in the liver, and the large surface area of the skin makes it a significant, detoxification process. Thus Safety and consumer assurance is an important issue (Maria, 2006). All essential oils should be sold in brown bottles or platinum containers; and stored in a cool, dark place. Do not expose the bottles to light or air for long periods, to prevent oxidation of essential oils components as this may make themmoretoxic. Storage areas must be out of reach for children because of their age of the person influenced by many essential oils. Some essential oils are not, orally, used, or external application or inhaled directly from the bottle or tissue.this is in fact their least effective mode of entry because it involves their passage through the digestive system (Jolene, 2012). thus Many, oils already, contain added anti-oxidants, but one can add vitamin E enzymes, amino acids and other natural herb extracts i.e. gum Arabic Production under conditions (squeezed from capsules) to the essential oils as a safe and efficient antioxidant; it also supposedly helps the skin to remain healthy (Maria, 2006).

1.9 Taxonomy and Botanical description of *Ocimum basilicum*:

Scientific Classification	
Kingdom	*Plantae*
Order	*Lamiales*
Family	*Lamiaceae*
Genus	*Ocimum*
Species	*O. basilicum*
Synonym	*O.lanceolatum*

Vern Names: (Arabic) Rehan (Engl) basil, Sweet or Garden basil

Ocimum basilicum L: It is a an oily evergreen plant, the stem is straight branched of the base its height (50-200 cm) its length may exceed three feet, leaves rounded on the edges hard and shiny feathery oval in shape look like a spear and the length range (1-3 cm) width (0.5 -1.5 cm) with opposite and petiolat; laminas ovate, 3-5.5x 1.5-2 cm. The flower holder grow from the armpit of the leaf and it is equal in length of the leaf or a little shorter, the flowers are small, whitish to lightly pinkish in colour with diameter of 2 cm, 6 mm across, with corollas twice as long as calyces. The fruits nutlet; rounded and in a ball shape looks like the bilberry with a good taste and very good odour , seeds ovate-globose, are many and small in size kidney shaped brown in colour(Gamal *et al*, 1997). *Ocimum basilicum L* is a green plant, some varieties, such as 'Purple Delight' has stem and furit that appears purple or green stem with white furit (Figure.1.6)

Figure.1.6: *Ocimum basilicum* Purple & green

1.9.1 Botanical Classifiction of Basil (*Ocimum basilicum L.*):

Belongs to the genus *Ocimum;* the word basil derived from the Greek oza which means strong odours (Hanan *et al*, 2010). Basileus is still considered the "king of herbs" by many cookery authors, recognized more than 150 species of *Lamiaceae* (Mohamed *et al*, 2015); however, Paton proposed that *Ocimum* had only 65 species and other attributions should be considered as synonyms. Among the species of the genus, *Ocimum basilicum L. (*basil) is the major essential oil crop world wide These species are highly aromatic and economically important(Salah *et al*,2009), 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. X citriodorum* vis. were identified using their seed characteristics, difference in shapes, size, colour, texture (Patel *et al*, 2015).

The aromatic oil of this species possesses a characteristic pleasant aroma with an appreciable note of cloves and sometimes licorice (Sims *et al*, 2014). The type used in Italian food is called sweet basil, as opposed to “Thai basil″ *O. basilicum* var. *thyrsiflora*. (Montasir *et al*, 2013). *Ocimum basilicum Lamiaceae* is commonly known as mint (Gabi *et al*, 2012). The species of *O.basilicum* is the most cultivated in Algeria is well known as "hbek" (Mario, 2012).

1.9.2 Planting of Basil (*Ocimum basilicum L.*):

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

1.9.3 Origin and Distribution of *Ocimum basilicum L*:

Ocimum basilicum L. is an annual and popular culinary herb belonging to the *Lamiaceae* family which grows in several regions all over the world (Ismail, 2006). Basil has a characteristic odour and sharp taste. The plant probably originated in India, Afghanistan, Pakistan, Northern India and Iran (Politeo *et al*, 2007).The genus *Ocimum* is distributed in regions of Asia, Africa and South America and all types are called Rihan in Sudan (Abduelrahman *et al*, 2009) and characterized by a great variability in its morphology and chemotype differences (Juliana *et al*, 2009). Basil is grown commercially all over Mediterranean region and in California (Özcan, 2002).

There are many cultivars of basil which vary in their leaf color (green or purple), flower color white, red, purple and aroma. But other species are also grown and there are many hybrids between species (Sims *et al*, 2014). Basil has many cultivars, often named by the type of aroma they emit. Basils may look almost identical but have distinct aromas, the plant part harvested depends upon projected use.

1.9.4 Chemical Components of *Ocimum basilicum L*:

Sudanese basils have several ornamental types containing a strong-scented essential oil composed primarily of chemical compounds such as eugenol, thymol and estragole. (Abduelrahman *et al*, 2009). The strong clove scent of sweet basil is derived from eugenol (Phyllis, 2012). *Ocimum basilicum L* the plant is rich with secondary metabolism components formed by aromatic plants eg alkaloids, glycosides, tannins, resins, saponines, flavones, phenols, terpens, steroids (Gabi Baba, 2012), essential oils of basil plant are characterised with strong aroma. The high economic value of basil oil is due to the presence of phenyl propanoids (Salah *et al*, 2009).

The main constituents of the essential oil of basil are produced by two different biochemical pathways, the phenylpropanoids (methyl chavicol, eugenol, methyleugenol and methyl cinnamate) by the shikimic acid pathway, and the terpenes (linalool and geraniol) by the mevalonic acid pathway, and they are usually obtained by steam or hydro-distillation methods (Özcan, 2002). The flavor, smell and bioactive properties of basil varieties are, largely, determined by their chemical components basil varieties contain the following oils in varying quantities: cinnamate, citronellol, geraniol, trans-α-bergamotene, 1,8-cinol (Seyed, 2006). Other chemicals that help to produce the distinctive scents of many basils, depending on their proportion in each specific breed, include: limonene, methyl chavicol, myrcene, ocimene (Hanan, 2010) and camphene fenchylic alcohol, borneol (Wierdak *et al*, 2013), geranyl acetate, nerol, terpinen-4-ol, octan-3-yl-acetate (Özcan, 2002), Carvon, E-methylcinnamate, β-Sebinene, camphor (Mohamed *et al*, 2015). β-caryophyllene, α-pinene, β-Pinene, α-phellandrene, Undec-4-ene, α–terpineol, chavicol methyether or stragol(Politeo, 2007) elemol , myrcene and neryl acetate(Hadj *et al*, 2012) linalyl acetate, geranyl acetate, allo-ocimen β-ocimene (Mario, 2012). Linalool, epi- α-cadinol, α-bergamotene cadinene (Hussain *et al*, 2008) gerianol (Nour *et al*, 2009), α-cubebeneas (Ismail *et al*, 2006) other constituents present in a significant quantity include: acetic acid, aspartic acid, beta-sitosterol, caffeic acid, caryophyllene, mucilage, oleanolic acid, p-methoxy cinnamialdehyde, phytosterols, rosmarinic acid, thymol and ursolic acid, linolen (Jolene, 2012).

The fragrance and chemical composition and quality of essential oil is effect by several factors (Sims *et al*, 2014), including genotype, environment, developmental stage and cultural factors (Mawada *et al*, 2005). According to the chemical composition and geographical origin, *Ocimum basilicum* types are classified into three groups:

European type, Exotic or Reunion type and African type (Abduelrahman *et al*, 2009).

The environmental factors, including day length, light intensity and ambient temperature influence on the quantity and quality of the essential oil content and, eventually, will affect the medicinal properties of the plant. It has been shown that in the family *Lamiaceae*, the day length increases the content of their extractions, and the duration of day light augments the quality of their active materials (Amir *et al*, 2011).

The Chemical composition, antioxidant and antimicrobial activities of essential oils from aerial parts of basil (*Ocimum basilicum L.*) are affected by the four seasons. The hydro-distilled essential oils content ranged from 0.5% to 0.8%, the maximum amounts were observed in winter while minimum in summer. The essential oils consisted of linalool as the most abundant component, followed by epi-α-cadinol, α-bergamotene (Hussain *et al*, 2008). The chemical composition of *O.basilicum L.* essential oil depends on chemotype, leaf and flower colors, aroma and origin of plants, and also numerous subtypes.

The highest value of phenolics, and flavonoids glycosides and antioxidant activity, anticancer activities were obtained in basil plants in the presence of bio-fertilizer (Hanan *et al*, 2010). The distillation method had a significant effect on the content of essential oil constituents and chemical composition of the essential oils (Zakiah *et al*, 2013). Higher amounts of essential oil constituents were found by hydro-distillating in comparison with water-steam distillating method (Zheljazkov *et al*, 2008). The influence of γ- and β-irradiation and storage time on the composition of volatile compounds in basil (Rimantas, 1996).

1.9.5 Culinary Uses of (*O. basilicum L.*):

Essential oils are widely used in pharmaceutical, cosmetic, and aromatherapy industries (Bhuwan, 2013). Basil (*Ocimum basilicum L)* is used as anti-malaria by Southern Sudanese, or as an insecticide in Western Sudan. The essential oils and oleo-resins may be extracted from leaves and flowers and used for flavoring in liqueurs and for fragrance in perfumes and soaps. For fresh market production, selected basil with good flavour and attractive, dark green or purple foliage is used. Scented basils, such as lemon, licorice and cinnamon basil are used fresh or dried in potpourri, jellies, honeys, vinegars and baked (Abduelrahman *et al*, 2009). Basil 'cinnamon' is commonly used in hot drinks, raw dishes, fruit salad. Due to its distinctive cinnamon taste and aroma, basil is considered a sweet herb with cooling and refreshing properties. The herb is also used as with vegetables forfish, sauces, soups, desserts, pickles, pizza, spagetti sauce, egg, cheese dishes, tomate juice as aflavouring agent (Juliana, 2009) confectionery, stews, dressing, and mixed drinks herbal. Its other main ingredients are olive oil, garlic, and pine nuts, and steeped in cream or milk to create an interesting flavour in ice cream or chocolates (Jolene, 2012).

1.9.6 Potential Health Effects and Medicinal Uses of Essential oils:

Essential oils from the *Lamiaceae* plants are rich in oxygenated terpenes and known to possess therapeutic properties (Wang, 2008). Recently, basil was shown to rank highest among species and herbal crops for phenolic compounds, essential oils from fresh or dry leaf, and seed which are associated with aging diseases (Hanan, 2010). Essential oils of basil have aromatic characteristics, and other non-volatile components, such as rosmarinic acid, has been used currently as a medicinal herb for a myriad of ailments and diseases (Sims *et al*, 2014), carcinogen and funigants, the plants are used for stomach ache, vomiting, and smooth muscle relaxant. The dried leaves have a sigificant anti-gardial, antimalarial, antioxidant, antiseptic activities and cytotoxicity (Jolene, 2012). Basil oil creams were prepared with light liquid paraffin and evaluated for in vitro antimicrobial activity, and were found to be consistent over 90 days (Jaya, 2013).

Some reports showed that *Ocimum* anti-depressant, stimulates the adrenal cortex, carminative, expectorant, sooth itching, reduce blood sugar levels, lowers blood pressure (Murray, 2001) and treatment of headaches (Gabi Baba, 2012), cough, diarrhea, constipation, warts, worms and kidney malfunctions (Hadj *et al*, 2012). Basil oil species are used as anti-carminative, tonic, and digestive (Politeo, 2007), also basil tea taken hot is good for treating nausea, flatulance and dysentery, its oil has been found to be beneficial for the alleviation of mental fatigue, colds, spasms,and treatment for wasp stings and snake, treat central nervous system (CNS) (Juliana *et al*, 2009). In traditional medicine the oil is used to relieve respiratory problems (Montasir *et al*, 2013), diarrhea, pneumonia, fever and cholera (Gabi Baba, 2012).

Externally, they are used to treat acne (Seyed, 2006). When adding basil oil to a warm bath it can help relieve gout and arthritis, as well as muscular and menstrual pains, basil oil it is used for skin care and hair care (Murray, 2001). Seed oils contains,fatty acid,lipid profile, Palmitic, stearic, oleic and linolenic acid, desirable for the potential industrial use of the oil as a drying oil (Salah *et al*, 2009). When the seeds are soaked in water, become gelatinous, and used as demulcents drink (Jolene, 2012). The root is used in the treatment of bowel complaints in children as a sedative for children and for its anticonvulsant activities (Juliana *et al*, 2009).

1.9.7 *Ocimum basilicum* is used as:

- **Anti-inflammatory:** the fixed oil and linolenic acid were tested in vivo rats and animal models with experimentally-induced gastric ulceration.
- **Activity:** mechanism seems to involve linolenic acid’s ability to block cyclooxygenase and lipoxygenase inhibiting pathways of arachidonate metabolism and histamine antagonistic effects.
- **Antiulcer:** Aqueous extract of aerial parts in vivo; rat.
- **Activity:** is comparable to Ranitidina (standard drug); prevented ulcerogenesis; administered intragastrically inhibited secretion of peptic-acid induced by aspirin; potently neutralized secretion of acid in the stomach
- **Ear infection treatment:** Essential oil and active constituents (thymol, carvacrol and salicylaldehyde) applied to ear canal, in vivo: rat’s experimentally-induced acute otitis media caused by pneumococci or Haemophilus influenza.
- **Activity:** healed 58% – 81% of animals infected with H. Influenzae and 6%-75% of those with pneumococci (by comparison, only 5.6%-6% of placebo group were cured); recommended as effective treatment.

- Glutathione S-transferase inhibition: Essential oil; dosage: 30 mg/to animal. In vivo: mouse.
- **Activity:** Effective in inhibiting enzyme transfer in the small intestine and liver, but not in the stomach.
- **Hypolipidemic and antioxidant:** Aqueous plant extract (0.5 g/100 g body weight) In vivo: rats with experimentally-induced hyperlipidemia.
- **Activity:** lowered plasma cholesterol (50%), triglycerides (83%) & LDL-cholesterol (79%); higher HDL-cholesterol (129%); stronger effect than fenofibrate; showed very high antioxidant activity (Jolene, 2012). The antioxidant activity of basil oil play role in reducing the risk of incidence of cardiovascular diseases as well as other chronic diseases (Hadj *et al*, 2012).

Basil essential oils have larvicidal activity towards anopheles larvae for about 9 hour (Azhari *et al*, 2009). Activity of basil essential oils (linalool), the most abundant component, against bacterial strains (Adam *et al*, 2015) i.e *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pasteurella multocida* and essential oil in vitro Antifungal against or pathogenic fungi *Aspergillus niger, Mucor mucedo, Fusarium solani, Botryodiplodia theobromae, Rhizopus solani* (Hussain *et al*, 2008) *aegyptiacus, Penicillium cyclopium, Trichoderma viride and Trichophyton mentagrophytes* (Jolene, 2012), antimicrobial (Politeo, 2007).

