Extraction of Essential Oil from Spearmint

(*Mentha spicata var. Viridis L.*)

By:

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الآية

بسم الله الرحمن الرحيم

قال تعالى:

لا يُظْلِمُ اللَّهُ نَفْساً إِلَّاً وَسَعَمَا لَهَا مَا حَسَبَهَا وَعَلَيْهَا مَا حُسِبَهُ

رَبَّنَا لاَ تُؤْخَذْنَا إِنَّ نَسِيَانَا أَوْ أَخْطَأْنَا رَبَّنَا وَلَا تَخْمِلْ عَلَيْنَا إِصْرًا كَثِّمَا

حَمْلَتُهُ عَلَى الْجَنِينِ من قَبْلَنَا رَبَّنَا وَلَا تَحمِلْنَا مَا لَانَ طَلَّقَةً لَّنَا بِهِ

وَاعْفَنَّا عَنْهَا وَاعْفُوْنَ عَنْ آخَرِينَ لَا وَارَكِنَّا أَنْتَ مَوْلُوْدَنَا فَانْصُرْنَا عَلَى الْهُجْرَةِ

الْحَافِرِينَ

صدق الله العظيم

سورة البقرة (286).
DEDICATION

TO

My parents
Sisters
Brother
& my lovely mother

With my great and deep love.
ACKNOWLEDGEMENT

I am deeply indebted to my supervisor PROF. ALI ABDALRHMAN RABAH Who helped with stimulating suggestions and encouragement in the research and writing this thesis.
Abstract:

A large number of herbal materials contain Essential Oils with extensive bioactivities. It is important to acknowledge plants and their medicinal value. There are a variety of methods for obtaining volatile oils from plants, such as extraction by organic solvent and steam distillation. In this study, steam distillation was used to extract oil from spearmint because this method is recommended for oil extracted for medical purposes. In addition, a reputable distiller will preserve the original qualities of the plant oil. Analysis of Essential oil was done using Gas Chromatography-Mass Spectrometry (GC-MS), in order to evaluate the essential Oil qualitatively and quantitatively. The GC-MS analysis showed twenty nine peaks each contained several components. The major components were: Carvomenthone (4.47%), myrcene (4.49%), piperitone (5.24%), limonene (19.99%) and carvone (42.26%) of the total component. Physico-chemical properties of the extracted oil were determined according to the British Standards Institute (BSI). Results were as follows: Acid value (6.45 mg), saponification value (42.08 mg), Peroxide value (6.6 mg), Iodine value (130.28 mg), Refractive index (at 25.1°C) (1.488) and Density was 0.93 at room temperature.
المستخلص:

عدد كبير من الاعشاب الطبيعية يحتوي على الزيوت العطرية التي لديها استعمال واسع في
الأنشطة الحيوية، من الضروري معرفة مدى أهميتها واستخداماتها الطبية. هناك عدة طرق للحصول
على الزيوت العطرية من النباتات مثل الاستخلاص بالمذيبات العضوية والتقطير البخاري، وفي هذه
الطريقة تم استخلاص زيت النعناع البلدي عن طريق التقطير البخاري لأنها الطريقة الموصى بها
للزيوت المستخلصة للأغراض الطبية. كما أن الزيت المستخلص بهذه الطريقة يحتفظ بالصفات الأصلية
للنبات. تم تحليل الزيت العضري (زيت النعناع) باستخدام جهاز اللوني الغازي-قياس الطيف الكتلي
(GC-MS) ، من أجل تقييم الزيت العضري كمياً وكيفاً. أظهرت تحاليل جهاز (GC-MS) تسع وعشرون
قيمة، كل منها يحتوي على مركبات عديدة. المركبات الأساسية هي: كارفونثون (4.47%)، مايرسين
(4.49%)، بيرتون (5.24%)، بيرمونين (19.99%)، والكارفون (42.26%). تم تحديد الخصائص
الفيزيميكائية طبقاً للمعايير البريطانية. كانت النتائج على النحو التالي: قيمة الحمض (6.45 مل)،
قيمة التصين (42.08 مل)، قيمة البيروكسيد (6.6 مل)، قيمة اليود (130.28 ملجم)، معامل الانكسار
 عند (25.1 °C) (1.488)، والكثافة (0.93).
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Chapter One: Introduction and Objective
1. Introduction and objective

1.1- Introduction

Plants have played an important role in the fields of drugs, perfumes and other chemicals of important economic values. Medicinal and aromatic plants were well known since ancient civilizations, Egyptian, Indian, Chinese and Japanese; and still many people continue to handle them using the primitive methods. Before the eighteenth century (1800) slow progress was made in photochemistry, while through the nineteenth century such progress became more rapid. The medicinal and aromatic plants have been collected from ancient times as roots, tree barks, leaves, blossoms and seeds. They were gathered from mountain tips, forests, rivers, and sea shores to satisfy people's need in many ways especially the folkloric medicines, spices and perfumes beside the recent pharmaceutical uses. The main sources of drugs now are the active ingredient obtained synthetic materials and natural plant products. Synthetic drugs increased with the development in technical know-how but human diseases and illness increased too because of the toxic and poisonous effects of synthetic drug.

On the other hand, natural medicinal and aromatic plants are not harmful to human body. Also the ingredients or active substances obtainable from medicinal and aromatic plants are important because of their association with human and the valuable substances cannot be supplied by single synthetic drug, rather one must resort to poly-pharmacy with its potential dangers. But the amounts of drugs obtained from medicinal plants are very small to satisfy the increasing world population hence more efforts should be done towards more production of medicinal and aromatic plants.

Sudan, with its variety of climatic zones and various environmental features, is very rich in flora which can serve as a large potential source for valuable natural products and substances obtained medicinal and aromatic plants. Such potential are marginally now to meet the demand for these natural products, locally and internationally, by collection and cultivation of the plants and flora on commercial basis.
Then Sudan can earn foreign currency and become self-dependent in the pharmaceutical, perfumeries, confectioneries and allied industries as well as being able to supply the foreign markets with finished or semi-finished products.

The most important Sudanese medicinal and aromatic plants containing essential oil for commercial production of high-return only marginally exploited are the following species: coriander, fennel, dill, anise, caraway, parsley and those are commercially cultivated, at present, mainly in the Northern region and few in central region and various parts of the Sudan. Nevertheless, culinary herbs such as wild basil (Ocimum basilicum), peppermint (Mentha piperita), spearmint (Mentha spicata), Japanese mint (Mentha arvensis) are of great potential and can be exploited. The other potential sources of essential oil are plants like lemongrass (Cymbopogon citratus) and camel’s hay “mahareb” (Cymbopogon proximis); and “Seida” (Cyperus rotundas), some of which are used as local perfumery products and in local medicine beside (Citrus spp.) and Eucalyptus “kapkor” specially (Eucalyptus citriodora) which was introduced from abroad. Further investigations in rose, thuja, jasmine, nal, mahareb, seida… est. Are needed in order to be established on commercial basis.

One of the major important essential oil crops are mint plants which exist widely in Sudan specially the local species namely common spearmint (Mentha officata L.), and the traditional type (Mentha officinalis) in Jebal Marra mountains few other species such as Japanese mint (Mentha arvensis) and peppermint (Mentha piperita) are available in research units only. Spearmint (M. spicata) is a perennial herb known as “Nanaa baladi”, it is known in Sudan and many countries widely for its medicinal, aromatic and flavoring properties.

Peppermint (Mentha piperita), was introduced to Sudan from abroad, and successfully cultivated to a limited experimental scale in Khartoum area and is expected to do even better in Northern Sudan and in Jebal Marra in Western Sudan. In contrast, it was a failure in the Gezira area because of environmental limitations.

