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# EXTRACTION OF VOLATILE OIL FROM SOME MEDICINAL AND AROMATIC PLANTS BY DIFFERENT METHODS

**Jury:**

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# Dedication

*To my dear father, who has stood by my side throughout my academic journey and been my biggest supporter. May God grant him a speedy recovery, InshaAllah.*

*To my mother, who has been my rock and pillar of strength for the past 23 years of my life. Your constant support and encouragement have been invaluable.*

*To my sisters, Kaouthar, Salsabil, and chaima, thank you for always being there for me and for your unwavering love and support. To my brothers, Nasro Allah and Mohammad, thank you for being my pillars of strength and for always pushing me to achieve my goals.*

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*To my entire family, thank you for your love, support, and encouragement throughout my academic journey.*

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# General Introduction

# Introduction

Medicinal plants have played a crucial role in traditional medicine practices across different cultures and societies throughout history. These plants have been revered for their therapeutic properties and have been extensively used to address various health conditions and promote overall well-being. The popular method of harnessing the beneficial properties of these plants is through the extraction of their essential oils.[1]

Essential oils (EOs) or vegetable essences are oily, volatile, odorous and colorless or slightly tinted products obtained by steam distillation, by expression, by incision or by enfleurage of the plant material. These plant essences are found in appreciable quantity in approximately 2000 species divided into 60 botanical families such as Lamiaceae (lavender, basil, mint …), Myrtaceae (eucalyptus), Lauraceae (cinnamon and sassafras), and the Apiaceae (coriander, cumin, fennel, parsley..). These volatile compounds belong to various chemical classes: alcohols, ethers or oxides, aldehydes, ketones, esters, amines, amides, phenols, and mainly the terpenes. EOs possess a wide range of biological activities including antimicrobial, antifungal, antioxidant, anti-inflammatory, and anticancer properties. Additionally, they have been investigated for their potential use in the treatment of various health conditions including respiratory disorders, skin diseases, digestive issues, and mental health problems (Aromatherapy). [2-4]

Nowadays, there is a growing interest in the scientific study of medicinal plants and their essential oils. Compounds of plant origin have been and still are an important source of compounds for drugs. Research has focused on identifying the active compounds, elucidating their mechanisms of action, and exploring their potential therapeutic applications in modern medicine. This interdisciplinary approach combines traditional knowledge with scientific advancements to maximize the benefits of medicinal plants and their bioactive compounds, such as essential oils. [5]

In this study, the effect of extraction methods on the yield and physico-chemical properties of essential oils from three different plants (Rosemary, Eucalyptus, and orange fruit) were investigated. These plants were selected since they are commonly used in culinary, industry and medicinal applications. It is important to note that both plant *Rosmarinus* *officinalis* L. and *Eucalyptus globulus* Labill. were collected in the region of Djemorah and Ain Zaatout.

On the other hand, the different essences obtained by simple hydrodistillation and by Clevenger apparatus are used in the preparation of aromatic soap. Soap played and still playing the most important role in infection prevention from bacteria and virus such as “Coronavirus”.

The first chapter of this manuscript, provides a review of literature on essential oils, their extraction methods, and some knowledge of aromatic plants which are chosen for this study.

The second chapter, present the experimental part of this work: laboratory experiments including essential oils extract technics, EOs characterization by using the physicochemical analysis and the different steps of soap making.

Finally, the last part will involve the discussion and analysis of the results obtained.

# Chapter I
# Literature review

# I.1. Topic 1: Essential Oils

## I.1.1. What is essential oil?

Essential oils are a concentrated hydrophobic liquid containing volatile aroma compounds from plants. They are also known as aromatic oils, fragrant oils, steam volatile oils, ethereal oils, or simply as the "oil of " plants material from which they were extracted, such as rosemary oil.[6]

The majority of them are fairly stable and contain natural antioxidants and natural antimicrobial agent as citrus fruits**.**[6]

Essential oil is used in perfumery, aromatherapy, cosmetics, incense, medicine, household cleaning products and for flavoring food and drink. [6, 7].