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 have toxicity effect, such as phenyl propanoids, or terpenoids (Hanan, 2010), linalool enantiomers as the main active agent responsible for antimalarial and antimicrobial activity against human pathogenic bacteria and fungi (Temel Özek, 2010). Benzene derivatives are generally more toxic and repellent to insects. Physiological,or chemoecological 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 (Phyllis, 2012). Estragole and safrole have shown mutagenic and carcinogenic effects in rats and cats (Jolene, 2012).

1.10 Mosquito Repellents:

Some diseases contracted by mosquitoes can be life threatening, such as malaria, yellow fever, dengue fever, filariasis, dengue, haemorrhagic fever and Japanese (Pin Yang, 2005).Anopheles mosquito is the major vector of malaria disease in Sudan (Mashitah *et al*, 2009) and two species of mosquito responsible malaria in Sudan (Gabi Baba, 2012). To control mosquito, any type of control should involve careful consideration of the biology of the mosquitoes and be based on scientific surveillance. A response to control mosquitoes may look very different from a response to control disease-vectoring mosquitoes (Nour *et al*, 2012). Disease prevention via prophylactic drugs and vaccines, the prevention and personal protection of mosquito bite using a mosquito repellent is a substance applied to skin (Koech, 2014). Over centuries, malaria is considered to be one of the long-standing public health issues in many of the tropical and subtropical regions of the world particularly in sub-Saharan Africa. Therefore, in the resource-poor settings, people are using various repellent plants to drive-away blood-sucking insects mainly mosquitoes to minimize the nuisance and disease transmission. The following factors have largely enabled them to apply repellent plants;

(i) Use of plant-based insect repellents is strongly blended with their tradition/culture,
(ii) Lack of accessibility and affordability to procure modern personal protective devices,
(iii) Easy accessibility and affordability of local repellent plants,
(iv) Non-availability of simple and cheap method of personal protection measures, when other vector control interventions are impossible and impracticable It is important to note that since time immemorial basil type has a rich tradition and practice of insect repellent plant usage to drive away insects particularly mosquitoes from their houses in the early evening hours (Apiwat *el at*, 2001).

1.10.1 Classification of Mosquito Repellents:

(i) Physical method.

(ii) Biological methods: by growing some fish species that feed on mosquito larvae in water bodies (Gupta, 2012).

(iii) Chemical methods: these are classified into:

Synthetic repellents i.e. N, N-diethyl-3-methylbenzamide (DEET), Permethrin, are chemical mosquito repellents which have remarkable safety profile, but in the mean time, thay have toxic effects that include uncomfortable sticky skin, damage of plastics, synthetic clothes or rubber (Gabi Baba, 2012).They have toxicity against nervous system like rashes, swelling, eye irritation, low blood pressure. DEET must be used with caution, especially with children. It has been known to cause dizziness. DEET may even cause cancer and defect in child birth. Another limitation is that it may be easily removed by perspiration and its efficacy decreases dramatically with rising out door temperatures (Koech *et al, 2*013) for disadvantages of synthetic repellents; most people choose natural repellents (Pin Yang, 2005), though they have advantages include: Synthetic repellents contain, (DEET) or picaridin are more effective than repellents with "natural" active ingredients. All synthetics gave almost all repellency for the first 2 hour, whereas some natural repellent products were most effective less than 2h, and required reapplication to be effective overseveral hours (Gupta, 2012).

Natural repellents: To avoid these adverse affects laboratories should try to replace DEET with repellents that are derived from plant extracts. A collagen membrane technique, was used to compare repellents against Aedes aegypti mosquitoes. Repellency was defined in terms of inhibition of probing after 5 min exposure on five male volunteers. Four repellent products were compared with technical DEET as the standard. DEET formulations required less active compound than citronellal for the same degree of repellency (Cosgrove, 1998). Development of natural substances as successful pests and vector control agents can also be economically feasible, especially if the sources of materials are abundant plants (Mashitah *et al*, 2009).

Natural repellents have several advantages and disadvantages:

Advantages:

- Non-sticky; non-toxic and environmentally friendly; safer on sensitive skins and some can be used on children as young as 3 months; reduced irritation; harmless to most plastics and fabrics (Jaya, 2013).

Disadvantages:

- Some types of essential oils repellents can be short-lived in their effectiveness, since they can completely evaporate and need frequent re-application.

-Some types of essential oils cannot be applied directly on the skin; if applied, they can cause rashes on skin (Gupta, 2012).

1.10.2. Preparations 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 application or to ensure long lasting effect. The following are the chief forms such preparations take. For a material to be valuable as a mosquito 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, taste and feel, it should also be harmless to clothing, and have a relatively low cost, effective against insects (Gabi Baba, 2012).

1.10.3 Type of Repellants:

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 etc.

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).

Gum Arabic preparations: Various creams or pastes of gum Arabic have been employed as vehicles more especially for pyrethrum. They dry leaving a thin adherent film which is not dislodged by sweating. Such preparations would be unsuitable for repellents of these preparations creams of the ointment type have frequently been noted as greasy and unpleasant in a hot climate though some are cosmetically excellent. The use of paraffin as a base has been common in basil, citronella preparations appears to have a reducing effect on repellency. Vanishing creams have not generally been found satisfactory. Owing to their 'disappearing' property, unless used in large amount, they are vulnerable to give patchy distribution of the repellent. A waxy cream would seem to be the most effective in prolonging repellent effect and if of suitable consistence such creams spread extremely well and are pleasant to use (Gupta *et al*, 2012).

Safety Measures to be taken with insect repellent when used for children or pregnant women:

- Children may be at greater risk for adverse reactions to repellants and should be kept out of their reach.
- Use only small amounts of repellent are used for children.
- Repellants shouldnot be applied to the hands of young children because this may result in accidental eye contact or ingestion.
- Try to reduce the use of repellents by dressing children in long sleeves and long pants. Use netting over strollers, playpens, etc. as with chemical exposures in general, pregnant women should take care to avoid exposures to repellants when applying them, as the fetus may be vulnerable (Jolene, 2012).

1.11 Natural Gums:

The term “gum” is used to describe a group of, naturally occurring, polysaccharides and/or proteins originating from different sources i.e. animal, plant and microbial. Many trees and shrubs are known to produce an aqueous thick exudation when the plant bark is injured. The solid exudation is, commonly, known as natural gum. Gums are found in large number of families Notable among them is Sterculiaceae and Leguminosae, which include Gums Arabic (Sunil, 2014).

1.11.1 Difintion of Gum Arabic:

Gum arabic occurs as a sticky liquid that oozes from the stems and branches of *Acacia senegal* and *Acacia seyal*, which is rich in non-viscous soluble fiber. Usually produced under stress conditions such as drought, poor soil fertility, and injury, the liquid dries in to form glassy nodules, which are hand picked by local farmers, the crude exudate of GA is processed by spray drying and roller drying (Mariana *et al,* 2012). In Sudan, the term 'gum Arabic' is used in a wider context to include two types of gum which are clearly separated in both national statistics and trade: "hashab" (*Acacia senegal*) and "talha" (*Acacia seyal*). Gum hashab, which accounts for about 90% of total production it is also known as Kordofan gum.this species of tree is about 15-20ft tall and has a life-span of 25-30 years (Murwan *et al*, 2008).

All gum exudates, from this group of *Acacia* species, have a laevorotatory (–ve) specific rotation in contrast to *Acacia seyal* (Talha) complex which produce gum exudates, that have a dextrorotary (+ve) specific rotation, other structural, botanical characteristics are noticeable even within the same species. The resulting solution is colourless, tasteless and does not interact easily with other chemical compounds (Abul Gasim, 1995).

1.11.2 History and Origins of Gum Arabic:

Gum Arabic is, certainly, the most ancient and the best known of all gums. The term ‘gum Arabic’ was coined by European merchants who imported it from arabion ports such as Jeddah and Alexandria. Egyptians referred to it as ‘kami’ and allegedly used it from the third dynasty onwards to secure bandages around mummies. This gum was supposedly used to fix pigments into hieroglyphic paintings. There are close to 900 – 1000 *Acacia* species capable of producing gum. These are primarily, located in tropical climates, with about 130 of them located, specifically, on the African continent (Abul Gasim, 1995). 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. Interestingly, close to 80% of gum Arabic is produced by the *Acacia senegal*. The remainder is produced either by the *Acacia seyal (Acacia laeta),* with each species contributing 10% of the total supply of gum. The gum produced by *Acacia senegal* is commonly referred to as "hard gum" and the gum from *Acacia seya*l, as "flaky gum" (Eqbal, 2013). Gums obtained from other *Acacia* species and, occasionally, from Albizia and Combretum, are also traded as "gum Arabic". African Gum Arabic an essential world forest product Figure 1.7, produced in the Gum Arabic belt extends in the Seven region north of the Equator and south of the Sahara desert, extending from west to east continuously from Mauritania, Senegal and Mali, through Burkina Faso, Niger, northern parts of Nigeria and Chad to Sudan, Eritrea, Ethiopia and Somalia in the east and northern parts of Uganda and Kenya. Sudan is predominately an agricultural country, with over 90% of its exports supplied by the agricultural sector (Abul Gasim, 1995).

Figure.1.7: African Gum belt.

The gum Arabic belt covers an area of about 500 – 520,000 square kilometers (120 million feddans). The Belt is home to roughly one-fifth of the population of Sudan.It spans twelve states; Western, Northern and Southern Darfur, White Nile, Sennar, Blue Nile and Gadarif. The belt covers parts of the clay and the sand plains. The species is drought resistant and also frost hardly. It can regenerate naturally from the seed, which is calle Gifar or by vegetative growth, locally known as Genain (Sitana, 2003), an important part of Sudanese exports since Sudanese Gum Arabic is said to be the best available quality. About half or more of the world's best quality Gum Arabic production is produced in Kordfan states (Ghada *et al*, 2015) (Figure 1.8).

Figure.1.8: Sudan Gum Arabic belt.

1.11.3 Structure of Gum Arabic:

Arabic gum is a branched chain (Figure 1.9), complex polysaccharide obtained, either neutral or slightly acidic. Found as mixed calcium, magnesium, and potassium salt of a polysaccharide acid. The side chains backbone 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 α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl, and 4-O-methyl β-D-glucuronopyranosyl, and the latter two mostly as end-units. Asmaller fraction is a higher molecular weight (Ghada *et al*, 2015).The attached arabinogalactan in the complex ending eventually in residues ofrhamnose or glucorinic acid .The smallest fraction having the highest protein content is a glycoprotein which differs in its amino acids composition (Mariana *et al*,2012)

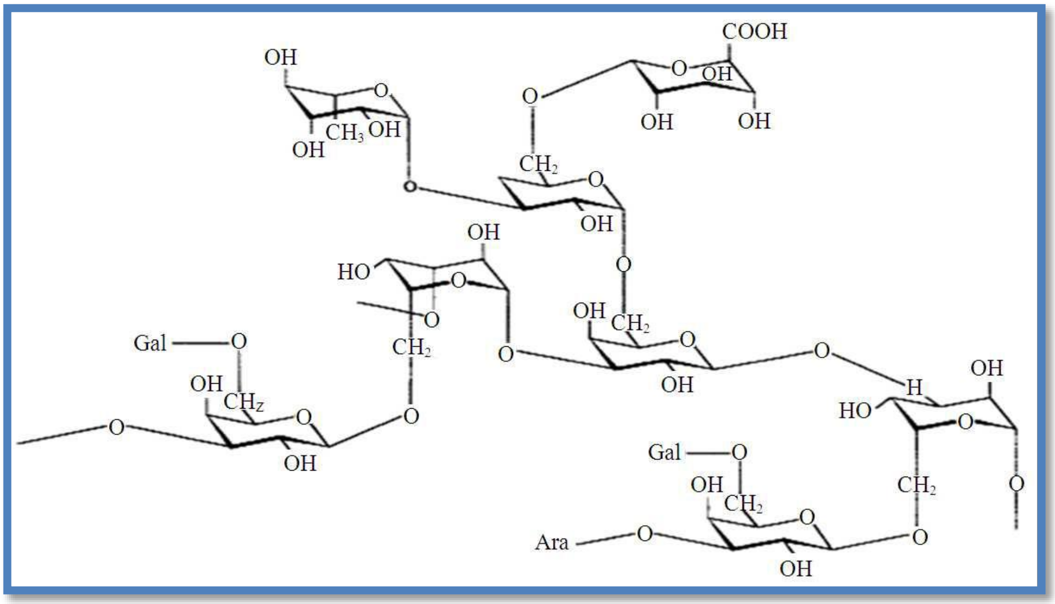


Figure 1.9: Structure of Gum Arabic

1.11.4. Chemical Composition:

Chemically, gum Arabic is a slightly acidic complex compound, made up of glycoprotein and polysaccharides and their calcium, magnesium and potassium salts. The principal polysaccharide is Arabic acid, a polysaccharide linking a D-galactose with branches composed of L-arabinose, L-rhamnose and D-glucuronic acid. The arabinogalactan, rich in hydroxproline and a low proportion of proteins, in additioncalcium, magnesium and potassium salt of a polysaccharidic acid and iron were found in only very minute quantities, other transition elements and heavy metals .The major amino-acids present in the protein of an ArabinoGalactan (AG) and an Arabinogalactan-Protein Complex (AGP) were hydroxyproline, serine and proline, whereas in GlycoProtein (GP), aspartic acid was the most abundant (Murwan *et al*, 2008). The biological functions of natural biopolymers from plant sources depend on chemical composition and molecular structure (Sunil, 2014).

1.11.5. Characteristics of Gum Arabic:

Gum Arabic of excellent quality is tear-shaped, round, with an orange-brown colour (Figure 1.10). After it is crushed or shattered, the spieces are paler in colour and have a vitreous appearance. Contrary to other vegetable gums, gum Arabic dissolves very well in water (up to50%). The viscosity of *Acacia Senegal* is weak. The resulting solution is colourless, tasteless and does not interact easily with other chemical compound (Mahasin, 1982).

Figure 1.10: Gum Arabic nodules

Therefore, there are some differences in chemical composition of GA from *Acacia senegal* and *Acacia seyal*. *Acacia seyal* gum contains a lower proportion of nitrogen, and has a+ve specific optical rotation (Michael. 2012). The two varieties of *Acacia* gum, *Acacia senegal* and *Acacia seyal*, are hyperbranched polysaccharides rich in arabinose and galactose, mainly formed by chains of 3, 6-linked β-D-Galp substituted in position 6 by side chains of 3-linked α-L-Araf. *Acacia senegal* presented the highest degree of branching (78.2% vs. 59.2%) with more branched galactopyranoses, shorter arabinosyl side branches, and more rhamnopyranoses in terminal position. Circular dichroism experiments evidenced that both *Acacia* gums were partially structured into polyproline type II helices but in a lesser extent for *Acacia seyal*. Analysis data suggested that macromolecules of both *Acacia* gums adopted an ellipsoid-like conformation in solution. The average molecular weight of *Acacia seyal* macromolecules are larger than those of *Acacia senegal* ones (8.2×10^5 g mol−1 vs. 6.8×10^5 g mol−1). Nevertheless, *Acacia seyal* macromolecules appeared more compact and less viscous (Rg: 17.1 nm; [η]: 16.5 mL g−1) than *Acacia senegal* ones (Rg: 30.8 nm; [η]: 22.8 mL g−1). The most compact structure of *Acacia seyal* gum can be, partly, explained by the lowest concentration of charged sugars that induces less electrostatic repulsion, and the highest content of long arabinose side chains that may self-organize and interact between them (i.e. hydrogen bonding, steric effect) etc. For both *Acacia* gums, the anisotropy of macromolecules increased with the molecular weight, however, *Acacia senegal* macromolecules were the most anisotropic ones(Lizeth *et al*, 2015). Gum from *Acacia s enegal* was reported to have high molecular weight average $2.2x10^5$- $4×10^5$ Mv (Taha *et al,* 2012).