Japanese mint (Mentha arvensis), which is a herbaceous perennial plant was introduced from abroad into Sudan and very limited scientific research abroad into done on it.
Although extensive studies were carried out in many countries on the cultivation of the two mint species, (the spearmint and Japanese mint), limited systematic research has been done to explore their herbal and essential oil content as well as their economic value in Sudan. (ELRasheed, 1977)

1.2 - Objective

The objective of the present study is to extract the spearmint oil and determine its constituents. As well as determine the physical and chemical properties of the extracted oil.
Chapter Two: Literature Review
Chapter Two

2 - Literature Review

2.1- Essential Oils

Essential oils are concentrated volatile aromatic compounds produced by plants the easily evaporated essences that give plants their wonderful scents. Each of these complex precious liquids is extracted from a particular species of plant life. Each plant species originates in certain regions of the world, with particular environmental conditions and neighboring fauna and flora. Essential oils are frequently referred to as the “life force” of plants. Unlike fatty oils, these "essential" oils are volatile, highly concentrated, substances extracted from flowers, leaves, stems, roots, seeds, bark, resin or fruit rinds. The amount of essential oils found in these plants can be anywhere from 0.01 percent to 10 percent of the total. That's why tons of plant material are required for just a few hundred pounds of oil. These oils have potent antimicrobial factors, having wide range of therapeutic constituents. These oils are often used for their flavor and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicines, and cosmetics. Beware of imitations. Essential oils cannot be substituted with synthetics. Only pure oils contain a full spectrum of compounds that cheap imitations simply cannot duplicate. (Rao., Pandey. 2006).

2.2 - Spearmint Oil

Plants possess a long therapeutic history over thousands of years and even considered to be a hopeful source of medicine in the traditional health care system (Hemalatha .Manish .2008).


So, the interest in a large number of traditional natural products has increased (Taylor ,Hudson,Towers.1996). Therefore, the growing interest, both in the industry and in the scientific research, for aromatic and medicinal plants because of their antimicrobial and antioxidant properties. These attributes are due to many active phytochemicals including flavanoids, terpenoids, carotenoids, coumarins, curcumines etc. These bioactive principles have also been confirmed using modern
analytical techniques. The role of medicinal plants for the treatment of various ailments as old as the history of man. Worldwide, about 80% of traditional medicines used in primary health care are derived from plants (Akerele, Guo .1985). Medicines derived from plants possess a potentially safer and more consistent medicine than synthetically produced drugs (Ripunjoy, S. 2013). Mint plants are important essential oil crops that exist widely in Sudan, specially the common spearmint (*Mentha spicata* L.). Spearmint species belong to the family Labiatae (Lamiaceae), which is considered as a famous medicinal and aromatic family (Guenther, E. 1975). Spearmint is a perennial herb known as ‘Nanaa baladi’. It is well known in Sudan and in many Arab countries, for its medicinal, aromatic and flavoring (Salim, E.A. 1997). Spearmint is cultivated in different parts of the world, in North America, England, Germany, Holland and in the Mediterranean region (Guenther, E. 1975). Total annual production is about 1500 tons, 30% – 50 % being of the native type and 50% – 70 % of Scotch type. Other minor cultivation areas for spearmint exist in China and India, where high quality oils with up to 80 % carvone are produced (Salim, E.A. 1997). The aim of this scientific review is to give good scientific background description highlighting theoretical and hypothesis focuses on that spearmint have great humanitarian values but so many agronomical and photochemical considerations must be considered for production, post-harvest food uses and therapeutic treatments are recommended to have efficient utilization and uses.

2.2.1 - Botany and description:
2.3 - Distillation Technology for Essential Oils

2.3.1 - Introduction to Distillation

Distillation is the most popular, widely used and cost-effective method for producing essential oils throughout the world. Distillation of aromatic plants simply implies vaporizing or liberating the oils from the plant cellular membranes in the presence of moisture, by applying high temperature and then cooling the vapor mixture to separate the oil from the water on the basis of the immiscibility and density of the essential oil with respect to water.

2.3.2 - Principles of Distillation

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

a) Sensitivity of the essential oil to the action of heat and water.
b) Volatility of the essential oil.
c) Water solubility of the essential oil.

Essential oils with high solubility in water and those that are susceptible to damage by heat cannot be steam distilled. Also, the oil must be steam volatile for steam distillation to be feasible. Most of the essential oils in commerce are steam volatile, reasonably stable to heat and practically insoluble in water; hence they are suitable for processing by steam distillation. Essential oils are a mixture of various aroma chemicals, basically monoterpenes, sesquiterpenes and their oxygenated derivatives, having a boiling point ranging from 150° to 300° C. When the plant material is subjected to heat in the presence of moisture from the steam, these oils are liberated from the plant. For the oil to change from the liquid to the vapor phase, it must receive latent heat that, within the tank, can only come from condensing steam. Consequently, the temperature of the steam within the still must be higher than the temperature at which the oil boils in the presence of water on the surface of the plant material, otherwise there would not be a temperature gradient to take the latent heat from the condensing steam to vaporize the oil droplet. Thus, the energy from the steam in form of heat as latent heat of vaporization converts the oil into a vapor. But, as the boiling point of the oil is higher than that of water, the vaporization takes place with steam on the basis of their relative vapor pressures.
It is imperative to note that a liquid always boils at the temperature at which its vapor pressure equals the atmospheric or surrounding pressure. For any two immiscible liquids, the total vapor pressure of the mixture is always equal to the sum of their partial pressures. The composition of the mixture in the vapor phase (in this case, oil and water) is determined by the concentration of the individual components multiplied by their respective partial pressures. For example, if a sample of an essential oil comprised of component A (boiling point, 190°C) and water (boiling point, 100°C) is boiled, after some time, once their vapors reach saturation, the temperature will immediately drop to 99.5°C, which is the temperature at which the sum of the two vapor pressures equals 760 mmHg. In other words, the oil forms an azeotropic mixture with water. Thus, any essential oil having high boiling point can be evaporated with steam in a ratio such that their combined vapor pressures equal the atmospheric pressure; the essential oil can be recovered from the plant by the wet distillation process.

2.4 - Methods of Producing Essential Oils

Methods for producing essential oils from plant materials are summarized in (Figure - 2.2). Regarding hydrodistillation, the essential oils industry has developed terminology to distinguish three types: water distillation; water and steam distillation; and direct steam distillation. These terms have become established in the essential oil industry. All three methods are subject to the same theoretical considerations which deal with distillation of two-phase systems. The differences lie mainly in the methods of handling the material. Some volatile oils cannot be distilled without decomposition and thus are usually obtained by expression (lemon oil, orange oil) or by other mechanical means. In certain countries, the general method for obtaining citrus oil involves puncturing the oil glands by rolling the fruit over a trough lined with sharp projections that are long enough to penetrate the epidermis and pierce the oil glands located within outer portion of the peel. A pressing action on the fruit removes the oil from the glands, and a fine spray of water washes the oil from the mashed peel while the juice is extracted through a central tube that cores the fruit. The resulting oil-water emulsion is separated by centrifugation. A variation of this process is to remove the peel from the fruit before the oil is extracted. Often, the volatile oil content of fresh plant parts (flower petals) is so small that oil removal is not commercially
feasible by the aforementioned methods. In such instances, an odorless, bland, fixed oil or fat is spread in a thin layer on glass plates. The flower petals are placed on the fat for a few hours; then repeatedly, the oil petals are removed, and a new layer of petals is introduced. After the fat has absorbed as much fragrance as possible, the oil may be removed by extraction with alcohol. This process, known as effleurage, was formerly used extensively in the production of perfumes and pomades.