## I.1.2. Distribution and location of essential oils in plants

Essential oils are volatile and aromatic compounds that are produced in specialized cells or glands (glandular trichomes) of certain plants used by them to protect themselves from predators and pests, but also to attract pollinators. In other words, essential oils are part of the immune system of the plant. These trichomes can be found in different parts of the plant, depending on the species. Oils are extracted from the leaves, petals, stems, seeds, and even the roots of the plants. Aromatic plants generally contain volatile oil in all their proportions in different concentrations.[8]

Here are some examples:

- **Leaves:** Some plants, such as peppermint, eucalyptus, and tea tree, produce essential oils in their leaves (the sub-dermal secretory cavities (glands) embedded within the leaves).
- **Flowers:** Many flowering plants, such as rose, jasmine, and lavender, produce essential oils in their flowers (the oil glands are usually located on the petals, sepals, or stamens).
- **Stems and bark**: Some plants, such as cinnamon, cassia, and ginger, produce essential oils in their stems and bark (the oil glands are typically located in the corky layer or resin canals of the bark).
- **Roots:** Some plants, such as ginger, vetiver, and angelica, produce essential oils in their roots (the oil glands are usually found in the root hairs or near the surface of the root).

Once produced, essential oils are stored in the plant tissues until they are used. When the plant is damaged or under attack, the essential oils are released into the air as a defense mechanism. [9]

### I.1.3. Chemical composition of essential oil

Essential oils are composed of a complex mixture of volatile organic compounds that are derived from plants. The chemical composition of essential oils can vary greatly depending on different factors affecting their content, such as exogenous and endogenous factors, postharvest treatment, extraction methods, and conservation conditions [10]. The **endogenous** factors are related to anatomical and physiological characteristics of the plants and to the biosynthetic pathways of the volatiles (**Fig I.1**), which might change in either the different tissues of the plants or in different seasons, but also could be influenced by DNA adaptation. The **exogenous** factors, over a long period, might affect some of the genes responsible for volatiles formation. Those factors lead to ecotypes or chemotypes in the same plant species [11, 12].