1.11.6 Physicochemical Properties of Gum Arabic:

The physical properties of gum Arabic, established as quality parameters include moisture, total ash, volatile matter and internal energy. Gum Arabic is a natural product complex mixture of hydrophilic carbohydrate and hydrophobic protein components emulsifier which adsorbs onto surface of oil droplets while hydrophilic carbohydrate component inhibits flocculation and coalescence of molecules through electrostatic and steric repulsions in food additives.

1.11.6.1 Moisture content:

Moisture content facilitates the solubility of hydrophilic carbohydrates and hydrophobic proteins in gum Arabic.

1.11.6.2 Total ash content:

Total ash content is used to determine the critical levels of foreign matter, acid insoluble and metal content, Matter, salts of calcium, potassium and magnesium. The cationic compositions of ash content are used to determine the specific levels of heavy metals in gum Arabic (Murwan *et al,* 2008).

The properties of gum Arabic are affected by the tree species, climate conditions at the time of exudation, and the conditions of storage. The properties are reported by the Gum Arabic Company (Allaoua, 2012).

1.11.6.3 -Nitrogen and protein content:

Gum Arabic is a polymer with about 3% protein. The protein fraction is responsible for the emulsification properties of the gum. An adsorbed layer of protein at oil/water interface provides the primary stabilizing structure in many food colloids (Murwan *et al*, 2008). The variability in the emulsifying properties of gums from different *Acacia* species is dependent not only on the total protein but also on the distribution of the protein-peptide between the low and high molecular weight fractions and on the molecular accessibility of the protein peptide for absorption. The main content of gum Arabic is Arabian (acid substance), and when it was decomposed, it givesarabinose. Thus gum arabic is called Arabic acid, therefore, the gum solution is slightly acidic with pH 4.66 and the acidity of which is due to uronic acids in their structures (Yusuf, 2012).

1.11.6.4 .Specific optical Rotation:

The specific optical rotation is considered as the most important criterion of purity and identity of gum Arabic, so it is used to differentiate between *Acacia senegal* gum and other botanically related *Acacia* gums.The specific optical rotation of *Acacia senegal* gum ranges between –26 to –34 (Mahasin, 1982).

1.11.7 Functional Properties:

1.11.7.1 Solubility:

Gum Arabic has high water solubility, dissolves in water up to 50% w/v in a concentration, forming a fluid. Highly branched structure of Gum Arabic its solution is acidic (pH 4.5). The Gum is insoluble in organic solvents and in oils, with which they form emulsions in aqueous suspension. The good solubility of these gums is also indicative of the absence of cross-linking between polymeric chains. This is because gums having cross-linked polymeric chains only swell in water without dissolving. The molecules leads to compact relatively small hydrodynamic volume and consequently, GA will only give viscous solutions at high concentrations. Solutions containing less than 10% of GA have a low viscosity and respond to Newtonian behavior. However, steric interactions of the hydrated molecules increase viscosity in those solutions containing more than 30% at 300C of GA resulting in an increasingly pseudoplastic behavior (Mariana *et al*, 2012).

1.11.7.2. Viscosity:

Most gum other than gum Arabic form highly viscous solutions at low concentrations of 1.5%. High viscosities are not obtained with gum Arabic until concentrations of about 40–50% are obtained; this ability to form highly concentrated solutions is responsible for the excellent stabilizing and emulsifying properties of gum Arabic when incorporated with large amounts of insoluble matters (Amalesh *et al,* 2010). Viscosity of gum solutions depend upon type and variety used in concentrations at about 40%, solutions took up pseudoplastic characteristics as denoted by decrease in viscosity with increasing shearing stress. Studies of flow of gum solution play an important role in identification and characterization of their molecular structure. The viscosity could be presented in many terms such as relative viscosity, specific viscosity, reduced viscosity and intrinsic viscosity it can be used to determine the molecular weight of *Acacia Senegal* gum (Mahasin, 1982). Solutions can be modified by the addition of acids or bases as these ones change the electrostatic charge on the macromolecule. In very acidic solutions, acid groups neutralize so induces a more compact conformation of the polymer which leads to a decreased viscossity; while a higher pH (less compact molecule) results in maximum viscosity around pH 5.0–5.5. In very basic solutions, the ionic strength increment reduces the electrostatic repulsion between GA molecules producing a more compact conformation of the biopolymer and thus reducing the viscosity of the solution (Mariana *et al,* 2012).

1.11.7.3 Emulsifying Properties:

The arabino galacto protein (AGP) component of Gum Arabic is responsible for its unequalled emulsifying properties, including the ability to form stable emulsion over a wide pH range and in the presence of electrolytes. Gum Arabic has a unique combination of excellent emulsifying properties and low solution viscosity, and water soluble/dispersible polysaccharides, termed hydrocolloids or gums, are known as viscosity builders and/or gelling agents in aqueous systems. Technologists call them stabilizers, gum Arabic, was known to exhibit emulsification properties. Hydrocolloids can form thick birefringent gel-like mechanical barriers at the oil-water interface of emulsion oil droplets (John, 2001) while the attached carbohydrate units stabilize the emulsion by steric and electrostatic repulsion. It was reported that stability of beverage Emulsions is influenced by a number of processing factors, such as pasteurization and demineralization, and by the pH of the emulsion (Eqbal, 2013). Fractionation studies show that, emulsifying properties generally improve with increasing molecular weight and protein content, the best results are obtained with mixtures of different fractions. Seemingly, the heterogeneous nature of the gum makes it an excellent emulsifier (Mariana *et al,* 2012). GA is well recognized as emulsifier used in essential oil and flavor industries.

Randall *et al,* 1998, was reported that the AGP is the main component responsible for ability to stabilize emulsions by the association of AG amphiphilic protein component with the surface of oil droplets, while the hydrophilic carbohydrate fraction is oriented toward the aqueous phase, preventing aggregation of the droplets by electrostatic repulsion (Murwan *et al,* 2008). The influence of the nature of the oil phase on the emulsifying behaviour of gum Arabic has been investigated at neutral pH. Time-dependent droplet-size distributions are reported for oil-in-water emulsions (1% wt gum, 10% oil) made with n-hexadecane, d-limonene and orange oil. The gum giving the most rapid lowering of the tension at the n-hexadecane-water interface also gives the most stable n-hexadecane-in-water emulsions as well as the smallest droplets with all three oils(Eric *et al,* 1991) and increased the average droplet size of the gum Arabic increasing the oil concentration to 10% increased the particle size with fairly similar proportions. The molecular weight of the gum Arabic sample effect of on the droplet-size distributions of n-hexadecane-in-water emulsions (1% wt gum, 10% vol. oil) at neutral pH. A high-molecular-weight fraction (0·87% nitrogen) corresponding to 10% of a natural gum (0·38% N) gives initially slightly larger droplets but better emulsion stability than the low-molecular-weight fraction (0·35% N) corresponding to the residual 90% of the original gum. Samples of a different gum Arabic (0·35% N) subjected to different degrees of controlled degradation give decreasing emulsion stability with reduction in weight-average molecular weight from 3.1×10^5 to 2.2×10^5Da (Vanda *et al,* 1991). Gum Arabic good stabilized emulsions over the 15 days storage period (Allaoua, 2012). The results show that the higher (AGP) content the more stable the emulsion as observed when GA is used alone, due to chemical composition (Séverine, 2012). GA Proteins have an essential role as emulsifying and stabilizing agents, whereas polysaccharides are mainly used for thickening and emulsifying. The overall stability and texture of food colloids depends on the functional properties of their ingredients, with the nature and strength of the protein–polysaccharide interactions (Ali, 2013). All emulsions exhibit shear thinning behavior, irrespective of oil emulsifier type and concentration. Gum Arabic emulsions showed good stabilities. These ingredients, however, may sometimes slowly degrade and lose their activity, undergo oxidation, react with components present in the food system which may limit their bio-availability, or change the colour or taste of a product, making it necessary that they be stabilized. One of the major concerns for emulsions is keeping the emulsion droplets uniformly distributed during storage and consumption (Murwan *et al,* 2008). This has led the food industry and many researchers to investigate the ability of hydro-colloids and proteins to stabilize emulsion droplets against creaming, flocculation and coalescence, depending on their intended application. Previous studies have also shown that the stability of oil-in-water (O/W) emulsions depends on both the type and concentration of ingredients contained in the emulsion as well as processing and storage conditions. Concentrated emulsions are a unique class of O/W emulsion in that they can be consumed in highly diluted form (low viscosity fluids such as milk and fruit juice beverages) or in their original concentrated form (such as in creams, margarine or butter). Thus, the emulsion must have a significant degree of stability in both the concentrated and diluted forms.At present, gum Arabic is one of the most widely used biopolymers in foods and beverages, its (AGP) component is responsible for its unequalled emulsifying properties, including the ability to form stable emulsion over a wide pH range (Michael, 2012). Stabilize beverage emulsions can be controlled by changing pH. The stabilizing effect of gum tragacanth was a result of the steric repulsion force and the stability can be controlled by changing pH (Sima *et al,* 2011).

1.11.7.4. Molecular Association:

The tendency of polysaccharides to associate in aqueous solution is well known. The Molecular association in fluids, greatly, influences their rheological behaviour. An increase in viscosity with concentration is probably due to increasing number of high molecular weight polymeric chains of the gums per unit volume and increased interaction between these chains in aqueous solution or dispersion. These are likely to increase cohesive density and therefore greater resistance to flow. These molecular associations can deeply affect their function in a particular application due to their influence on molecular weight, shape and size, which determines how molecules interact with other molecules and water. There are several factors such as hydrogen bonding, hydrophobic association, an association mediated by ions, electrostatic. Interactions depend on the concentration and the presence of protein components that affect the ability to form supermolecular complexes. Gum Arabic is used as a functional ingredient, which means that the typical functions of GA are: flavoring agent, humectants, thickener, surface-finishing agent and retards sugar crystallization (Eqbal, 2013).

1.11.8 Applications of Gum Arabic:

1.11.8.1 Food Preparations:

Some of the physicochemical and biochemical properties of GA suggest possible applications in liquid formulas and solid food preparations (Ghada *et al*, 2015). These properties and features of GA have been as a stabilizer as suspending agent for insoluble drugs, a thickener, and an emulsifier to a lesser extent in textiles, ceramics, lithography, cosmetics and pharmaceuticals, in the food industry. GA is primarily used in confectionery (Elsevier, 2012). GA is used in bakery, adhesive and comparatively water absorption properties (Francisco, 2014).

GA is a natural product complex mixture of hydrophilic carbohydrate and hydrophobic protein components emulsifier which adsorbs onto surface of oil droplets while hydrophilic carbohydrate component inhibits flocculation and coalescence of molecules through electrostatic and steric repulsions in food (Mariana *et al*, 2012). GA has several domestic uses namely in manufacturing, crafts making and in foodstuff. GA is an excellent dietary product. GA is also used in the preparation of etching, and in the chemical industry and as a preserving agent for inks. It is also used to sensitize lithographic plates, to stiffen cloth, coat certain paper types and for coating metals to prevent corrosion. And it is used as a dispersant in paints and insecticidal emulsions, respectively keeping the pigments and active components uniformly distributed throughout the product (Williams *et al*, 2011).

Due to its stability in acid conditions and its high solubility, GA is used in soft drinks and well suited for use in citrus and cola flavor oil emulsions; gum prevents sucrose crystallization, provides a controlled flavor. It also provides the appropriate texture to these candies, GA is used to compensate for the loss of texture, mouth feel and body, resulting from there placement of sugars by artificial sweeteners. It is also used in chewing gum as a coating agent and as a pigment stabilizer (Eqbal, 2013). To maintain a uniform distribution of the fat across the product high levels of gum are used to ensure complete coverage of the interface and to prevent flocculation and coalescence of oil droplets. Normally, a weighting agent is added to increase the oil-phase density, inhibiting destabilization due to creaming. GA is used, increasingly, as a source of soluble fiber in low-calorie and dietetic beverages (Amalesh *et al*, 2010). In powdered beverage mixes; GA is added to produce the same opacity, appearance, mouth feel and palatability as in natural fruit juices. In microencapsulation (Lorena *et al*, 2012) liquid, solid or gaseous substances are coated with a protective layer to prevent chemical deterioration and the loss of volatile compounds (Sergio, 2013).

1.10.7.2 Pharmacological Uses:

GA is utilized to stabilize emulsions, as a binding agent and for coating medications. Not more than 5% of the GA is used for pharmaceutical industry as a carrier of drugs since it is considered a physiologically harmless substance (Sunil, 2014). *Acacia* gum forms a stable complex coacervate at pH of 4.0. A coacervate is used to microencapsulate some vegetable oils because of its high water solubility, low viscosity and emulsification properties; certain drugs can also be conjugated to *Acacia* gum microspheres. Periodate oxidation of *Acacia* gum offers a convenient means of producing stable microspherical conjugates between the gum and certain types of drug. Thus, the investigators developed *Acacia* gum microspheres as carrier for sustained release of drugs such as primaquine and ampicillin (Michael, 2012).

GA has been, extensively, tested for its properties as non-digestible polysaccharide which can reach the large intestine without digestion; in the small intestine, it can be classified as dietary fiber. Due to its physical properties, it reduces glucose absorption, increases fecal mass, bile acids and has the potential to beneficially modify the physiological state of humans. GA is slowly fermented by the bacterial flora of the large intestine producing short chain fatty acids. Therefore, its tolerance is excellent and can be consumed in high daily doses without intestinal complications. In addition, GA is able to, selectively; increase the proportion of lactic acid bacteria and biphidus bacteria in healthy subjects (Mariana *et al*, 2012). Human dietary intake studies have indicated a reduction in blood cholesterol levels when above average amounts of gum Arabic (25 grams/day) are ingested in solution for 21 to 30 days it reduceds total cholesterol by 6 to 10.4%, respectively also reduces total sugars. The addition of a 7% gum Arabic solution reduces the dissipation rate of sodium chloride solution (Taha *et al*, 2012) and 5% gum Arabic have some positive effect on decreasing glucose level in the blood stream of the animals studied (Sabahelkhier, 2013). It is also used in the traditional treatment of patients with chronic kidney disease (CKD). Addition of GA to the diet has been shown to increase fecal nitrogen excretion and decrease serum urea nitrogen concentration in patients with CKD (Ali *et al*, 2013). GA contains many types of enzymes such as oxidases, peroxidases, and pectinases, some of which have antimicrobial properties (Mahasin, 1982), and antioxidant properties, anticancer properties, are used intreatments for several degenerative diseases such as kidney failure cardiovascular and gastrointestinal (Sunil, 2014). GA protects lipids from the attack of lipoxygenase and free radicals by adsorbing at the oil droplet surface. GA has been reported to exert a protective effect against gentamicin and cisplatin nephrotoxicity and doxorubicin cardiotoxicity used as biological models in rats (Mariana *et al*, 2012).