![Diagram of Plant organs containing essential oils](image)

Figure -2.2: Plant organs containing essential oils.
In the perfume industry, most modern essential oil production is accomplished by extraction, using volatile solvents such as petroleum ether and hexane. The chief advantages of extraction over distillation is that uniform temperature (usually 50°C) can be maintained during the process. As a result, extracted oils have a more natural odor that is unmatched by distilled oils, which may have undergone chemical alteration by the high temperature. This feature is of considerable importance to the perfume industry; however, the established distillation method is of lower cost than the extraction process. Destructive distillation means distilling volatile oil in the absence of air. When wood or resin of members of the Pinaceae or Cupressaceous is heated without air, decomposition takes place and a number of volatile compounds are driven off. The residual mass is charcoal. The condensed volatile matter usually separates into 2 layers: an aqueous layer containing wood naphtha (methyl alcohol) and pyroligneous acid (crude acetic), and a tarry liquid in the form of pine tar, juniper tar, or other tars, depending on the wood used. This dry distillation is usually conducted in retorts and, if the wood is chipped or coarsely ground and the heat is applied rapidly, the yield often represents about 10% of the wood weight used.

2.4.1 - Hydrodistillation

In order to isolate essential oils by hydrodistillation, the aromatic plant material is packed in a still and a sufficient quantity of water is added and brought to a boil; alternatively, live steam is injected into the plant charge. Due to the influence of hot water and steam, the essential oil is freed from the oil glands in the plant tissue. The vapor mixture of water and oil is condensed by indirect cooling with water. From the condenser, distillate flows into a separator, where oil separates automatically from the distillate water.

2.4.1.1 - Mechanism of Distillation

Hydrodistillation of plant material involves the following main physicochemical processes:
- i) Hydrodiffusion
- ii) Hydrolysis
- iii) Decomposition by heat
2.4.1.1.1-Hydrodiffusion

Diffusion of essential oils and hot water through plant membranes is known as hydrodiffusion. In steam distillation, the steam does not actually penetrate the dry cell membranes. Therefore, dry plant material can be exhausted with dry steam only when all the volatile oil has been freed from the oil-bearing cells by first thorough comminution of the plant material. But, when the plant material is soaked with water, exchange of vapors within the tissue is based on their permeability while in swollen condition. Membranes of plant cells are almost impermeable to volatile oils. Therefore, in the actual process, at the temperature of boiling water, a part of volatile oil dissolves in the water present within the glands, and this oil-water solution permeates, by osmosis, the swollen membranes and finally reaches the outer surface, where the oil is vaporized by passing steam. Another aspect of hydrodiffusion is that the speed of oil vaporization is not influenced by the volatility of the oil components, but by their degree of solubility in water. Therefore, the high-boiling but more water-soluble constituents of oil in plant tissue distill before the low-boiling but less water-soluble constituents. Since hydrodiffusion rates are slow, distillation of uncomminuted material takes longer time than comminuted material.

2.4.1.1.2 – Hydrolysis

Hydrolysis in the present context is defined as a chemical reaction between water and certain constituents of essential oils. Esters are constituents of essential oils and, in the presence of water, especially at high temperatures, they tend to react with water to form acids and alcohols. However, the reactions are not complete in either direction and the relationship between the molal concentrations of various constituents at equilibrium is written as:

**Equation (1)**

\[ K = \frac{(\text{alcohol}) \times (\text{acid})}{(\text{ester}) \times (\text{water})} \]

Where K is the equilibrium constant. Therefore, if the amount of water is large, the amounts of alcohol and acid will also be large, resulting in a decreased yield of essential oil. Furthermore, since this is a time-dependent reaction, the extent to which hydrolysis proceeds depends on the time of contact between oil and water. This is one of the disadvantages of water distillation.
2.4.1.1.3 - Effect of Heat

Almost all constituents of essential oils are unstable at high temperature. To obtain the best quality oil, distillation must be done at low temperatures. The temperature in steam distillation is determined entirely by the operating pressure, whereas in water distillation and in water and steam distillation the operating pressure is usually atmospheric. All the previously described three effects, i.e. hydrodiffusion, hydrolysis and thermal decomposition, occur simultaneously and affect one another. The rate of diffusion usually increases with temperatures as does the solubility of essential oils in water. The same is true for the rate and extent of hydrolysis. However, it is possible to obtain better yield and quality of oils by:

(1) Maintaining the temperature as low as possible.
(2) Using as little water as possible, in the case of steam distillation.
(3) Thoroughly comminuting the plant material and packing it uniformly before distillation.

2.4.2 - Three Types of Hydrodistillation

There are three types of hydrodistillation for isolating essential oils from plant materials:
1. Water distillation
2. Water and steam distillation
3. Direct steam distillation

2.4.2.1 - Water Distillation

In this method, the material is completely immersed in water, which is boiled by applying heat by direct fire, steam jacket, 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. When the still is heated by direct fire, adequate precautions are necessary to prevent the charge from overheating. When a steam jacket or closed steam coil is used, there is less danger of overheating; with open steam coils this danger is avoided. But with open steam, care must be taken to prevent accumulation of condensed water within the still. Therefore, the still should be well insulated. The plant material in the still must be
agitated as the water boils, otherwise agglomerations of dense material will settle on the bottom and become thermally degraded. Certain plant materials like cinnamon bark, which are rich in mucilage, must be powdered so that the charge can readily disperse in the water; as the temperature of the water increases, the mucilage will be leached from the ground cinnamon. This greatly increases the viscosity of the water-charge mixture, thereby allowing it to char. Consequently, before any field distillation is done, a small-scale water distillation in glassware should be performed to observe whether any changes take place during the distillation process. From this laboratory trial, the yield of oil from a known weight of the plant material can be determined. The laboratory apparatus recommended for trial distillations is the Clevenger system (Figure 2.3). During water distillation, all parts of the plant charge must be kept in motion by boiling water; this is possible when the distillation material is charged loosely and remains loose in the boiling water. For this reason only, water distillation possesses one distinct advantage, i.e. that it permits processing of finely powdered material or plant parts that, by contact with live steam, would otherwise form lumps through which the steam cannot penetrate. Other practical advantages of water distillation are that the stills are inexpensive, easy to construct and suitable for field operation. These are still widely used with portable equipment in many countries. The main disadvantage of water distillation is that complete extraction is not possible. Besides, certain esters are partly hydrolyzed and sensitive substances like aldehydes tend to polymerize. Water distillation requires a greater number of stills, more space and more fuel. It demands considerable experience and familiarity with the method. The high-boiling and somewhat water-soluble oil constituents cannot be completely vaporized or they require large quantities of steam. Thus, the process becomes uneconomical. For these reasons, water distillation is used only in cases in which the plant material by its very nature cannot be processed by water and steam distillation or by direct steam distillation.
Floral attars are defined as the distillates obtained by hydrodistillation of flowers (such as saffron, marigold, rose, jasmine, pandanus) in sandal wood oil or other base materials like paraffin. Attar manufacturing takes place in remote places because the flowers must be processed quickly after collection. The apparatus and equipment used to manufacture attar are light, flexible, easy to repair, and have a fair degree of efficiency. Keeping in view these facts, the traditional “deg and bhapka” process has been used for centuries and is used even now with the following traditional equipment (Figure -2.4).