1.11.8.3 In cosmetic Industry: GA acts as astabilizer in lotions, it has been recommended for use as fixative and binder in hair care, cream and as a stabilizer and film former in protective creams and an emulsions, it increases the viscosity and assists in imping spreading, adds a smooth feel to the skin and acts as binding agent in the formulation of compact cakes and rouges and as an adhesive in the preparation of face masks, the gum is also used as afoam stabilizer in liquid soap (Mariana *et al*, 2012).

1.12 Emulsions:

The word "emulsion" is derived from the Latin word *emulgeo* meaning to "milk", (Khan *et al*, 2006). An Emulsion is a liquid preparation containing two immiscible liquids, one of which is dispersedas minute globule into other. The liquid that is broken up into globule is called the dispersed phase or internal phase and the liquid in which the globules are dispersed is known as continuous or external phase, which are made miscible by the addition of a third substance known as a surfactant or emulsifying agent (Srinu, 2012). Emulsions are thermodynamically unstable systems that have a tendency to break down over time. The breakdown of an emulsion may manifest itself through different physicochemical mechanical i.e. creaming, cracking, phase inversion, flocculation, gravitational separation, Ostwald ripening (Murwan *et al*, 2008). There are several methods to detect stability of prepared emulsion.

- Rheological assessment.
- Macroscopic examination.
- Globule size analysis and accelerated stability test many type of emulsifying agents are interfacial tense to film formation and electric potential and emulsion rheology (Vani Madaan *et al*, 2014).

1.12.1. Type of Emulsion:

Emulsions are classified in two groups:

1.12.1.1 Oil/ water emulsion: A system that consists of oil droplets dispersed as globules and the water phase serve as the continuous phase, emulsion is term as oil/water (O/W) emulsion (Figure 1.10). These are most used internally; emulsifying bases used in this type are *Acacia* gum, *tragacanth* gum, methyl cellulose, saponins, sodium dioctyl sulfosuccinate, and polymers known as the Spans® and Tweens®, sodium laurel sulfate, sodiumoleate glyceryl monstearte synthetic substance, etc. An O/W emulsion is formed if the aqueous phase constitutes more than 45%of total weight if ahydrophilic emulsifier is (William, 2006).

1.12.1. 2. Water/oil emulsion: A system that consists of water droplets dispersed as globules and the oil phase serve as the continuous phase, emulsion is term as water/oil (W/O) emulsion (Figure 1.10).These mostl used extrnally emulsifying bases used in this type is wool fat, resins, bees wax, soap, spans, cholesterols, are used as creams, lotion (Ahmadi *et al*, 2013).

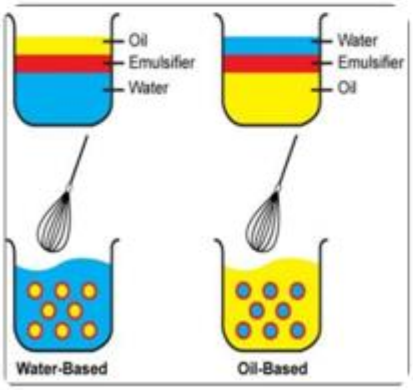


Figure 1.11: Types of water/oil emulsions

1.12.2. Mechanisms of emulsification:

The principal of emulsifying agents include;

1.12.2. 1. Interfacial Tension:

Emulsion have tendency to attain a thermodynamically stable staste by way of coalesoence in the dispersed phase and ultimately separation of two phases. Interfacial tension is one way to decrease the free surface associated with the formation of droplets. Emulsion behavior and stability are affected by the properties of the adsorbed layers that stabilize the oil-water interfaces. In this case the adsorbed layers consist of molecules of surfactants (Vani Madaan et al, 2014).

1.12.2..2. Surface Tension Theory:

According to this theory, emulsification takes place by reduction of interfacial tension between two phases, determination of the surface tension may give some ideas on the possibility of emulsification but insufficient to understand stability, phase separation or the rheological behavior (Sevrine *et al*,2012).

1.12.2. 3. Repulsion theory:

The emulsifying agent creates a film over one phase that forms globules, which repel each other. This repulsive force causes them to remain suspended in the dispersion medium.Viscosity modification emulgents like gum *Acacia*, gum *tragacanth*, polyethylene glycol, glycerine, caboxymethyl cellulose, all increase the viscosity of the medium (Wiley, 2013).

1.12.3. Stability of Emulsion:

Emulsion stability refers to the ability of an emulsion to resist change in its properties over time (Silvestre *et al*, 2005). Emulsion stability is significantly affected by the type of oil used and increase in the length of the stirring time is significantly increased stability of the emulsion. Other factors affect on the formation and stabilization of emulsions is:

-The physical nature of the interfacial film.
-The steric or electric barrier.
-Viscosity and rheological behavior.
-Oil droplet viscosity.
-Particle size distribution and polydispersity.

-Electrical charge and zeta potential.

-Phase volume ratio of the dispersed phase.

-Temperature.

-Emulsifying agents (Murwan *et al*, 2008).

Emulsion stabilized by electric charge, emulsions in which the oil is present in very small amounts belongs in the group stabilized by an electric charge. The oil particles are negatively charged i.e. mineral oil. Hydrophilic are stabilized by hydration and an electric charge. There are a number of factors that have been shown to be important in determining emulsion stability. First, the nature of the oil phase (Mostafa *et al*, 2011). Emulsions stabilized by colloids. The following are commonly used i.e eggs, gelatin, flour, starch, milk, Gum *Acacia*, gum *tragacanth*, other substances (Amalesh *et al*, 2010). Emulsion stabilized by powder (the most stable emulsions are formed with more polar compounds oil-in-water emulsions can also be stabilized by water-soluble polymers). Thus, in commercial emulsions, often the surfactant system is not a single surfactant but consists of a mixture of surfactants, proteins and water-soluble polymers. The composition of the surfactant system, thickness and viscoelasticity of the adsorbed layer stabilizing the oil-water interface, and the strength and nature of the interactions between adsorbed layers on different droplets affect the emulsion structure, stability and rheological properties. The strength of this interfacial film will prevent coalescence, which is the merging of the droplets when the interfacial film is broken. The naturally occurring surface active compounds found in heavy oils are in large amount and would definitely influence emulsion characteristics (Michael, 2009). It depends not only on the oil/water/surfactant system types and concentrations but also on homogenization condition (Lorena *et al*, 2012).

1.12.4. Instability of Emulision:

Physical instability of emulision: can, probably, be the most important consideration with respect to pharamaceutical and cosmetic emulision in the stability of the finished product. Some workens define instability of emulision only in terms of agglomeration of internal phase and its separation from product. Creaming resulting from flocculation and concentration of the globules of the internal phase sometimes is not considered as a mark of emulision (Vani Madaan *et al*, 2014).

1.12.4.1. Creaming of Emulsion:

Creaming may be defined as the upward movement of dispersed globules to form a thick layer at the surface of the emulsion. Creaming is temporary phase because it can be redistributed by mild shaking or stirining to get again a homogenous emulsion, as far as possible creaming of an emulsion should be avoided because it may lead to craking with complete separation in two phases. Under the influence of gravity suspended particles or globules tend to upward movement known as creaming while downward movement of the droplets is known as sedimentation. According to Strok's law the rate of creaming depeneds on the number of factors which can be explained by the following equation (Hassan, 2013).

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

V: Rate of creaming,

r = Radius of glolubles

g = Gravittion consant.

Δp = the density differences between the oil and water phase.

η = Viscosity of the continuous phase

1.12.4.2. Flocculation and Its Prevention:

Flocculation involves the aggregation of the dispersed gloubles into loose clusters within the emulusion. Indiviual droplets retain their identities but each cluster behaves physically as a single unit. This would increase the rate of creaming .As flocculation must precede coalescence, any factor preventing or retarding flocculation would therefore maintain the stability of the emulusion. Flocculation is, potentially, a reversible process under the influence of shear flow or Brownian motion, or by manipulation of the interparticle forces, a partially, flocculated emulsion may be converted into partially dispersed emulsion. In concentrated emulsions a perceptible increase in viscosity occurs with flocculation. Bridging flocculation occurs when a polyelectrolyte adsorbs to the surface of more than one droplet and links them together. Bridging flocculation by, strongly, adsorbing polymers may be, effectively, irreversible (Stanislaw *et al*, 2011).

1.12.4.3. Coalescence:

It is a physical phenomenon whereby two or more droplets combine together to form a single large droplet. It is due to the tendency of the emulsion to achieve a, thermodynamically, more stable state by a decrease in contact area between oil and water phase (Wiley, 2013).

1.12.4.4. Ostwald ripening:

Larger droplets grow at the expense of the smaller ones; this happens because of the transport of dispersed phase molecules from the smaller to the larger droplets through the dominant continuous phase. The mechanism of emulsion destabilization with time in these systems is attributed to Ostwald ripening (Arora *et al,* 2009).

1.12.4.5. Creacking:

Creacking of an emulsion refers to separation of dispersed phase and continuous phase, due to coalescence of dispersed phase globules which are difficult to re dispersed by shaking. Coalescence is a growth process during which the emulsified particles join to form large particles. This process is also known as craking. A, physical, chemical or biological effects that change the nature of the interfacial film of an emulsifying agent may cause craking, coalescence, the complet, fusion of droplets leads to a dcrease of the two immiscible phases (Stanislaw *et al*, 2011).

1.12.4.6. Phase inversion:

Means the change of one type of emulsion into other type that is oil-in-water emulsion changes into water in oil type and vice versa. The temperature at which the inversion occurs depends on the emulsifier concentration and is called phase inversion temperature. An O/W emulsion stabilized by a non-ionic polyoxyethylene derived surfactant contain oil-swollen miscelles of the surfactant as well as emulsifying oil inversion often can be seen when an emulsion prepared by heating and mixing the two phases, it is being cooled, this changes in the solubilitys of the emulsifying agent i.e. O/W emulsion with sodium stearate as the emulsifier can be inverted by adding calcium chloride because calcium stearate formed is a lipophillic emulsifier and favors the formation of W/O emulsion. The phase inversion can be minimized by keeping the concentration of dispersed phase between 30 to 60 percent storing the emulsion in a cool phase and by using proper emulsifying agent in adequate concentration (Stanislaw *et al*, 2011).

1.12.5. Chemical Instability:

Chemical instability of an emulsion causes coalescence of particles of the 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 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 an electrolyte can strip emlusifying agents of thei hydrated layers and cause their precipitation.

Change in PH may also lead to the breaking of emulsion. Soap satbilized emulsions are therefor usually formulated at an alkaline pH. Enviromental conditions, such as the presence of light, air, contaminating microbes, adversely, affect the stability of an emulsion. For light sensitive emulsion, light resistance containers are used. For emulsion susceptible to oxidative decomposition, anti-oxidants may be included in the formulation and adequate lable waming provided to ensure that the container is, tightly, closed after use. Many molds, yeasts, and bacteria can decompose the emulsifying agent, disruptingvthe system. Even if the emulsifier is not affected by microbes, the product can be rendered unsightly by their presence and growth and will not off cours not be efficacious from a pharmaceutical or therapeutic point. Because fungi 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.

Chemical instability is of three types namely: Oxidation, Microbial contamination, adverse storage condition.

1.12.5.1. Oxidation:

Many of the oil and fats used in emulsion formulation are of animal or vegetable origin and can be suscaptible to oxidation by atmospheric oxygen or by the action of micro organisms. Oxidation, of microbiological origin, is controlled by the use of anti-microbial preservative and atmospheric, agent, oxidation by the use of reducing agents or more, usually, anti- oxidants like butylatedhdroxyanisole (up to 0.02 % for fixed oil), and for some essential oil(up to 0.1%)

1.12.5.2. Microbial Contamination:

Microbial contamination of emulsion occurs by micro-organisms can, adversely, affect the physicochemical properties of product, causing such problem as gas production, color changes, hydrolysis of fats and oils, pH changes in aqueous phase and breaking of the emulsion. An emulsion contains, many, bacteria and may constitue a serious health hazard and most funig 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 (Vani Madaan *et al*, 2014). Species of the genus *pseudomonas* can utilize polysorbates, aliphatic hydrocarbons and compound. Some fixed oil such as arachiz oil; can be used sum *aspercillus* and rhizopus species, and liquid paraffin by some species of penicillium. Water in oil product have less chances of microbial spoilage as comared 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 micro organisms that might contamination the product (Vani Madaan *et al*, 2014) .

1.12.5.3. Adverse Storage Conditions:

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 increaseed kintic motion, both of the dispersed droplet and of the motion of the emulgent will result in a more exepended monolayer and so coalesecence is more likely. Certain macro molecules emulsifying agents may also be coagulated by an increase in temperature. 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 producted kept as far as possible from the ingress of micro organisms during manufacture, storage and use (Vania Madaam *et al*, 2014).

1.13. Method of Preparation Primary Emulsion:

Emulsions can be prepared by various methods:

1.13.1. **Dry gum method**: oil: water: gum (4:2:1).

The dry gum method is used to prepare the primary emulsion of liquid.

1.13.2. **Wet gum method:** taking oil: water: gum (4:2:1) with *Acacia* gum powder as an emulsifying agent. Some wet gum technique taking oil: water: *Acacia* gum (4:2:0.5) (Amalesh *et al*, 2010).

1.14. Emulistion Type and Means of Detection:

Several tests are available for distinguishing between O/W and W/O type emulsions and means of detections are:

- Dilution test: O/W emulsion can be diluted with water, W/O emulsion can be diluted with oil.

- Conductivity test: Pair of electrodes connected to a lamp and an electric source is dipped in emulsion. If the emulsionis O/W type then lamp glows if lamp does not glow then the emulsion is W/O type.

- Dye Test:

Red dye is used when the red dye is mixed with emulsion. One drop of emulsion placed on microscope slide. If globules appear red is colorless a ground is, then the emulsion is W/O type (Vani Madaan *et al*, 2014).

1.15. Emulsifying Agents:

Emulsifying agents are substances which stabilize an emulsion and can be classified based on this dispersed phase and size of liquid droplets. All emulsifying agents concentrate at and are adsorbed onto the oil/water interface to provide a protective barrier around the dispersed droplets. Emulsifiers stabilize emulsion by reducing the interfacial tension of the system. Some agents enhance stability by imparting a charge on the droplet surface thus reducing the physical contact between the droplets and decreasing the potential for coalescence. They can also be classified according to:

- Chemical structure is synthetic, natural, finely dispersed solids, and auxiliary agents.
- Action mechanism (monomolecular, multimolecular, and solid particle films).

Regardless of their classification, all emulsifying agents must be chemically stable in the system with other emulsion components and nontoxic and non-irritant (William, 2006). The emulsifier molecules can form films at the droplets surface and impart mechanical stability. Presence of the emulsifier molecules at the oil-water interface is accompanied by a decrease in the interfacial tension between the two phases (water and oil).

1.16. Eletric Properties of the Emulsifiers:

The simplest type is ions such as OH^- that can be, specifically, adsorbed on the emulsion droplet thus producing a charge. An electrical double layer can be produced, which provides electrostatic repulsion. This has been demonstrated with very dilute O/W emulsions by removing any acidity. Clearly that process is not practical. The most effective emulsifiers are non-ionic surfactants that can be used to emulsify O/W or W/O. In addition, they can stabilize the emulsion against flocculation and coalescence. Ionic surfactants such as sodium dodecyl sulfate (SDS) can also be used as emulsifiers (for O/W), but the system is sensitive to the presence of electrolytes. Surfactant mixtures, for example, ionic and non-ionic, or mixtures of non-ionic surfactants can be more effective in emulsification and stabilization of the emulsion. Non-ionic polymers sometimes referred to as polymeric surfactants, the surfactant molecules lower the interfacial tension between the oil and water and form a stable phase. For example, Pluronics are more effective in stabilization of the emulsion, but they may suffer from the difficulty of emulsification (to produce small droplets) unless high energy is applied for the process (Hans, 2007). Cationic-lipophilic portion is positively charged (Benzalkonium chloride). Cationic emulsions are also used in certain products due to their antimicrobial properties. Non-ionic emulsions are most popular due to their low toxicity, ability to be injected directly into the body, and compatibility with many drug ingredients like stearyl alcohol, acetyl alcohol and glyceryl monostearate. These are mostly used as thickening agents and stabilizers for O/W emulsions (Mostafa *et al*, 2011).

1.17 Mechanisms of Emulsion Formation:

1.17.1. Film Formation:

The concept of an oriented film of emulsifier on the surface of the internal phase of an emulsion is of fundamental importance to the understanding of most theories of emulsification. The ability to reading form a film around each dispersed globule is one of the most important requriments of an efficient emulsion agent. Film produces additional repulsive forces between droplets to contract attractive Vander Wall Forces and inhibits the close approach of droplets. A film may be defined as a thin (25–500 µm) layer of coherent solid matter adhered to a substrate. In most cases the film is derived from a liquid. Viscosity is an important factor in producing a good film, since the polymer needs to flow and cover the substrate. The viscosity depends on the substrate to be coated and application method employed, but is generally in the range of 0.05–1.0 Pa.s (Ahmadi *et al*, 2013) (Figure 1.12: Films Formation).

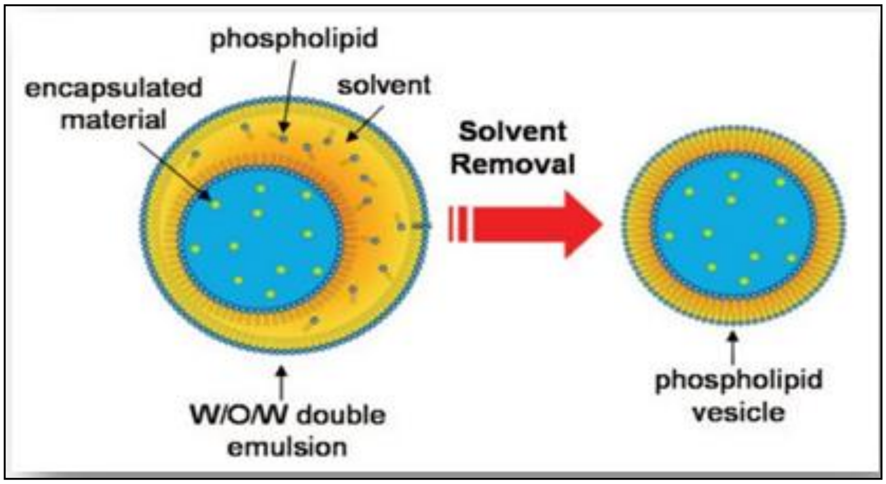


Figure 1.12: Films Formation

1.17.1.1 Type of Films Formation:

-Monomolecular Films:

Surfactant absorbs at oil in water interface and form monomolecular film. The film rapidly envelopes droplets as soon as they are formed. Monomolecular film should be compact and strong enough so that it cans not easil distributed or broken. Combination of emulgents is used higher today. Combination consists of a pre dominantly hydrophilic emulgent in aqueous phase and hydrophilic agent in the oily phase to form acomplex film. Surface active agents or amphiphiles; reduce interfacial tension because of their absorption O/W interface to form monomolecular film (Figure 1.14). The reduction in surface free energy is of itself probably not the main factor involved. The dispersed droplets are surrounded by a coherent monolayer of the surfactant which prevents coalescence between two droplets ch one other. If the emulsifier is ionized, the presence of strong charge may lead to repulsion in droplets and hence increasing stability.

-Multimolecular films:

Emulsifying agents such as hydrophilic colloids (*Acacia* and gelatin) tend to form multimolecular films around the globules and prevent then from coalescence (Figure 1.13). The stability is improved by adding viscosity agents such as *tragacanth* gum, methyl cellulos etc (Ahmadi *et al*, 2013).

-Solid particle films:

The finelly divided solid particles that are wetted to some degree by both oil and water (can act as emulsifying agents). This is result from their being concentrated at the interface where they produce particles films around the dispersed droplets so as to prevent the coalescence globules (Figure.1.13). Powder particles are wetted preferentially by water form oil in water emulsion. Powder particles were more easily wetted by oil form water in oil emulsion and are absorbed at oil water interface and form a rigid film of closely packed. Solid films act as mechanical barriers and prevent the coalescence globules these tend to produce coarse emulsion depending on affinity of emulsifier to a particular phase (magnesium aluminum silicate) is called Veegum, O/W emulsion (Wiley, 2013).

All types of films reduce the interfacial tension they are effective at high concentration. They also have affinity towards the oil phase and facilitate interfacial absorption.

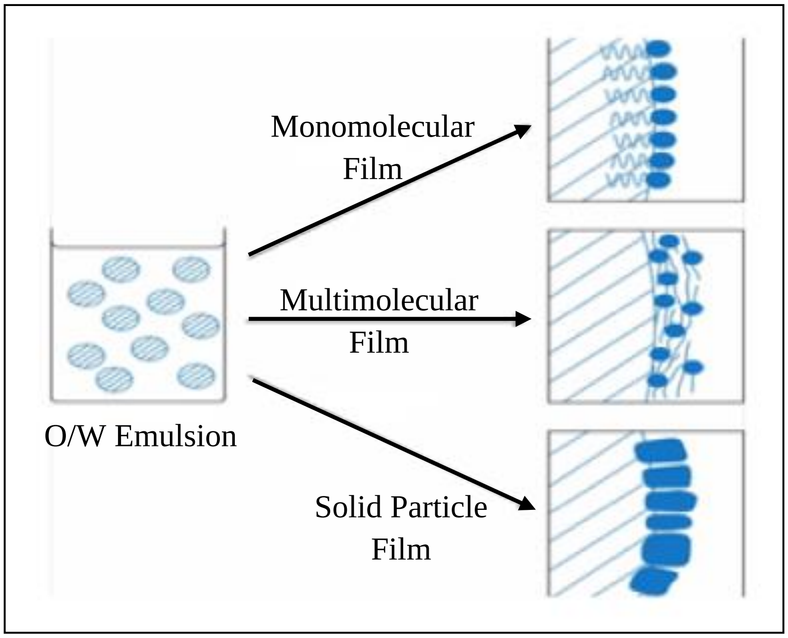


Figure.1.13: Type of Film Formation.

1.18. Electrical Potential:

The same or similar film can produce repulsive electrical forces between approaching droplets. Such repulsion is due to an electrical double layer, which may arise from electrically charged groups oriented on the surface of emulsified globules. The potential produced by the double layer creates a repulsive effect between the oil droplets and thus hinders coalescence (Figure.1.14). Although the repulsive electric potential at the emulsion interface can be calculated, it can not be measured directly for comparison with theory. The dispered phase globules are known to acquire an electric charge during the process of globules emulsifcation. The globules gather to charge layers around themselves called Helmholtz double layer, zeta potential in which the difference in charge potentials between points on the fixed charge layer on the globules to the points where both positive and negative charge exists. Zeta potentials can be measured by zeta meter and helps in the prediction of flocculation behavior of the system. Dispersed phase systems having a zeta potential value higher than 0.25mV are likely to be stable because the intensity of the charge on the particle repels them from each other. Zeta potential for the surfactant stabilized emulsion compares favorably with the calculated double layer potential .the chang in zeta potential parallels rather parallels rather satisfactorily the change in double layer potential as electrolyte is added. These and related on the magrnitude of the potential at the interface can be used to calculate the total repulsion between oil droplets as the fuction of the distance beween them (Vani Madaan *et al*, 2014).

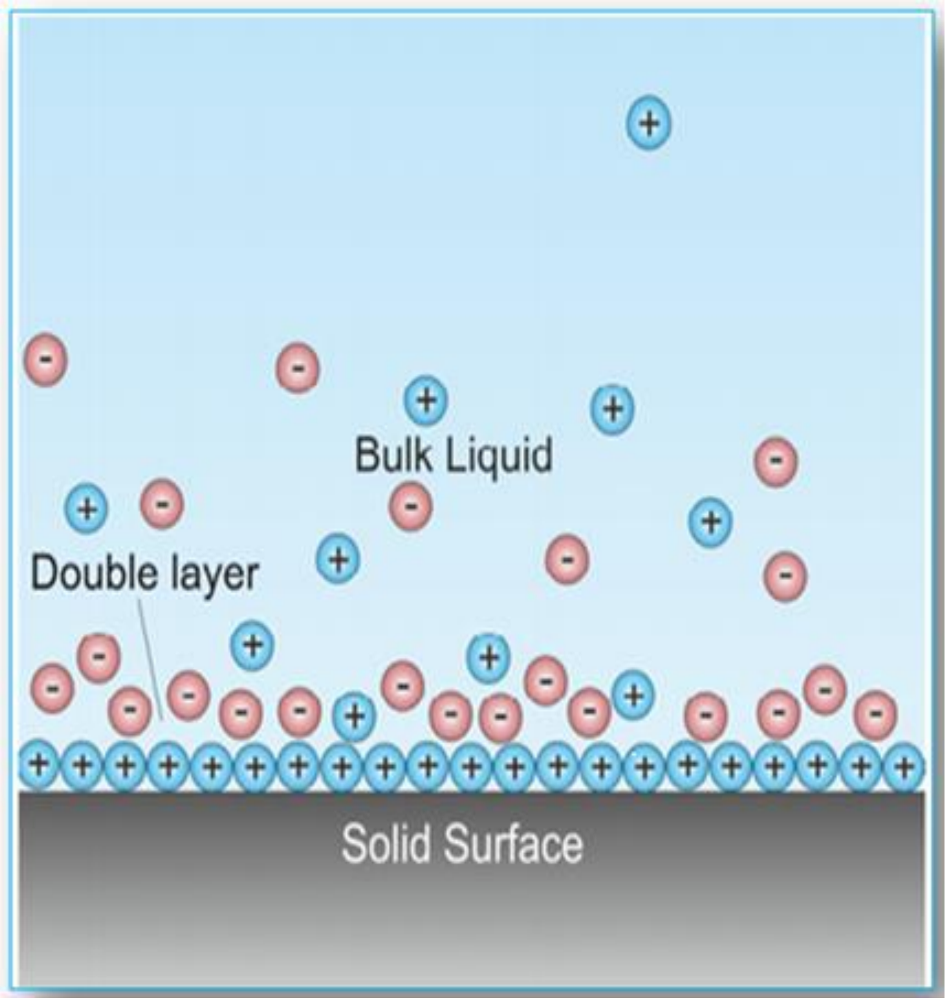


Figure 1.14: Electric potential

1.19. Emulision Rheology:

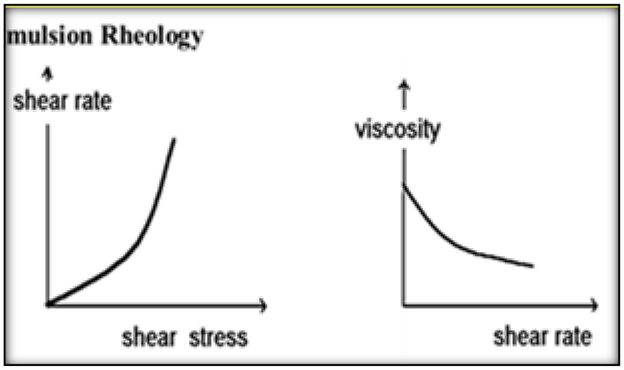


Figure 1.15: Emulision Rheology

Rheology is defined as the science of deformation and ligueds. Emulisfied products undergo a wide variety of shear stress during either preparation in many of these processes (Figure 1.15). The flow properties of the product will be vital for the proper performance of the emulision under the condition of use or preparation, thus spreadability of dermatologic and cosmetic products must be controlled to achieve a satisfactory preparation (Wiley, 2013). The flow of parenteral emulsion through a hypodermic needl, the removed of an emlsion from a bottle or tube, and the behavior of an emulsion in the various operations employed in large scale manufacture of these products, all indicate the need for correct flow characteristic. All emulsion, except dilute ones, exhibit non-newtonian flow, which complicates interpretation of data and quantitative comparison with different systems and formulations (Wiley, 2013).

Rheological characterization of polysaccharides is important as it provides fundamental information required for assessment of some of the final properties of a product, such as quality, storage stability, effect of formulation variables on product characteristics, mainly factors affecting emulsion rheology, known as properties of emulsion, inclulde: viscosity of the dispersed droplets, droplet size distribution, dispersed phase volume fraction, continuous phase, droplet charge, and interfacial film (Ahmadi *et al*, 2013).

1.20 Properties of Emulsions System:

The Structure of Emulsions System**:**

1.20.1. Macro Emulsions:

Emulsion in which the particle dispersed phase has a diameter of (1 to 100um). Macro-emulsions comprise large droplets and thus are “unstable” in the sense that the droplets sediment or float, depending on the densities of the dispersed phase and dispersion medium. Separation of the dispersed and continuous phases, usually, occurs within time periods from few seconds to a few hours, depending upon the viscosity of the fluid medium and the size and density of droplets.

Macro-emulsions usually contain low molecular weight or polymeric surfactants that decrease the rates of coalescence of dispersed droplets. Droplets of the dispersed phase may be also stabilized by adsorption of solid particles onto their surface so-called Pickering stabilization.