- Deg (still)
- Bhapka (receiver)
- Chonga (bamboo condenser)
- Traditional bhatti (furnace)
- Gachchi (cooling water tank)
- Kuppi (leather bottle)
2.4.2.1.2 - 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, so 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, 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 either water and steam distillation or direct steam distillation.

2.4.2.2 - Water and Steam Distillation

In water and steam distillation, the steam can be generated either in a satellite boiler or within the still, although separated from the plant material. Like water distillation, water and steam distillation is widely used in rural areas. Moreover, it
does not require a great deal more capital expenditure than water distillation. 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. In fact, it is common that persons performing water distillation eventually progress to water and steam distillation. It follows that once rural distillers have produced a few batches of oil by water distillation, they realize that the quality of oil is not very good because of its still notes (subdued aroma). As a result, some modifications are made. Using the same still, a perforated grid or plate is fashioned so that the plant material is raised above the water. This reduces the capacity of the still but affords a better quality of oil. If the amount of water is not sufficient to allow the completion of distillation, a cohobation tube is attached and condensate water is added back to the still manually, thereby ensuring that the water, which is being used as the steam source, will never run out. It is also believed that this will, to some extent, control the loss of dissolved oxygenated constituents in the condensate water because the re-used condensate water will allow it to become saturated with dissolved constituents, after which more oil will dissolve in it.

2.4.2.2.1 – Cohobation

Cohobation is a procedure that can only be used during water distillation or water and steam distillation. It uses the practice of returning the distillate water to the still after the oil has been separated from it so that it can be re-boiled. The principal behind it is to minimize the losses of oxygenated components, particularly phenols which dissolve to some extent in the distillate water. For most oils, this level of oil loss through solution in water is less than 0.2%, whereas for phenol-rich oils the amount of oil dissolved in the distillate water is 0.2%-0.7%. As this material is being constantly re-vaporized, condensed and re-vaporized again, any dissolved oxygenated constituents will promote hydrolysis and degradation of themselves or other oil constituents. Similarly, if an oxygenated component is constantly brought in contact with a direct heat source or side of a still, which is considerably hotter than 100° C, then the chances of degradation are enhanced. As a result, the practice of cohobation is not recommended unless the temperature to which oxygenated constituents in the distillate are exposed is no higher than 100° C. In steam and water distillation, the plant material cannot be in direct contact with the fire source beneath the still; however, the walls of the still are good conductors.
of heat so that still notes can also be obtained from the thermal degradation reactions of plant material that is touching the sides of the still. As the steam in the steam and water distillation process is wet, a major drawback of this type of distillation is that it will make the plant material quite wet. This slows down distillation as the steam has to vaporize the water to allow it to condense further up the still. One way to prevent the lower plant material resting on the grid from becoming waterlogged is to use a baffle to prevent the water from boiling too vigorously and coming in direct contact with the plant material.

![Distillation unit with cohabation.](image)

Figure - 2.5: Distillation unit with cohabation.

### 2.4.2.2.2 - Advantages of Water and Steam Distillation over Water Distillation

- Higher 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 and water distillation is faster than water distillation, so it is more energy efficient.
2.4.2.3 - Disadvantages of Water and Steam Distillation

- Due to the 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 the water from boiling too vigorously and coming in direct contact with the plant material.

2.4.2.3 - Direct Steam Distillation

As the name suggests, direct steam distillation is the process of distilling plant material with steam generated outside the still in a satellite steam generator generally referred to as a boiler. As in water and steam distillation, the plant material is supported on a perforated grid above the steam inlet. A real advantage of satellite steam generation is that the amount of steam can be readily controlled. Because steam is generated in a satellite boiler, the plant material is heated no higher than 100°C and, consequently, it should not undergo thermal degradation. Steam distillation is the most widely accepted process for the production of essential oils on large scale. Throughout the flavor and fragrance supply business, it is a standard practice. An obvious drawback to steam distillation is the much higher capital expenditure needed to build such a facility. In some situations, such as the large-scale production of low-cost oils (e.g. rosemary, Chinese cedar wood, lemongrass, litsea cubeba, spike lavender, eucalyptus, citronella, cornmint), the world market prices of the oils are barely high enough to justify their production by steam distillation without amortizing the capital expenditure required to build the facility over a period of 10 years or more.

2.4.2.3.1 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.

2.4.2.3.2 - Disadvantage of Direct Steam Distillation

- Much higher capital expenditure needed to establish this activity than for the other two processes.

2.4.3 - Modern (Non-traditional) Methods of Extraction of Essential Oils

Traditional methods of extraction of essential oils have been discussed and these are the methods most widely used on commercial scale. However, with technological advancement, new techniques have been developed which may not necessarily be widely used for commercial production of essential oils but are considered valuable in certain situations, such as the production of costly essential oils in a natural state without any alteration of their thermo sensitive components or the extraction of essential oils for micro-analysis. These techniques are as follows:

- 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)
- Thermo micro distillation
- Micro distillation
- Molecular spinning band distillation
- Membrane extraction
2.5 - Purification of Crude Essential Oils

Essential oil as obtained from the oil separator is in crude form. It may have suspended impurities and appreciable moisture content. It might even contain some objectionable constituents which degrade its flavor quality. The presence of moisture and impurities adversely affects the keeping quality of oil and accelerates polymerization and other undesirable reactions. Addition of a drying agent like anhydrous sodium sulphate to the oil, standing overnight followed by filtration will remove the moisture and free the oil of suspended impurities. Use of high-speed centrifugation to clarify the essential oils is common. Essential oils are frequently rectified or re-distilled to remove objectionable constituents. In order to keep the temperature of re-distillation within permissible limits, the process is carried out under vacuum or with the help of steam distillation.

2.6 - Chemical Constituents of Essential Oils

Pure essential oils are mixtures of more than 200 components, normally mixtures of terpenes or phenylpropanic derivatives, in which the chemical and structural differences between compounds are minimal. They can be essentially classified into two groups:

**2.6.1 Volatile fraction:** Essential oil constituting of 90–95% of the oil in weight, containing the monoterpenes and sesquiterpenes hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

**2.6.2 Nonvolatile residue:** that comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids.

2.6.3 - Hydrocarbon:

Essential Oils consist of Chemical Compounds that have hydrogen and carbon as their building blocks. Basic Hydrocarbon found in plants are isoprene having the following structure.

\[
\text{CH}_2=\text{C} \equiv \text{CH} \equiv \text{CH}_2 \\
\text{CH}_3
\]

(Isoprene)
2.6.4 - Terpenes:
Generally have names ending in “ene.” For examples: Limonene, Pinene, Piperene, Camphene, etc. Terpenes are anti inflammatory, antiseptic, antiviral, and bactericidal. Terpenes can be further categorized in monoterpenes, sesquiterpenes and diterpenes. Referring back to isoprene units under the Hydrocarbon heading, when two of these isoprene units join head to tail, the result is a monoterpene, when three join, it’s a sesquiterpene and four linked isoprene units are diterpenes.