1.20.2. Mini-Emulsion:

Emulsion in which the particle dispersed phase has a diameter of 50nm to 1um. Mini-emulsions are, usually, stabilized against diffusion degradation Ostwald ripening by a compound, insoluble, in the continuous phase. The dispersed phase contains mixed stabilizers, i.e., an ionic surfactant, such as sodium dodecylsulfate (n-dodecyl sulfate sodium) and a short aliphatic chain alcohol (co-surfactant) for colloidal stability, such as a hydrocarbon (“co-stabilizer” frequently and improperly called a “co-surfactant”) limiting diffusion degradation. Mini-emulsions are stable for at least several days (Stanislaw *et al,* 2011).

1.20.3. Microemulsions:

May be, defined as dispersion of insoluble liquids in second liquid that appear clear and homogenous to naked eyes. Microemulsions are, frequently, called solubilizeds system because on microscopic basis they seem to, behave as true solution. Microemulsions should not become fused with solution formed by co-solvency i.e.water, benzene, and ethanol. Blending of a small amount of oil with water, result in a two-phase system because water and oil do not mix. If the same small amount of oil is added to an aqueous solution of suitable surfactant in the micelle state, the oil may, preferentially, dissolve in the interior of the micelle because of its hydrophobic character. The three basic types of microemulsion are direct, reverse, and bicontinuous. Microemulsion dispersion made of water/oil, and surfactant(s) that are isotropic and thermodynamically stable system with dispersed domain diameter varying approximately from 1 to 100 nm (Figure 1.16). In a microemulsion the domains of the dispersed phase are either globular or interconnected to give a bicontinuous microemulsion. The average diameter of droplets in macroemulsion is close to one millimeter. Therefore, since micro means10^{-6}m and emulsion implies that droplets of the dispersed phase have diameters close to 10^{-3}m.

The term “microemulsion” has come to take on special meaning. Entities of the dispersed phase are usually stabilized by surfactant systems (i.e., aliphatic alcohol). The term “oil” refers to any water-insoluble liquid (Stanislaw *et al,* 2011). The high percentage of emulsifiers causes a very fast penetration, respectively, permeation of the active agents into the skin due to its powerful impact on the skin barrier. This is an advantage especially for the pharmaceutical sector. In the cosmetic sector W/O microemulsions are, mostly, used in skin cleansing, e.g. in form of fat-containing cleansing gels, shower gels and foam bath products (Hans, 2007). In addition, the stability of a microemulsion is, often, easily compromised by dilution, by heating, or by changing pH.

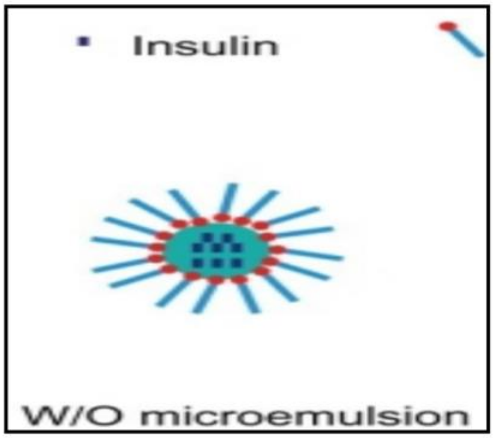


Figure 1.16: Micro-emulsions

1.20.4. Nano Emulsions:

These usually have a size range of 20–100 nm. Similar to macroemulsions, they are only kinetically Stable (Mason, 2006).

2.23.5. Double or Multiple Emulsions:

Dispersed phase in these emulsions contain smaller droplets that is miscible with the continuous phase. Thus, emulsions of O/W/O, W/O/W may be formed i.e (Corboxy methyl cellulose sodium). Multiple Emulsions are used in prolonged action, test masking, enzyme entrapment etc (Wiley, 2013). In these systems, both hydrophobic and hydrophilic emulsifiers are used O/W/O – are formed better by lipophilic, non-ionic surfactants using gum *Acacia*-emulsified systems. W/O/W – are formed better by non-ionic surfactants in a two-stage emulsification procedure (Figure.1.17) (Stanislaw *et al*, 2011).

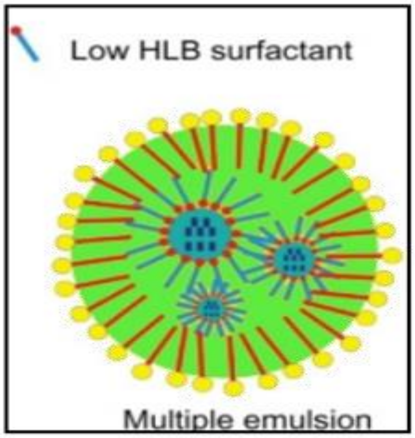


Figure .1.17: Multiple Emulsions

1.21 Emulsion Polymerizatio:

The name emulsion polymerization is arise from a historical “misconception” rather than occurring in emulsion droplets, polymerization takes place in the latex particales that form spontaneously, in the first few minutes of the process. These latex particales are, in to range of 100nm in size and are made of many individual polymer chains; the particles are stopped from coagulating with each other because is surrounded by the surfactant; the charge on the surfactant repels other particlly electro- stattically. When water soluble polymers are used as stabilizer instead of soap, the repulsion between particales arises because these water soluble polymers form a hairy layer around a particale that repels other particales because pushing particales together would involve compressing these chains (Figure.1.18). Emulsion polymer usually starts with an emulsion “incorporating” water, monomer and surfactant. The most common type of emulsion polymerization is oil in water emulsion in which droplets of mommer are emulsified in continuous phase water. Water soluble polymers, such as gum Arabic, polyvinyl achohols or hydroxyl etheyl cellulose, can also be used to act as emulsifier/stabilizer. Dispersion resulting from emulsion polymerization is often called latex or an emulsion. These emulsion finl applications in adhesives, paint, paper coating and textile coating (Vani Madaan *et al*, 2014)

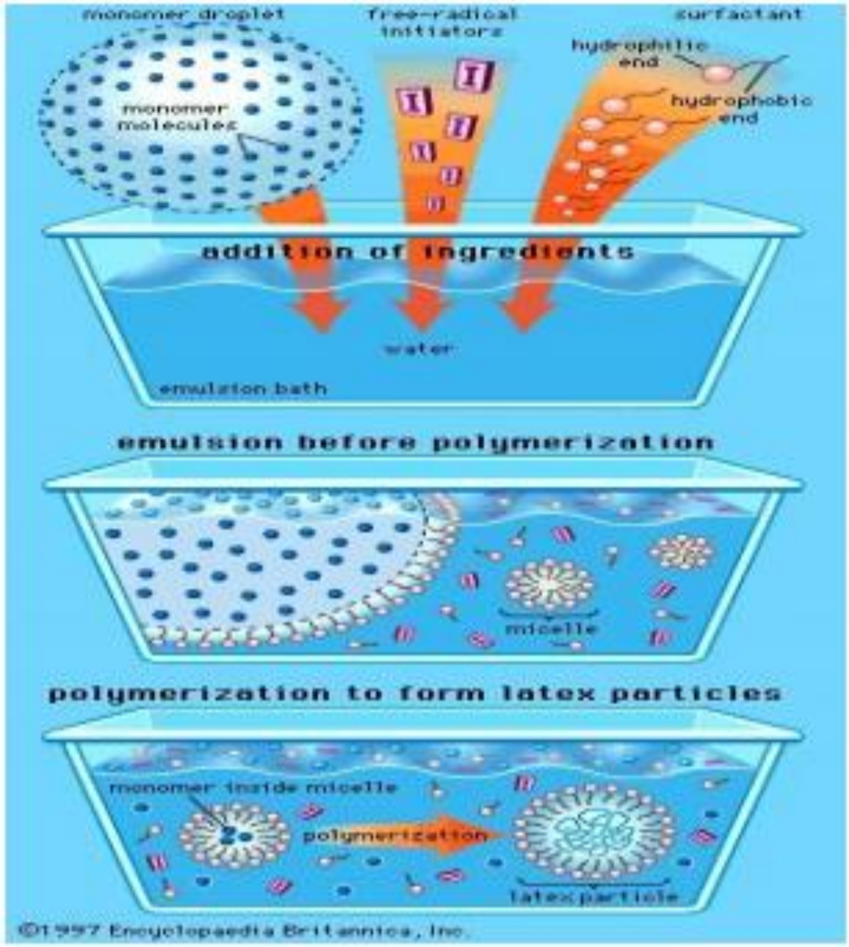


Figure 1.18: Emulsion Polymerizatio

1.22 Industrial Applications of Emulsions:

Emulsions are, widely, used in industry for encapsulation, solubilization, entrapment, and controlled delivery of active ingredients. In order to answer the increasing demand for clean label excipients, natural polymers can replace, potentially, irritative synthetic surfactants used in emulsion formulation. Indeed, biopolymers i.e gum Arabic are, currently, used in food industry i.e, mayonnaise, salad creams, deserts, and beverages (Eqbal, 2013) to stabilize emulsions, and they appear as promising candidates in the pharmaceutical field too. Pharmaceutically, acceptable emulsifiers must also be stable, compatible with other ingredients, non-toxic, odourless, tasteless, or colorless, and do not interfere with the stability of the active agent. All proteins and some polysaccharides are able to adsorb at a globule surface, thus decreasing the interfacial tension and enhancing the interfacial elasticity (Eléonore *et al*, 2012). Due to their texturing properties, polysaccharides and their mixtures are good thickening agents of aqueous solutions that are, widely, employed to control the rheological properties of oil-in-watere emulsions. Proteins and polysaccharides may also be associated either through covalent bonding or electrostatic interactions. Proteins can emulsify and stabilize emulsion by decreasing surface tension and provide electrostatic repulsion on surface of the oil droplet. Alternative layers of, oppositely, charged biopolymers can also be formed around the globules to obtain multi-layered “membranes”. These layers can provide electrostatic and esteric stabilization, thus improving thermal stability and resistance to external treatment. Novel biopolymer-stabilized, emulsions have a great potential in the pharmaceutical field for encapsulation, controlled digestion, and targeted release although several challenging issues such as storage and bacteriological concerns still need to be addressed (Eléonore *et al*, 2012). Their efficiency for enhancing both the viscosity and the stability of emulsion depends on the polymer concentration and structure (Séverine *et al*, 2012). Thus, the emulsion must have a significant degree of stability in both concentrated and diluted forms (Srinu, 2012). Emulsions also offer potential in the design of systems capable of giving controlled rates of drug release and of affording protection to drugs susceptible to oxidation or hydrolysis due to the presence of hydrophilic and lipophilic domains (Michael, 2009). Other use for emulsions: paints industry, i.e. emulsions of alkyd resins and latex emulsions, biological fluids, petrochemicals. Dry cleaning formulations – this may contain water droplets emulsified in the dry cleaning oil, which is necessary to remove soils and clays. Bitumen emulsions: these emulsions prepared stable in the containers, but when applied to road chippings, they must coalesce to form a uniform film of bitumen. Many crude oils contain water droplets and these must be removed by coalescence followed by separation (Hans, 2007). Emulsions play an important role in pharmaceutical preparations such as personal care and cosmetics industry. In contrast to pharmaceutical ointments which must penetrate deep into the skin, cosmetic products are meant only for use on the skin surface. In the last few years physical products have started to gain the interest of consumers, because of the inconsiderate use of chemical components and the harmful consequences of them on peoples, health. Scientists started to look for alternative findings to surpass these problems. For this reason herbs study has gained attention. Traditional herbs provide an interesting, largely, unexplored source for development of potential new cosmetics (Eforia *et al,* 2012). In the present study were combined all these beneficial components in order to make O/W emulsions. The use of mineral oil such as paraffin oil is very common in pharmaceuticals and in cosmetics emulsions due to having antioxidation properties and emulsifying agent.

1.23 Objectives:

In this study, the stability of concentrated and dilute emulsions were studied by using gum Arabic, and other stabilizers such as liquid paraffin oil, and white paraffin wax and Vaseline (pure petroleum jelly).

The main objective of this study was to:

-To extract the essential oils of two types *Ocimum basilicum* cultivated in Sudan.

-To identify components Basil oil by GC-MS.

-To study physicochemical characteristic of essential oil of *Ocimum basilicum L.* green and *Ocimum basilicum L.* purple.

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

-To study the effectiveness of basil oil creams as a mosquito repellent under laboratory conditions.

CHATER TWO

MATERRIALS AND METHODS

2.1 Materials:

Basil oil, light liquid paraffin, white petroleum jelly, paraffin wax, gum Arabic, ethanol, potassium iodide, chlorform solution, sodium thiosulphate.

-Instrument: water bath, reflux condenser, Clevenger-type apparatus,

2.2 Methods:

2.2.1 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 fertilizers), the leaves were collected from Omdurman, Khartoum State. All plant materials were collected during March 2015. And the plant material were, taxonomically, authenticated at the herbarium of medicinal and aromatic plants at Traditional Medicinal Research Institute (National Center for Research), Khartoum, Sudan. Herbarium specimens were deposited there for further future reference.

2.2.2. Prepartion of Meterials:

Fresh leaves 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 (29 ± 1°C) for three days. The dried leaves powdered material of two type *O.basilicum* (50 g) with (500ml) distilled water were subjected to hydro distillation for 5 hours using a clevenger-type apparatus (Medicinal and Aromatic Plants Research Institute). 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 the volume of the obtained essential oil was calculated as a percentage volume per weight (Hussain *et al*, 2008). The oils were collected in glass with air-tight cover and were dried over anhydrous Na_2SO_4 (Figure 2.1), and stored in a dark glass bottle in light resistant at 4 – 6°C (Nour *et al*, 2009) until analyzed by GC/MS to determine oil constituents.

Figure 2.1: Basil oil extract

2.2.3 Phytochemical screening:

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

2.2.4. Identification of components Basil oil by GC-MS:

Essential oils composition was determined using Gas Chromatography-Mass Spectrometry instrument (2010). The gas chromatography-Mass Spectroscopy (GC-MS) of essential oil was analysed on a Shimadzu (QP-2010) instrument at 70 eV and 250°C. GC Column: ULBON HR-1equivalent to OV-1. The GC conditions used were as follows: capillary column: DB-5MS; fused silica (polydimethylsiloxane, 30m length, 0.25mm diameter 0.25µm film thickness); injection temperature program: 250°C (5 min), column oven temperature 40°C; for 1min to 275°C (5 min); hold time1– 11min and rate 5cm injection mode split ; carrier gas (helium) purge flow was 3ml/min, linear velocity of 48.1cm/min; injection port split less at 250°C; and split ratio 50 under flow control model pressure 100kpa, total flow 93.8 ml/min and column flow 1.78ml/min, injection volume, 0.1 µL. The identification of the essential oil constituents was based on a comparison of their retention times, and mass spectra with those present in the National Institute for Standard Technology computer data bank (NIST:2009s. LIB) (Ismail, 2006)

2.2.5. Physicochemical characteristics of oil extract:

In this study physicochemical properties of oil were determined moiture, peroxide, color, percentage of oil yield, density, optical activity, refractive index, viscosity, total acid number, iodine number and saponification value.

2.2.5.1. 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.

2.2.5.2. 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).

2.2.5.3 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 the display screen (Barkatullah et al, 2012).