2.6.5 - Monoterpenes \([C_{10} H_{16}]\)

**Properties:** Analgesic, Bactericidal, Expectorant, and Stimulant. Monoterpenes are naturally occurring compounds, the majority being unsaturated hydrocarbons (C10). But some of their oxygenated derivatives such as alcohols, Ketones, and carboxylic acids known as monoterpenoids. The branched-chain C10 hydrocarbons comprises of two isoprene units and is widely distributed in nature with more than 400 naturally occurring monoterpenes identified. Moreover, besides being linear derivatives (Geraniol, Citronellol), the monoterpenes can be cyclic molecules (Menthol – Monocyclic; Camphor – bicyclic; Pinenes (α and β) – Pine genera as well. Thujone (a monoterpene) is the toxic agent found in Artemisia absinthium (wormwood) from which the liqueur, absinthe, is made. Borneol and camphor are two common monoterpenes. Borneol, derived from pine oil, is used as a disinfectant and deodorant. Camphor is used as a counterirritant, anesthetic, expectorant, and antipruritic, among many other uses

**Example:**
- Camphene and pinene in cypress oil.
- Camphene, pinene and thujhene in black pepper.

2.6.6 - Sesquiterpenes

**Properties:** anti-inflammatory, anti-septic, analgesic, anti-allergic. Sesquiterpenes are biogenetically derived from farensyl pyrophosphate and in structure may be linear, monocyclic or bicyclic. They constitute a very large group of secondary metabolites, some having been shown to be stress compounds formed as a result of disease or injury.
2.6.6.1 - Sesquiterpene Lactones:

Over 500 compounds of this group are known; they are particularly characteristics of the Composite but do occur sporadically in other families. Not only have they proved to be of interest from chemical and chemotaxonomic viewpoints, but also possess many antitumor, anti-leukemia, cytotoxic and antimicrobial activities. They can be responsible for skin allergies in humans and they can also act as insect feeding deterrents. Chemically the compounds can be classified according to their carboxylic skeletons; thus, from the germacranolides can be derived the guaianolides, pseudoguaianolides, eudesmanolides, eremophilanolides, xanthanolides, etc. A structural feature of all these compounds, which appears to be associated with much of the biological activity, is the α, β-unsaturated-γ-lactones.

**Example:**

- Farnesene in chamomile and lavender.
- Beta-caryophyllene in basil and black pepper.

2.6.7 – Diterpenes:

**Properties:** anti-fungal, expectorant, hormonal balancers, hypotensive. Diterpenes are made of up four isoprene units. This molecule is too heavy to allow for evaporation with steam in the distillation process, so is rarely found in distilled essential oils. Diterpenes occur in all plant families and consist of compounds having a C20. skeleton. There are about 2500 known diterpenes that belong to 20 major structural types. Plant hormones Gibberellins and phytol occurring as a side chain on chlorophyll are diterpenic derivatives. The biosynthesis occurs in plastids and interestingly mixtures of monoterpenes and diterpenes are the major constituents of plant resins. In a similar manner to monoterpenes, diterpenes arise from metabolism of geranylgeranyl pyrophosphate (GGPP). Diterpenes have limited therapeutical importance and are used in certain sedatives (coughs) as well as in antispasmodics and antioxolytics.

**Example:**

- Sclareol in clary sage is an example of a diterpene alcohol.
2.6.8 – Alcohols

**Properties:** anti-septic, anti-viral, bactericidal and germicidal. Alcohols are the compounds which contain Hydroxyl compounds. Alcohols exist naturally, either as a free compound, or combined with a terpenes or ester. When terpenes are attached to an oxygen atom, and hydrogen atom, the result is an alcohol. When the terpene is monoterpene, the resulting alcohol is called a monoterpenol. Alcohols have a very low or totally absent toxic reaction in the body or on the skin. Therefore, they are considered safe to use.

**Example:**
- Linalool found in ylang-ylang and lavender.
- Geraniol in geranium and rose.

2.6.9 - Aldehydes:

**Properties:** anti-fungal, anti-inflammatory, anti-septic, anti-viral, bactericidal, disinfectant, sedative. Medicinally, essential oils containing aldehydes are effective in treating Candida and other fungal infections.

**Example:**
- Citral in lemon.
- Lemongrass and lemon balm.
- Citronellal in lemongrass, lemon balm and citrus eucalyptus.

2.6.10 - Acids

**Properties:** anti-inflammatory. Organic acids in their free state are generally found in very small quantities within Essential oils. Plant acids act as components or buffer systems to control acidity.

**Example:**
- Cinnamic and benzoic acid in benzoin.
- Citric and lactic.
2.6.11- Esters

Esters are formed through the reaction of alcohols with acids. Essential oils containing esters are used for their soothing, balancing effects. Because of the presence of alcohol, they are effective antimicrobial agents. Medicinally, esters are characterized as antifungal and sedative, with a balancing action on the nervous system. They generally are free from precautions with the exception of methyl salicylate found in birch and wintergreen which is toxic within the system.

Example:
- Linalyl acetate in bergamot and lavender.
- Geranyl formate in geranium.

2.6.12 – Ketones:

Properties: anti-catarrhal, cell proliferant, expectorant, vulnerary. Ketones often are found in plants that are used for upper respiratory complaints. They assist the flow of mucus and ease congestion. Essential oils containing ketones are beneficial for promoting wound healing and encouraging the formation of scar tissue. Ketones are usually (not always) very toxic. The most toxic ketone is Thujone found in mugwort, sage, tansy, thuja and wormwood oils. Other toxic ketones found in essential oils are pulegone in pennyroyal, and pinocamphone in hyssops. Some non-toxic ketones are jasmone in jasmine oil, fenchone in fennel oil, carvone in spearmint and dill oil and menthone in peppermint oil.

Example:
- Fenchone in fennel, carvone in spearmint and dill
- Menthone in peppermint.

2.6.13- Lactones

Properties: anti-inflammatory, anti phlogistic, expectorant, febrifuge. Lactones are known to be particularly effective for their anti-inflammatory action, possibly by their role in the reduction of prostaglandin synthesis and expectorant actions. Lactones have an even stronger expectorant action then ketones .( Josip, O. Politeo and I. Jerkovic .2008).
2.7 - Storage influences

The composition or characteristics of most essential oils are modified by exposure to air and sunlight and affected by temperature (Aweiss, E. 1997). Chemical changes in volatile compounds are known to occur due to light, oxygen, moisture, heat and storage period resulting in the formation of artifacts or polymerization (Gopalakrishnan, N. 1994) figure (2.10). Essential oils composition changed when subjected to oxidative changes in the presence of air and light, and during storage, and less stable compounds may also change due to chemical interactions with other constituents (Choi, H. and Swamura, M. 2002) changes in essential oil components during storage could occur by rearrangement, hydrogenation or dehydrogenation of other components (Njoroge, S. M.; Ukeda, H. and Sawanura, M. 1996). We can come to say that storage of essential oil even in refrigerator condition (10 C) affected oil composition of spearmint oil; through changes in the quality, presence and absence of the components in the oil (Salim, E. A. 2014).

![Diagram of major factors affecting storage of essential oils](image)

Figure - 2.6 major factors affecting storage of essential oils.

2.7.1 - Photochemistry:

Essential oil of oil bearing plants contains mixture of hydrocarbons as shown in (figure - 2.11). The major compounds are terpenic hydrocarbon and oxygenated compounds each one of which undergoes successive classification as shown below. Sangwan postulated that essential oils production and composition are highly
integrated with the physiology of whole plant and so depend on the metabolic status of the synthesizing tissues. Their effect is reflected in biogenic process for synthesis of different components by affecting the efficiency of converting enzymes, beside mint oil formation and collection in oil glands (Aflatuni, A. 2005).

2.8 - Biosynthesis of carvone:

Biosynthesis of essential oil under goes a subsequent bioprocess as shown in (figure - 2.12). Monoterpenes (C_{10}) comprise the major components of the essential oils of the mint of Lamiaceae family, including peppermint (Mentha piperita) and spearmint (Mentha spicata) (Lawrence, B. M. 1981).