2.2.5. 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 into the oil inside the container. The meter was turned on and adjusted to a 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 meter’s display screen. The viscosity value of the oil was measured in centipoises (Barkatullah et al, 2012).

2.2.5. 5. Determination of total Acid Number (TAN):

1.25g of oil were placed in a flask. 50 mls of fat solvent were added to the flask, well shake and titrated against 0.1N KOH solution, using 1ml phenolphthalein as indicator. Alkali was added till a pink color was established for (20 – 30s) were noted that the number of milliliters of standard alkali required and calculated the acid value of the fat.0.1M KOH contain 5.6g/l or 5.6 mg/ml. acid value was calculated using the following formula: Vx Nx56.1/ W.

Whereas:

V = volume of potassium hydroxide used.

N = normality of Potassium hydroxidell.

W = weight in g of the sample.

2.2.5. 6. Determination Iodine Value:

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

Iodine value = (B-T) x12.7/1000 x 100/0.2 = (B -T) x 6.35.

Where as B = 0.1N Na_2SO_3 requir by blank.

T = 0.1N Na2SO3 required by Sample.

2.2.5. 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 25mls 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 colourless. A blank titration was carried out at the same time and under the same condition. The difference between the blank and test reading gives the number of millitres 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 atriglyceride then:

Saponification value(S) = 3x56x1000/Average mol.wt of sample.

Average Mol.Wt of sample (fat) = 3x 56 x1000/S (plummed, 1978).

2.2.5. 8. Determination of the Peroxide Value:

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

2.2.6. Preparation of cream base (aqueous base):

Vaseline, Pure Petroleum Jelly® paraffin oil and white wax were melted in a water bath at 80°C, to the desired concentrations. Different combinations that consisted of 10g basil oil extracts and mixture of pure vaseline with paraffin oil and paraffin wax in the ratio 50:20:30 were then mixed wtih crude gum solution the ratio is (9:0.5) water:gum (180 :20) and the resultant stock solution was serially diluted to the desired concentration. 50g of the jelly and 30g of the white paraffin wax were scooped using a spatula and transferred into a clean 500ml beaker. The beaker was then partially immersed in a water bath at 80°C then stirred, continuously, using a stirring rod until it fully melted (for 5 minutes). 20g of paraffin oil were added and stirred, continuously, until a homogenized solution was obtained. The gum solution were added to form the primary emulsion oil: water: gum *Acacia* (4:2:0.5) (Amalesh *et al*, 2010), then 0.032g of sodium benzoate (antifung, antimicroblia) was dissolved and added. Final volume was stirred slowly until the product became a soft cream of high viscosity and very soomth to the skin (Figure 2.2), the cream were stored in glass tube with air-tight cover under 27C°. The test extracts Emulsion in which the particle dispersed phase has a diameter were used with basil essential oil and emulsified in the gum Arabic solution, each test has different concentrations of cream and basil oil, the ratio is (cream:oil 9:1) and stored at 15 – 20 C° until tested for repellent activity.

Figure 2.2 Cream Prepared by the researcher

2.2.7 Preparation of the test materials:

2.2.7.1 Preparation of mosquitoes:

Mosquitoes used in this study were laboratory reared anopheles females (malaria vector), was reared according to the standard protocol of the Medinical Insecticide Section, National Central Institute of Health (STACK Laboratory) Khartom 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 – 26°C 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 2mg of food in a 24 well polystyrene plate. The larvae were reared continuously for several generations and kept under 25 – 30°C, 60 – 70% relative humidity and photoperiod of 13:11 hours (light/dark). Larvae were fed on ground biscuit. At larval stage, they were fed daily with 100mg of active dry yeast that was sprinkled on the water 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 pipetteand transferred in a container, three quarters full of water that was then inserted in a wooden cage (Gupta, 2014). 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 a 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 3-5-day-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 ±2ºC, 60 ±10% relative humidityand 12h: 12h (light: dark) (Xin Chao Liu , 2014)

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

The technique involves counting of the repellency tests:

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 15 female mosquitoes 3-5 day-old (without water and sugar for 18-24 hours) was aspirated randomly using a mouth aspirator introduced into (40x40x40 cm) net cage. Evaluations were carried out by placing the arms of volunteers inside cages. The temperature was maintained at 25-30°C and the relative humidity at 55-60%. Three human volunteers were employed for one repellency tests. Both arms of volunteers, which would later be placed one in each cage, were, carefully, covered with thick paper, except for ar area of 3x10 cm exposing the arm to mosquito bites. This area (of 3x10 cm) was not treated with anything for control (one arm) approximately 0.1 cm^3 of cream base was applied to the one arm and 0.1 cm^3 of basil oil cream was applied to the other arm. Each arm of a volunteer was placed inside the cage containing 15 female mosquitoes for 5 min. each test was repeated three times. After that the arms of the test person were cleaned with ethanol, ethanol served as a control. After air drying the arm approximately 0.1 cm^3 of basil oil cream (repellent) was applied to one arm of a volunteer (5%) and 10% concentrations to each arm. Each arm of a volunteer was placed inside the cage containing 15 female mosquitoes for 5 min each test was repeated three times.The step two at the same condition of step one, each arm of a volunteer was placed inside the cage containing 15 female mosquitoes for 5min exposure was repeated every 30 min without renewing the basil oil creamtreatments. During the 5 min exposure the number of mosquitoes landing on each 3x10 cm 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 6 h 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. This test repeated for under 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 (Nour *et al*, 2009). The number of mosquitoes landed during a 5-minutes interval was recorded. The repellency of basil oil cream was calculated by the formula:

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

3.1Physio-chemical Properties of Essential Oils:

The appearance of essential oils was pale yellow, transparent and clear with a 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) and (0.8%) for *Ocimum basilicum L.* green (whit flower). The results similar to those previously reported on this species (Özcan, 2002, Hussain *et al*, 2008, Hadj *et al*, 2012, Mawada, 2005) were reported that they studied the parts of *Ocimum basilicum L*. the oil content varied from (0.1–1.98%) and it depends also on the seasonal factor and locality. A study by Vieira and Simon (2006) showed that oil contents of essential oil in 15 studied varieties of *Ocimum basilicum L.* ranged from 0.58% to 1.64%, other previously was reported by (Abduelrahman *et al*, 2009), the essential oil content of the leaf in ninteen types in Sudan ranged from 0.1 to 01.54%.

In this study (Table.3.1) show the yield obtained and physicochemical properties of two types of Basil *Ocimum* oils the resultsice showed that the percenteg of oil yield of *O.basilicum* purple is high (1.5±0.1%) the oil yield of *O.basilicum L.* green (0.8±0.1%). The difference appeared, might be due to ecological factors, and the influence of storage time on volatile oil (Rimantas, 1996). The oil extract of *Ocimum basilicum L*. was found that have a similar value in physicochemical characteristics (Table.3 1), almost the same numerical values.

Refractive index: the range is 1.51±0.4 for *O.basilicum L. cv*. green and is 1.49±0.6 for *O.basilicum L. cv*.purple. It is a ratio found within the range of essential oils.

Density ranged: the percentage of *O.basilicum L. cv*.purple is 0.91g cm^{-3} *O.basilicum L. cv*. Green 0.86 g cm^{-3}. These values are similar to the results of *Ocimum basilicum L. essential* oil which studied by (Hadj *et al*, 2012).

Viscosity value: *O.basilicum L. cv*.purple is 3.37 *O.basilicum L. cv*. Green 3.18. All essential oil characterized by low viscosity value**.**

Table.3.1: Show Physical and chemical Properties of two types of *Ocimum basilicum:*

Properties		
Latin name	***Ocimum basilicum* Purple**	***Ocimumbasilicum* White**
Yield oil%	1.5%	0.8%
Colour	Yellow	pale yellow
Refractive index	1.49±0.6	1.51±0.4
Density	0.91g cm^{-3}	0.86g cm^{-3}
Viscosity value	3.37	3.18
Saponification value	109	137
Iodine value	31.73	38.07
Acid value	2,80	4.48
Peroxide value	0.0	0.0
Moisture	19.5±1	20±0.2

Saponification value: Saponification value for extracted oil was 109 for *O.basilicum L. cv.*purple, 137 for *O.basilicum L. cv*. green which is considered a high value indicating that the content of oil extracted from fatty acids is high when compared with other essential oils, i.e. roses (8 – 21). The saponification values showed richness in long fatty acid chain (Barkatullah *et al,* 2012).

Iodine value: Iodine value was ranged from 31.72 for *O.basilicum L. cv.*purple to 38.07, the iodine value is the indicative of who described that most of the oils are were ranged from 118±0.11 to157±0.65 this value indicat on number of the unstaureted bonds

The acid number: Acid value for *O.basilicum L. cv.*purple is 2.9. This value indicate the molecular decomposition for oil components during distillation, but acid value for *O.basilicum L.cv*. green 4.4, this value lies within the range of volatile oils. Oil with low free fatty acids, has more significant usage.

Peroxide value: In both oils it was found that equal zero means the oil extract in doesn't contain any peroxidy substance.

In the results study have almost the same numerical values for viscosity ranged from 3.37 for *O.basilicum L. cv.*purple to 3.18 for *O.basilicum L. cv*. Green respectively. All volatile oils were characterized by low viscosity than fixed oils (Barkatullah *et al*, 2012).

Moisture range: In the study have almost the same numerical values for moisture ranged from 19.40 for *O.basilicum L. cv.*purple to 20 for *O.basilicum L. cv*. green respectively; this value is highedue to several environmental factors.

The results of the chromatographic analyses obtained for the essential oil is shown in (Figure.1, Table.2). 39 and 33 constituents of essential oil composition for *O.basilicum L.cv.* purple and *O.basilicum cv*. green respectively.

3.2 Chemical Compositions by GC-Mas Spectrometry.

The Chemical compositions of essential oils of *Ocimum basilicum L.* are shown in (Table 3.2.). In the order of the retention times of the constituents 39 constituents were identified in *O. basilicum L.* Purple, representing (100% of the total oil).

Linalool (30.10%), methyl eugenol (20.56%), geraniol (11.35%), methyl cinnamate (10.01%), 1,8-cineole (6.55%), E-bergamoten (3.72%), amophrene (2.35%), 2-propenoic acid (1.47%), camphor (1.01%),m-Xylene(1.15%), alph-terpineol (1.15%), Z-ocimene (0.84 %), bicyclogermacrene (0.78%), Borneol (0.76%), was found as the major compounds for *Ocimum basilicum L.* purple. The essential oil of *O. basilicum L.* green constituents was 33 compounds that identified representing (100% of total oil) (Table 3.2.b). Methylcinnamate (35.21%), linalool (16.38%), methyl eugenol (12.87%), methyl chavicol (9.3), 1, 8-cineole (4.92%), 2-propenoic acid (3.95%), E-bergamoten (2.88%), Eugenol (2.95%), tau-cadinol (1.56%), m-xylene (1.15%) camphor (1.10%), Z-ocimene (0.85 %), fenchyl alcohol (0.80%) was found as the major compounds for *O. basilicum L.* green.

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

No.	Compound Name	Retention Time	Area (%)	Height (%)
1.	Etheybenzen	5.218	0.30	0.49
2.	o-xylene	5.407	1.15*	1.73
3.	m-xylene	5.987	0.48	0.75
4.	α-pinene	7.020	0.10	0.16
5.	β-Phellandrene	8.149	0.15	0.23
6.	β-pinene	8.237	0.30	0.44
7.	β-Myrcene	8.644	0.34	0.51
8.	(-)-Limonene	9.775	0.16	0.24
9.	1,8 Cineole	9.854	6.55*	9.43
10.	(E)-Ocimene	10.353	0.84	1.20
11.	trans-sabinene hydrate	10.991	0.08	0.12
12.	E-Linalool oxide	11.118	0.12	0.15
13.	L-Fenchone	11.596	0.52	0.67
14.	β-Linalool	12.039	30.10*	23.97
15.	Fenchol	12.454	0.56	0.79
16.	L-Camphor	13.337	1.01	1.28
17.	Borneol	14.045	0.76	0.76
18.	Terpinen-4-ol	14.350	0.21	0.27
19.	α-Terpineol	14.774	1.15	1.42
20.	Chavicolmethyl ether (anisol)	14.958	0.42	0.52
21.	(E)-Geraniol	16.630	11.35*	11.65
22.	Z-Methylcinnamate	18.104	1.47	1.75
23.	p-Eugenol	19.588	0.35	0.35
24.	Gernyl acetate	20.156	0.35	0.48
25.	2-propenoic acid	20.323	10.01*	10.17
26.	β-Elemene	20.480	0.59	0.82
27.	Methyl eugenol	20.874	20.56*	17.99
28.	trans Caryophyllene	21.272	0.16	0.21
29.	α-bergamonten	21.622	3.72*	4.85
30.	α-Guaiene (azulene)	21.726	0.18	0.27
31.	α-Humulene	22.185	0.15	0.17
32.	β-Cubebene	22.423	0.16	0.21
33.	Germacrene-D	22.907	0.53	0.45
34.	Biocyclogermacre	23.304	0.78	0.91
35.	Azulene(delta Guaie)	23.511	0.54	0.57
36.	α-Amorphene	23.740	0.99	1.17
37.	(+) β-Funebrene	23.912	0.21	0.19
38.	Cubenol	26.286	0.26	0.28
39.	Tau-Cadinol	26.910	2.35	2.38
Total			**100.00%**	**100.00%**

Table 3.2b: Show Chemical **Composition by GC-MS of *Ocimum basilicum L.* green:**

NO.	**Compound Name**	**Retention Time**	**Area (%)**	**Height (%)**
1.	Etheybenzen	5.208	0.3	0.48
2.	o-xylene	5.397	1.15	1.64
3.	m-xylene	5.977	0.51	0.77
4.	α-pinene	7.007	0.11	0.16
5.	Camphen	7.425	0.03	0.06
6.	Sabinene	8.133	0.10	0.16
7.	β-pinene	8.224	0.19	0.30
8.	β-Myrcene	8.632	0.15	0.22
9.	(-)-Limonene	9.758	0.15	0.24
10.	1,8Cineole	9.833	4.92*	7.34
11.	E-ocimene	10.336	0.85	1.19
12.	γ-Terpinene	10.668	0.07	0.10
13.	trans-sabinene hydrate	10.972	0.07	0.09
14.	E-Linalool oxide	11.099	0.08	0.11
15.	Fenchone	11.578	0.77	0.97
16.	β-Linalool	11.989	16.38*	17.18
17.	Fenchol	12.427	0.80	1.07
18.	L-Camphor	13.319	1.10*	1.39
19.	Isoborneol	14.030	0.41	0.42
20.	Terpinen-4-ol	14.335	0.61	0.74
21.	α-Terpineol	14.765	0.78	0.89
22.	Methy Chavical	14.968	9.31*	11.33
23.	Geraniol	16.581	0.32	0.34
24.	2-propenoic acid	18.101	3.65*	4.43
25.	Eugenol	19.587	2.95*	2.89
26.	Methyl cinnamate	20.372	35.21*	24.75
27.	Methyl eugenol	20.842	12.87*	13.65
28.	α-bergamonten	21.609	2.88*	3.70
29.	Germacrene-D	22.894	0.43	0.36
30.	Biocyclogermacrene	23.292	0.30	0.35
31.	Delta Guaiene	23.499	0.28	0.30
32.	α-Amorphene	23.728	0.72	0.86
33.	Tau-Cadinol	26.896	1.56	1.52
			100.00	**100.00**

Among the oxygenated compounds such as linalool, 1,8-cineole, methyl chavicol, methyl eugenol, geraniol, (Except for *O. basilicum L. cv*. green), terpineol in samples was found to be low, geranylacetate (0.48%), terpinen-4-ol (0.27%), eugenol (0.35%). α-pinene, sabinene, β-pinene, myrcene, limonene, ocimene, thujene, terpinene as the most important monoterpenes hydrocarbons. The essential oil of *O. basilicum L.*In this study the contents of monoterpenic hydrocarbons were found in a low ratio in both types. These results are similar to results of a study by (Wesolowska *et al*, 2012) were they reported chemical composition of three sweet basil oils (*Ocimum basilicum L.).* Forty-eight compounds (95.08% of the total oil) were identified in the essential oil of *O. basilicum L.* 'Thai Siam' the main components found in the oil linalool ranged from (24.60 to 36.60%, E-methyl cinnamate (18.73 to 21.90%) and methyl chavicol (5.57 to 7.50%). In the essential oil of *O. basilicum L. cv*. 'Bolloso Napoletano', forty constituents were identified, representing 97% of total oil. Linalool (41.09 to 47.75%), methyl chavicol (14.34 to 20.21%) and 1,8-cineole (7.21 to 10.23%) were the major components. Similarly, linalool (37.51 and 48.65%), methyl chavicol (13.41 and 18.55%) and 1,8-cineole (7.72% and 12.59%) were found to be the main constituents of the oil of *O. basilicum L.* 'A Foglie di Lattuga'. A total of 35 compounds were identified (Seyd, 2006) was observed twenty constituents (98.5% of the total oil) were identified in the volatile oil of *O. basilicum L.* purple, the main constituents of oil methyl chavicol (52.4%), linalool (20.1%), epi-α-cadinol (5.9%) and trans-α-bergamotene (5.2%). But in oil of *O. basilicum L. cv*. green, twelve components were characterized representing (99.4% of the total oil), methyl chavicol (40.5%), geranial (27.6%), neral (18.5%) and caryophyllene oxide (5.4%). The oil of green basil was characterized by a high content (46.1%) of citral (neral and geranial). Other previously was reported by (Abdurlrahman *et al*, 2009). The essential oil compositions of 19 types of basil, 14 of them grow in Sudan. GLC separations of the essential oils of basil types contained between 5 and 12 components, all are monoterpenes except for some sesquiterpenes, present in small amounts. Linalool was present in amounts above 3%. Geraniol was detected in amounts up to 27.3%. Methyl eugenol was detected in the essential oil, it represents as much as 9.0%, eugenol from 0.05 to 43.3%, methyl cinnamate content from 1.9 to 42.4%. According to the major constituent the basil types were classified into seven groups, namely, high methyl chavicol, high linalool, high geraniol, linalool- methyl cinnamate, linalool-geraniol, methyl cinnamate-linalool and eugenol-linalool, some types have methyl eugenol in trace amounts (Sanni *et al*, 2008) repoted the analysis of the various parts of the plant showed that *Ocimum basilicum* leaf contains the following eugenol that is responsible for its clove scent. The dried leaves of *O. basilicum* contain 45 compounds are found in volatile oils of this plant with the major compounds being linalool (1.1-65.4%), eugenol, methylchaviol (13.5 to 87.2%), methylcinnam (l0-11.2%), linolen, olimene, α-Pinene (0.2-0.4%), β-Pinene (<1.0%), cineol, anethol, estragol, thymol, citral and camphor, myrcene (0.1-2%), and α-Terpinene (0.03-0.2%).

(Hadj *et al*, 2012) reported that *Ocimum basilicum* essential oil (Algerian species), has been investigated by GC/MS and GC/FID. Fourty compounds have been identified accounting for 97.4%. The major compounds were: Linalool (32.83%), linalyl acetate (16%), elemol (7.44%), geranyl acetate (6.18%), myrcene (6.12%), allo-ocimen (5.02%), α-terpineol (4.9%), (E)-β-ocimene (3.68%) and neryl acetate (3.45%). (Mohamed *et al*, 2015) were analyzed the volatile oil of Egyptian *Ocimum Basilicum L.* Forty two volatile compounds were identified, the major chemical constituents linalool (33.9%), eugenol (8.31%) and 2, 6-dimethyl-6-(4-methyl-3-pentenyl)-bicyclo (3.1%) hept-2-ene (8.04%). Other reports were reported by (Ismail, 2006) that linalool (44.18%), 1,8-cineol (13.65%), eugenol (8.59%), methyl cinnamate (4.26%), iso caryophyllene (3.10%), and a-cubebene (4.97%) are the dominant compounds of the *Ocimum Basilicum L.* essential oil.

The results of this study showed that small amount of the eugenol (0.35%) in *Ocimum basilicum L.* purple, but in *O. basilicum L.cv*. green was found to be (2.89%), this is due to that the latter has stronger scent than the former. And low amounts of some sesquiterpenes are also observed in both types of *Ocimum* oils. The amount of limonene is the same in both types. It should be noted that the major components of essential oils in *O. basilicum L.* are in the order linalool, methyl eugenol, geraniol, methylcinnamate, 1,8-cineole, bergamoten, amophrene, 2-propenoic acid, camphor, alph-terpineol, ocimene, germacrene, methyl chavicol, eugenol, cadinol, fenchyl alcohol. These essential oils of *O. basilicum L.* has high value biological activity, thus, the bioactivity of this oil should be investigated.

(Wierdak *et al*, 2013) reported that the *Ocimum basilicum* essential oils show an impressive chemical variability, with compositions characterized by the predominance of various compounds (chemotypes) related to the sample described in this work. Some studies in the literature suggest linalool, methylcinnamate, 1, 8-cineole, methyl eugenol as the main active agent responsible for repellenting mosquito (Mawada, 2005).

The results of this study shows the chemical composition of the essential oils of two varieties of basil, *Ocimum basilicum L.* purple and *O. basilicum L.* green the dominant component in both varieties was linalool, methyl cinnamate. Horeover, the green variety was characterized by a high content (9%) of methyl chavicol. Amorphen and geranly acetate was not detected in the oil of green basil. The chemical composition of the essential oils of this study suggested that the presence of one chemotype of *Ocimum basilicum*, *Ocimum basilicum L. (*Purple) rich in (linalool), and *O. basilicum L* green rich in methyl cinnamate, which is similar to Wesolowska *et al*, 2012; in which *Ocimum basilicum L.* linalool content.

In this study showed high chemical composition of *O. basilicum* oil is different, according to literature findings, the observed differences may be probably due to different several environmental factors. Harvest time has been observed to affect the chemical content and composition of essential oil, different chemotypes and the nutritional status of the plants as well as other factors that can influence the oil composition (Cedric, 2014).

3.3 Repellent Test Results:

Table 3.3: shows repellent 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 5% to 10% concentration of basil oil creams offered the complete and total percent of protection as 85%, 99% respectively up to 2± 0.03 h, 3.10 ± 0.03h, respectively. Though, the repellency effects of basil oil creams is relatively inferior with the lower concentrations, at higher concentrations the basil has offered similar protection like *C. citrates* (Akono Ntonga, 2014). This could be due to increased volatilization of the applied essential oil with time, reducing efficacy.

The present investigation results suggest that the basil oil cream has displayed various degree of repellency at various concentrations against A*nopheles* (Table 3.3). It could be possibly explained that the speedy loss of repellent activity is due to the significantly faster volatilization rate of bio-active compounds of the essential oil rather than the lack of efficacy (Gabi Baba, 2012). All two tested concentrations of basil oil cream offered significant protection and student's test results show statistically significant difference between treated and control groups. In Kenya, essential oils of *Ocimum basilicum*, different concentrations were evaluated for repellency on forearms of human volunteers against anopheles, and were found to be more effective than DEET (Koech *et al*, 2013).

This finding is in line with a study conducted in Sudan by (Nour *et al*, 2009) it was reported that when the essential oil of four types of *Ocimum basilicum* was used at 10% concentration, the mixture offered protection against mosquito only for 2h with 92–100% of repellency. However, while increasing the concentration of this oil blend to 20% the complete protection was increased dramatically to 2½h with nearly 100% of repellency.

The percentage of protection in relation to the dose method was performed and found that the basil oil cream shown various degrees of repellency impact against anopheles. It has provided the maximum total percentage of protection of about (100%) repellency.

(Apiwat Tawatsin *el at*, 2001) reported the protection based on type plant, the volatile oil obtained from hairy basil exhibited the least repellency among the oils from five *Ocimum spp.*, being only fifteen minutes against anopheles Aegypti, but those of

O.gratissimum, O.basilicum, O.basillicum L. fa. *Citratum* and *O. tenuiflorum* were 135, 75, 75 and 105 minutes respectively. The oils from turmeric, citronella grass and hairy basil, especially with the addition basil as topical repellents against both day- and night-biting mosquitoes, and repellents providing at least four hours of protection under mosquito cage conditions were then tested for efficacy under large room conditions.

Table 3.3: Show Repellent test result:

TEST	**Time/hour**	**Treatment 5%**		**Treatment 10%**		**Control**	
		Landing	Biting	Landing	Biting	Landing	Biting
1	0	0	1	0	0	9	9
2	0.5	0	2	0	0	9	9
3	1	0	2	0	0	12	12
4	1.5	2	2	0	0	13	13
5	2	2	2	0	0	14	14
6	2.5	3	2	0	0	14	14
7	3	4	3	2	1	14	14

Percentage of repellency	**Treatment of oil 5%**	**Treatment of oil 10%**
	85%	99%

(Opender *et al.,* 2008) showed that in the results investigations the essential oil of *citronella, cedar, verbena, pennyroyal, geranium, lavender, pine, cinnamon, rosemary, thyme, basil*and *peppermint, Eucalyptusoil of turmeric, kaffir lime, citronella grass* and *citronella* cream were shown to possess repellency against mosquitoes less than 2h but in the results investigation by (Aipwat Tawatsin *et al*, 2001), these oils possess repellency against mosquitoes above 3h, and the repellency increase with concentration.

In this study, we tested the repellent activity of basil oil creams (two type's O. *basilicum* essential oil) against Anopheles mosquito. These results, somewhat, same with some earlier studies in repellent activity of basil essential oil against mosquito; but these also differ with some reported studies (Mawada, 2005), the differences can be attributed to many factors; perhaps the most obvious is the difference in concentration of essential oil, also some researchers were observed the *Ocimum basilicum* oil which have high mosquito repellent activity with the emergence of mosquito paralysis at concentrations of 3% giving 100% protection. This activity is similar to the reported effect of DEET (Koech *et al*, 2013). Other reported by (Gabi Baba, 2012) showed that the 50% concentration of *O. basilicum* oil exhibited higher repellant potential on anopheles gambiae with protection time of 183 and 120 min, respectively.

The results in this work indicated that the repellent potential of the oils is concentration dependent. The paralytic activity is due to the action of terpenon oil and peripheral nervous system resulting in disruptions of signal transmission along the nerveaxon (Koech, 2014). The exact mode of action of sweet basil oil is still unknown and its activity is most likely due to camphor and limonene, methyl chavicol, linalool, p-cymene, terpinene, interestingly 2-decenol and decanal were the most predominant constituents in leaf oil known for repellent activity (Nour *et al*, 2012). Thus sweet basil oil serves to paralyze mosquitoes and block them on the skin surface since it acts in a vapour-phase. These results indicated that basil oil cream have potential control mosquito.

Formulation in form of a jelly might have helped in improving its efficacy due to the activity of Vaseline (pure petroleum jelly). This could be due to the presence of liquid paraffin and paraffin wax (whit wax) gum Arabic, gum Arabic is a natural emulsifier obtained from *Acacia Senegal.* It is an acidic polysaccharide with important applications in pharmaceutical formulations. The polysaccharide structure and hydrophilic nature enable gum Arabic to be used as a suspending agent or viscosity modifier. Other components present in the jelly (Koech, 2014). From knowledge of the basil oil creams components; mosquito repellent activity can be use.

Basil oil creams based repellents are safe for humans. Nevertheless, it did not cause any adverse effect in terms of discomfort or skin irritations on the human volunteers during and after the study period. Basil oil cream based products are generally regarded to be safe, compared to the synthetic repellents.

In these perspectives, plant-based insect repellents could be extremely helpful in avoiding mosquito bites and ultimately disease transmission, when other vector control interventions are impossible. Hence, cream formation from the essential oil of basil medicinal plant could serve as a repellent agent against mosquito. The majority of the existing modern synthetic chemical repellents are relatively expensive. Besides their toxicity and adverse side effects, a few of them require electricity for their usage (Gupta, 2012). Plant-based repellents are extremely useful in the in accessible rural areas, wherever there is a lack of electricity. Furthermore, plant-based repellent products are culturally acceptable, economical and locally known and available. This study could not describe the rearing details, e.g., soil, water. And nutritional conditions of the plant materials used for oil extraction because the study plants were purchased from the local area. The quality of volatile oils depends on many factors, e.g., plant species, rearing conditions, maturation of harvested plants, plant storage, plant preparation and methods of extraction. Thus, these foctors should be carefully considered and standardized when the extraction of volatile oils is being planned. Basil oil creams were prepared with light liquid paraffin and evaluated for in vitro antimicrobial activity, skin irritation test, cream was found to be constant over 90 days, no reports are available about any possible side-effects on external usage of basil oil. Basil oil creams, externally, usage have antimicrobial activity.

3.4 Conclusion:

In conclusion, this study clearly demonstrated the potential of cream preparation with volatile oils derived from *O.basilicum*, for use as topical repellents against mosquitoes. *O.basilicum.cv.* green has strong odour like lemon and *O. basilicum.cv* purple like clove odour. To improve their repellent efficacy, the oil should be formulated with vanillin or other oil have repellent activity and could replace DEET, currently the most common chemical repellent available.

3.5 Recommendation:

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

- Producing the basil oil cream industrially and registering it as a pharmaceutical product for public use.
- Further studies should emphasize planting *O. basilicum* under appropriate agricultural conditions as well as required testing in a wider range.
- Conducting Study the effect of the basil oil cream with the passage of time.
- Determing nutritional value of minerals in basil oil.
- Planting *O. basilicum* under appropriate agricultural conditions in order to obtain the best yield.
- Conduct research using basil oil in the treatment of wounds and cosmetic products (soap, Hand Lotion).
- Identify and isolate the most effective material to repel mosquitoes.
- Study other options of mulsifiers to use them with *O. basilicum* to make the repellent.

- Study the stability properties of the cream and its shelflife.
- Evaluate the health effects of the cream.
- Feasabilty studies for products.
- Blending the fixed oil with essential oil to prepare the repellent.

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