![Figure - 2.7 Biosynthesis of carvone.](image)

The monoterpenes diverse from primary metabolism by conversion of isopentenyl diphosphate and dimethylallyl diphosphate, via the action of the prenyltransferase geranyl diphosphate synthase, to geranyl diphosphate, which undergoes subsequent cyclization by limonene synthase to (4S)-(−)-limonene. (−)-Limonene serves as the common olefinic precursor of the essential oil terpenes of both peppermint and spearmint by way of a series of secondary, largely redox,
transformations, then limonene undergoes hydroxylation to form trans-carveol which dehydrogenated to form Carvone. (Alonso, W. R.; Rajaonarivony, J. M.; Gershenzon, J. and Croteau, R. 1992)

2.9 - Physico-chemicals of spearmint:

In general, relative density of spearmint is less than one and values vary between 0.697 and 1.188. And spearmint relative density at 20/20°C is 0.920 – 0.937 according to ISO 3033:1988 and Surburg and Köpsel reported that relative density of spearmint oil range between 0.921 and 0.938 and the American spearmint specification is in the range of 0.91700 to 0.93400. Refractive index of Italian spearmint is 1.4650 and Indian spearmint is 1.458 and Surburg and Köpsel reported that other spearmint refractive index at 20 °C is 1.489 – 1.491 while Furia and Bellanca reported that it ranged between 1.410 – 1.4830 at 20 °C (Furia, E. T. And Bellanca, N. 1975). ISO 3033:1988 mentioned that refractive index at 20 °C is in the range of 1.4850 – 1.4910. The optical rotation of an essential oil is the angle through which the plane of polarized light obtained by steam distillation, shows an optical rotation of -40 to -60 degrees at 20°C (Furia, and Bellanca, 1975. ISO 3033:1988 mentioned that refractive index at 20 °C is in the range of 1.4850 – 1.4910. American specification showed that spearmint refractive index in the range of 1.47900 – 1.48900.

2.9.1 Carvone and spearmint oil constituents:

Carvone, the major component of spearmint oil, can be spectro photometrically determined (Small et al., 1952). Also Carvone can be detected by using ultraviolet absorption techniques which is considered as easier, faster and more reliable than old traditional bisulfate assay (Guenther, 1975; Abu-Zeid, 1992). The composition of the redistillation residue of Scotch spearmint oil (type midwest, Mentha cardiaca Ger.) was investigated. In addition to a number of known sesquiterpene alcohols the new natural compounds - bourbonen-13-ol and endo-1-bourbonanol were identified, as well as 1,5-epoxysalvial- 4(14)-ene (4) and its exo configurated isomer 5 (Surburg and Köpsel, 1987). Salim used GC/MS investigated Carvone content and spearmint oil constituents and found the that spearmint oil contain Carvone, D-limonene, Limonene, Eucalyptol, Trans-Cardeyl acetate,

2.10 - Spearmint as a nutritional profile:

The mint plants can be used with diet, confectioneries and other uses. The fresh and dried leaves are used as spice and flavoring agents. **Fresh spearmint (Mentha spicata) nutritional value per 100 g contain;** Energy: 44 Kcal, Carbohydrates: 8.41 g, Energy: 44 Kcal, Carbohydrates: 8.41 g, Protein: 3.29 g, Fat: 0.73 g, Minerals; Iron: 11.87 mg, Manganese: 1.118 mg, Copper: 0.240 mg, Potassium: 458 mg; Pyridoxine: 0.158 mg, Riboflavin: 0.175 mg, Vitamin C: 13.3 mg, Cholesterol: 0 mg, Pantothenic acid (vitamin B5): 0.061 mg, Vitamin B6: 0.041 mg, Folate (vitamin B9): 3 mg and Vitamin C: 4.6 mg. They are added to diets to be made into mint sauces or jellies, served as condiments with lamb, potatoes, peas and carrots or scattered finely as chopped leaf salads, squash or cucumber to add refreshing bite. The fresh leaves of the spearmint contain various nutritive elements.

2.11- Medicinal profile:

2.11.1 - Health effects:

The oil is used in carminative, pharmaceutical, antiseptic, tooth paste, soap, insecticide, and perfumery. The infusion of leaves and flowering branches are antispasmodic, antiseptic chologne and carminative. Infusions, cataplasms or inhaling materials are used for colds, catarrh, infection of throat and brochia of lungs (Wern, R. C. 1989). Administration of spearmint tea to rats causes dose-dependent, temporary or permanent negative effects on the reproductive system of the male rat and leads to lipid peroxidation, that results in histopathologies in the
kidney, liver, and uterine tissues. It’s essential oil did not show any evidence of mutagenicity in the Ames test beside other treatments.

- **Antioxidant activity:**

A report, published in the *Journal of Chemistry*, revealed that spearmint extract has "good total phenolic and flavonoid contents. It exhibited excellent antioxidant activity, as measured by β-carotene bleaching and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays." Spearmint has also been described as having excellent antioxidant activity, comparable to BHT (Kanatt, 2007). Due both to its antioxidant activity and its common use to season lamb in South Asian cuisine, it has been studied as an additive to radiation-processed lamb meat, and was found effective in delaying oxidation of fats and reducing formation of harmful substances, which can be detected using thiobarbituric acid as a reagent.

- **Antifungal properties:**

Spearmint essential oil is associated with a number of antifungal properties. Spearmint has been studied for antifungal activity; its essential oil was found to have some antifungal activity, although less than oregano.

- **Antimicrobial activity:**

Microbiological activities against gram positive and gram negative bacterial. Salim found that spearmint had inhibitory effect against staphylococcus aureus, Klepsella sp., E. coli and Pseudomonas aeruginosa.

- **Relieving symptoms of digestive problems:**

Spearmint may help relax the stomach muscles, reducing symptoms of nausea and other digestive problems. According to a review of the potential health benefits of spearmint tea, published in the journal *Phytotherapy Research*, "human studies on the GI, respiratory tract and analgesic effects of peppermint oil and its constituents have been reported. However, more research is necessary to back this claim."
- **Treating hirsutism (abnormal hair growth):**
- There is very promising data published in the journal *Phytotherapy Research* showing that drinking two cups of spearmint tea a day for five days could reduce the level of androgens in women with hirsutism. Women with hirsutism grow hair on their face, breasts, and stomach. This can cause a great deal of distress. The hair grows because of excessively high levels of the 'masculinizing' androgen hormones.

- **Spearmint and rosemary extracts found to improve memory:**

  Prof. Susan Farr, from Saint Louis University School of Medicine, presented findings to suggest that antioxidants from spearmint and rosemary made into an enhanced extract may help improve learning and memory, which could help with age-related cognitive decline.

- **Other health conditions that spearmint may be effective at treating:**

  Some people consume spearmint to help alleviate symptoms of nausea, indigestion, gas, headache, toothache, cramps, sore throat, Arthritis, Colds, Diarrhea, Muscle pain, fatigue, and stress. Spearmint is also applied topically (to the skin) to help reduce swelling due to nerve or muscle pain.
Chapter Three: Material and Methods
3.1 - Material

3.1.1- Experimental site:

A field experiment was conducted at ‘Halfaya’ area, Khartoum North (15.6935869N, 32.5263182E). The climate was a semi – arid, tropical, with seasonal annual rainfall of 120 mm during the period mainly between January to March. The mean maximum and minimum temperatures are as high as 47.5 °C during summer and as low as 7.5 °C during winter (SMAR .2010).

3.1.2 - Experimental Material:

Spearmint (Mentha spicata var. Viridis L) plants were raised from underground runners (rhizome-like rootstock) obtained from fully mature plants grown in spearmint growing area at ‘Halfaya’, Khartoum North, Sudan which was suggested to be brought from Egypt from ancient time through river movement. The other species was obtained from Administration of Horticulture, Ministry of Agriculture, Sudan.

3.2 - Methods:

3.2.1 - Determination of mint oil content (v/w)

To determine the oil content of mint plants, hydro- distillation technique for oil extraction was use by taking 100 dried samples of shades, stems and flowers) separately from each plot, placed in a bottomed flask. Water was added so that the sample immersed. A lass apparatus for distillation volatile water equipped with a condenser.
3.2.2 - Gas Chromatography-Mass Spectrometer

Mass spectrometry (MS) can be defined as the study of systems through the formation of gaseous ions, with or without fragmentation, which are then characterized by their mass-to-charge ratios ($m/z$) and relative abundances. The analyze may be ionized thermally, by an electric field or by impacting energetic electrons, ions, or photons. During the past decade, there has been a tremendous growth in popularity of mass spectrometers as a tool for both, routine analytical experiments and fundamental research. This is due to a number of features including relatively low cost, simplicity of design and extremely fast data acquisition rates. Although the sample is destroyed by the mass spectrometer, the technique is very sensitive and only low amounts of material are used in the analysis. In addition, the potential of combined gas chromatography-mass spectrometry (GC-MS) for determining volatile compounds, contained in very complex flavor and fragrance samples, is well known. The subsequent introduction of powerful data acquisition and processing systems, including automated library search techniques, ensured that the information content of the large quantities of
data generated by GC-MS instruments was fully exploited. The most frequent and simple identification method in GC-MS consists of the comparison of the acquired unknown mass spectra with those contained in a reference MS library. A mass spectrometer produces an enormous amount of data, especially in combination with chromatographic sample inlets. Over the years, many approaches for analysis of GC-MS data have been proposed using various algorithms, many of which are quite sophisticated, in efforts to detect, identify, and quantify all of the chromatographic peaks. Library search algorithms are commonly provided with mass spectrometer data systems with the purpose to assist in the identification of unknown compounds. However, as is well known, compounds such as isomers, when analyzed by means of GC-MS, can be incorrectly identified; a drawback which is often observed in essential oil analysis. As is widely acknowledged, the composition of essential oils is mainly represented by terpenes, which generate very similar mass spectra; hence, a favorable match factor is not sufficient for identification and peak assignment becomes a difficult, if not impracticable.

In order to increase the reliability of the analytical results and to address the qualitative determination of compositions of complex samples by GC-MS, retention indices can be an effective tool. The use of retention indices in conjunction with the structural information provided by GC-MS is widely accepted, and routinely used to confirm the identity of compounds. Besides, retention indices when incorporated to MS libraries can be applied as a filter, thus shortening the search routine for matching results, and enhancing the credibility of MS identification. All provided data contained in mass spectral libraries have been recorded using authentic samples, it can be observed that the mass spectrum of a given sesquiterpene is usually sufficient to ensure its identification when associated with its retention index obtained on methyl silicone stationary phases. Indeed, for the aforecited class of compounds, there would be no need to use a polyethylene glycol phase, which could even lead to misinterpretations caused by possible changes in the retention behavior of sesquiterpene hydrocarbons as a result of column aging or deterioration. Moreover, according to the authors, attention should be paid to the retention index and the mass spectrum registration of each individual sesquiterpene, since many compounds with rather similar mass spectra elute in a narrow range; more than 160 compounds can elute within 100 retention index units on a methyl silicone-based column, for example, 1400–1500 (Joulain, D. and W.A. König, 1998).
Figure -3.2 GC-MS device.

3.3 - Physicochemical Properties of Spearmint Oil

3.3.1- Determination of saponification value
The saponification value is the number of mg of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1 g of the substance. In the procedure described, a 50-mL burette should preferably be used for titration, as in the blank titration the volume of hydrochloric acid (0.5 mol/l) VS used is exactly 35.5 mL when the concentration of ethanolic potassium hydroxide is exactly 40 g/l, (WHO. ECSPP,2015).

**Procedure:**

weight 2 g of the sample (1), was put in a flask with a capacity of about 200 mL, add 25 mL of potassium hydroxide/ethanol, was then added attach a reflux condenser to the flask, after that put it in the a boiling water-bath for 30 minutes, frequently rotating the contents of the flask, immediately add 1 mL of phenolphthalein/ethanol and titrate the excess of alkali with hydrochloric acid (0.5 mol). Note the number of mL of hydrochloric acid (0.5 mol) required to titrate the sample (a). Repeat the operation without the sample and note the number of mL of hydrochloric acid (0.5 mol) required for neutralization (2). Calculate the saponification value from the following formula:

Equation (2) \[ sv = \frac{(v_2 - v_1 \times 0.02805 \times 1000)}{\text{weight (in g) of sample}} \]

3.3.2 - Determination of acid value
The acid value is the number of mg of potassium hydroxide required to neutralize the free acid in 1 g of the substance, (WHO. ECSPP, 2015).

**Procedure:**
Accurately weigh 2 g of the sample, into a 250-mL flask, and add 50 mL of a mixture of (25 ml ethanol and 25 ml of ether), add 1 mL of phenolphthalein, titrate with potassium hydroxide (0.1 mol/l), constantly shaking the contents of the flask until a pink color, which persists for 15 seconds, is obtained. Note the number of mL required (a). Calculate the acid value from the following formula:

\[
\text{Equation (3)} \quad \text{Av} = \frac{(a \times 0.00561 \times 1000)}{\text{weight (in g) of sample}}
\]

**3.3.3 - Determination of Peroxide value**
Dissolve 3 g(v2) of the oil in conical flask, and add in 10 mL of chloroform and 15 mL of glacial acetic acid. Add 1 mL of a freshly prepared solution of 1.3 g of potassium iodide in 1 mL of water, mix by gentle swirling and set aside in the dark for 5 minutes. And titrate with sodium thiosulphate (0.1 M), using starch as indicator. Repeat the operation without the substance (blank(v1)) being tested and calculate the difference between the titrations.

\[
\text{Equation (4)} \quad \text{pv} = \frac{((v_2 - v_1) \times 0.1 \times 1000)}{\text{weight (in g) of sample}}
\]

**3.3.4 - Determination of iodine value**
The iodine value of a substance is the weight of halogens expressed as iodine absorbed by 100 parts by weight of the substance. The quantity of substance used in the determination should be such that at least 70% of the iodine added, as provided in the recommended procedure, is not absorbed. Unless otherwise specified in the monograph, the quantity of the substance indicated in the following table should be used for the determination (WHO. ECSPP, 2015), depending on the expected iodine value:

Table -3.1 shows the expected iodine value.

<table>
<thead>
<tr>
<th>Iodine value</th>
<th>Quantity of substance in g</th>
</tr>
</thead>
<tbody>
<tr>
<td>less than 20</td>
<td>1.0</td>
</tr>
<tr>
<td>20 – 60</td>
<td>0.5 - 0.25</td>
</tr>
<tr>
<td>60 – 100</td>
<td>0.25 - 0.15</td>
</tr>
<tr>
<td>more than 100</td>
<td>0.15 - 0.10</td>
</tr>
</tbody>
</table>

**procedure:**
Place a quantity of the test substance, accurately weighed, as specified in the monograph, in a dry 300-mL to 500-mL stoppered flask, add 15 mL of carbon tetrachloride R and dissolve. Add 25 mL of iodine bromide TS, insert the stopper, previously moistened with potassium iodide (80 g/l) TS, shake the flask gently, and keep in the dark for 30 minutes, unless otherwise specified in the monograph. Add 20 mL of potassium iodide (80 g/l) TS and 150 mL of water, and, whilst shaking the contents of the flask, titrate with sodium thiosulfate (0.1 mol) VS, adding starch TS as indicator towards the end of the titration. Note the number of
mL required (a). At the same time carry out the operation in exactly the same manner, but without the substance being tested, and note the number of mL of sodium thiosulfate (0.1 mol/l) VS required (b). Calculate the iodine value from the following formula:

\[
IV = \frac{((b-a) \times 0.01269 \times 100)}{\text{weight (in g) of sample}}
\]
Chapter Four: Results and Discussions
4-Results and discussions

4.1- Physicochemical Properties of Spearmint Oil

Table - 4.1 Physicochemical Properties of Spearmint Oil.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of oil in a 100 g</td>
<td>0.05 ml of spearmint oil</td>
</tr>
<tr>
<td>Acid value</td>
<td>6.45 mg</td>
</tr>
<tr>
<td>saponification value</td>
<td>42.08 mg</td>
</tr>
<tr>
<td>Peroxide value</td>
<td>6.6 mg</td>
</tr>
<tr>
<td>Iodine value</td>
<td>130.28 mg</td>
</tr>
<tr>
<td>Refractive index (at 25.1 °C)</td>
<td>1.488</td>
</tr>
<tr>
<td>Density (at room temperature)</td>
<td>0.93</td>
</tr>
<tr>
<td>Refractometer (at room temperature)</td>
<td>1.844</td>
</tr>
<tr>
<td>Color (Yellow Slant to reddish)</td>
<td></td>
</tr>
<tr>
<td>Yellow</td>
<td>8</td>
</tr>
<tr>
<td>Red</td>
<td>2.30</td>
</tr>
<tr>
<td>Blue</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Figure - 4.1 Chromatogram of spearmint essential oil.
### Table - 4.2.1 Peak Report (TLC) from GC-MS.

<table>
<thead>
<tr>
<th>Peak</th>
<th>R.Time</th>
<th>I.Time</th>
<th>F.time</th>
<th>Area</th>
<th>Area%</th>
<th>Height</th>
<th>Height%</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>10.260</td>
<td>10.200</td>
<td>10.308</td>
<td>5919063</td>
<td>0.54</td>
<td>2387585</td>
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</tr>
<tr>
<td>2</td>
<td>10.781</td>
<td>10.725</td>
<td>10.833</td>
<td>17353273</td>
<td>1.58</td>
<td>6578171</td>
<td>3.22</td>
</tr>
<tr>
<td>3</td>
<td>12.153</td>
<td>12.100</td>
<td>12.192</td>
<td>12521001</td>
<td>1.14</td>
<td>4856720</td>
<td>2.38</td>
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<tr>
<td>4</td>
<td>12.243</td>
<td>12.192</td>
<td>12.300</td>
<td>19393772</td>
<td>1.76</td>
<td>7482518</td>
<td>3.66</td>
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<tr>
<td>5</td>
<td>12.780</td>
<td>12.692</td>
<td>12.833</td>
<td>49499611</td>
<td>4.49</td>
<td>15649888</td>
<td>7.66</td>
</tr>
<tr>
<td>6</td>
<td>13.060</td>
<td>13.017</td>
<td>13.100</td>
<td>20641597</td>
<td>1.87</td>
<td>8455558</td>
<td>4.14</td>
</tr>
<tr>
<td>8</td>
<td>14.310</td>
<td>14.275</td>
<td>14.342</td>
<td>4370588</td>
<td>0.40</td>
<td>2037269</td>
<td>1.00</td>
</tr>
<tr>
<td>9</td>
<td>14.639</td>
<td>14.608</td>
<td>14.675</td>
<td>2772341</td>
<td>0.25</td>
<td>1321837</td>
<td>0.65</td>
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<tr>
<td>10</td>
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<td>16.292</td>
<td>16.367</td>
<td>5549784</td>
<td>0.50</td>
<td>2410548</td>
<td>1.18</td>
</tr>
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<td>17.005</td>
<td>16.967</td>
<td>17.050</td>
<td>6612323</td>
<td>0.60</td>
<td>2637918</td>
<td>1.29</td>
</tr>
<tr>
<td>12</td>
<td>18.723</td>
<td>18.667</td>
<td>18.775</td>
<td>3260105</td>
<td>0.30</td>
<td>927706</td>
<td>0.45</td>
</tr>
<tr>
<td>13</td>
<td>19.336</td>
<td>19.242</td>
<td>19.375</td>
<td>30166303</td>
<td>2.74</td>
<td>7912750</td>
<td>3.87</td>
</tr>
<tr>
<td>14</td>
<td>19.466</td>
<td>19.408</td>
<td>19.508</td>
<td>10503333</td>
<td>0.95</td>
<td>3372243</td>
<td>1.65</td>
</tr>
<tr>
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<td>20.250</td>
<td>20.375</td>
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<td>0.74</td>
<td>2086183</td>
<td>1.02</td>
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<td>20.617</td>
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<td>465358371</td>
<td>42.26</td>
<td>35376713</td>
<td>17.31</td>
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<tr>
<td>18</td>
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<td>22.942</td>
<td>23.075</td>
<td>52262331</td>
<td>4.75</td>
<td>15577222</td>
<td>7.62</td>
</tr>
</tbody>
</table>
Discussions

The aim of this study was to extract and describe the chemical composition of essential oils of Mentha spicata oil from mint and examine the physiochemical properties of the oil. The essential oils, obtained from Stem and leaves by hydro-distillation, were analyzed by gas chromatography-mass spectrometry (GC/MS).

Discussions: Mint plant are one of the most interesting research plants. They are between medicinal and aromatic plants. There is numerous research on mint, however, only few reports are available on the Physicochemical Properties of Spearmint Oil in Sudan.
Mint essential oil contains: Acid value (6.45 mg), saponification value (42.08 mg), Peroxide value (6.6 mg) and Iodine value (130.28 mg). Also the GC-MS device show Twenty nine peak and each peak have three hit in the appendix.
Chapter Five: Conclusions and Recommendations
5.1 - Conclusions

Steam distillation method was found to be one of the promising techniques for the extraction of Essential Oil from plants as this process will preserve the original qualities of the plant. Steam distillation is a special type of distillation or a separation process for temperature sensitive materials like oils, resins, hydrocarbons, etc. which are insoluble in water and may decompose at their boiling point (which can be prevented using Steam Distillation method). Analysis using Gas Chromatography-Mass Spectrometer was found to be the best method to identify even the minor components of particular oil along with major components. Extraction of Essential Oils using Steam Distillation can be used on industrial scale to make various finished products which includes body oils, cosmetic lotions, baths, hair rinses, soaps, perfumes and room sprays.

5.2 - Recommendations

- Doing more studies on the essential oils in order to know the physical and chemical properties. In order to take advantage of the chemical components used in the medical and industrial field.
- Furthermore to apply simulation production line to simulate the process by the chemical software's.
- Establish peppermint oil industry in order to make it from local industries that bring in hard currency for Sudan.
- Doing studies on the possibility of using waste as feed for animals naturally. To get to zero waste "cleaner production."
Chapter Six: References
References


7- Rao., Pandey. 2006.” Extraction of Essential Oil and its applications”. Department of Chemical Engineering, National Institute of Technology, Rourkela.


Appendixes
Appendix A (pdf fil)
Appendix B

The extracted oil from spearmint