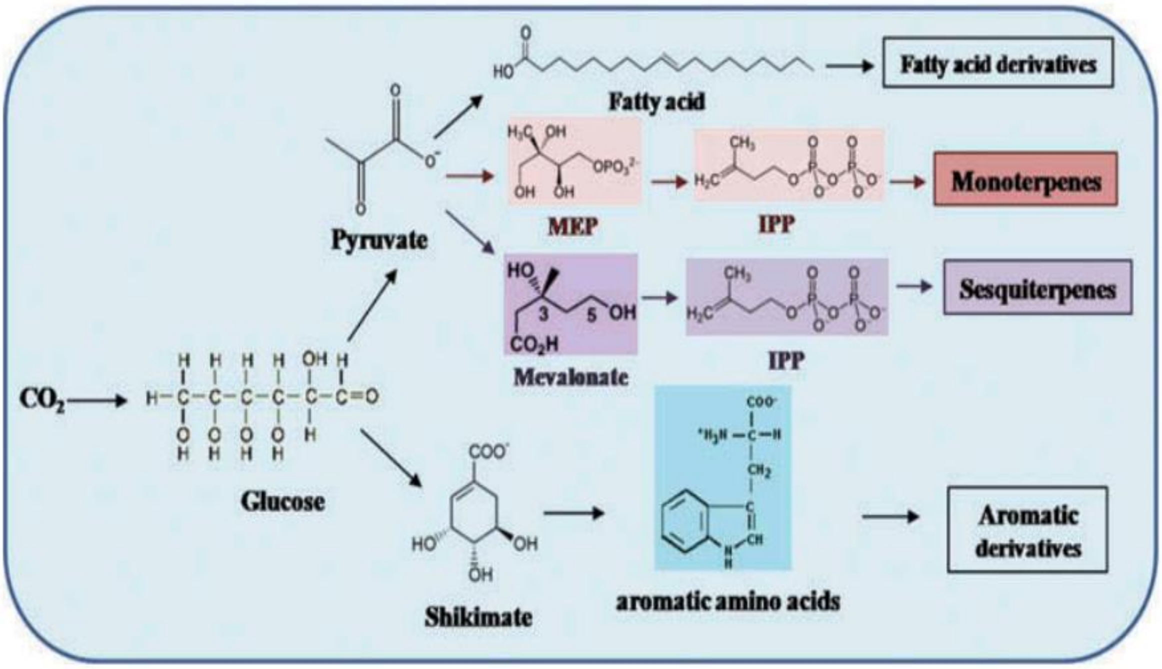
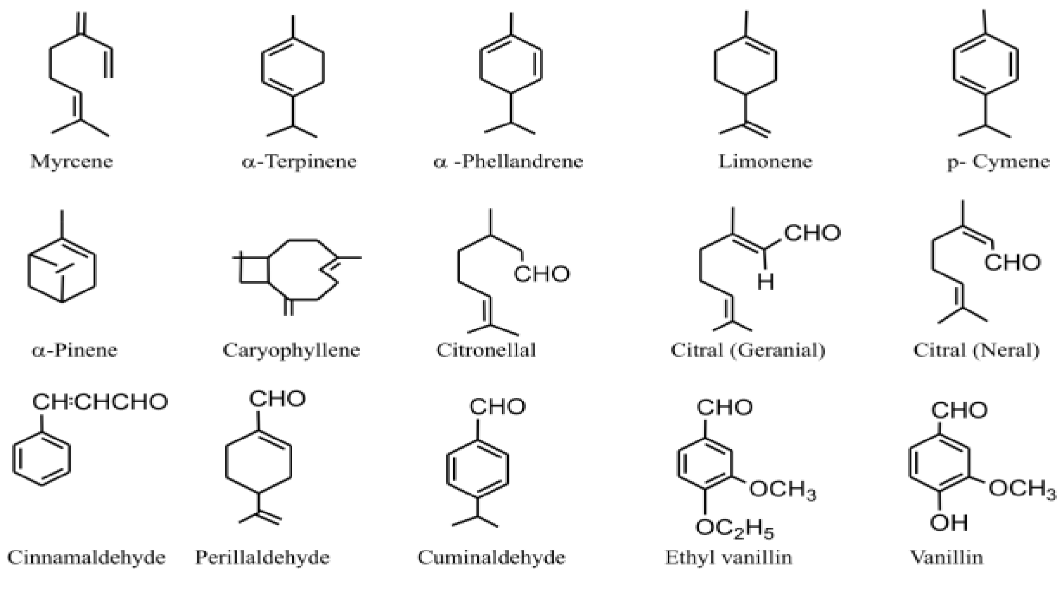



**Figure 0.1:** Biosynthesis pathways of major volatile organic compounds (MEP, 2-methyl-erythritol-4- phosphate; IPP, isopentyl pyrophosphate)

Essential oils can be classified based on their dominant chemical constituents. Some of the major classes of essential oil compounds include [13, 14]:

- **Monoterpenes:** These are composed of two isoprene units and are the most common class of essential oil compounds, as examples: limonene, pinene, and terpinene.
- **Sesquiterpenes:** These are composed of three isoprene units and have a more complex structure than monoterpenes, for examples: caryophyllene, farnesene, and bisabolol.
- **Phenylpropanoids:** These are derived from the amino acid phenylalanine and include compounds such as eugenol and safrole.
- **Aldehydes:** These are characterized by the presence of a carbonyl group (-CHO) and include compounds such as citral and cinnamaldehyde.
- **Ketones:** These are characterized by the presence of a carbonyl group (-C=O) and include compounds such as menthone and carvone.
- **Esters:** These are derived from an acid and an alcohol such as linalyl acetate and geranyl acetate.
- **Oxides:** These are characterized by the presence of an oxygen atom and include compounds such as 1,8-cineole (eucalyptol) and bisabolol oxide.

These classes of compounds can be used to classify essential oils based on their chemical composition (**Fig I.2**), and each class has its own characteristic aroma and therapeutic properties. [15]



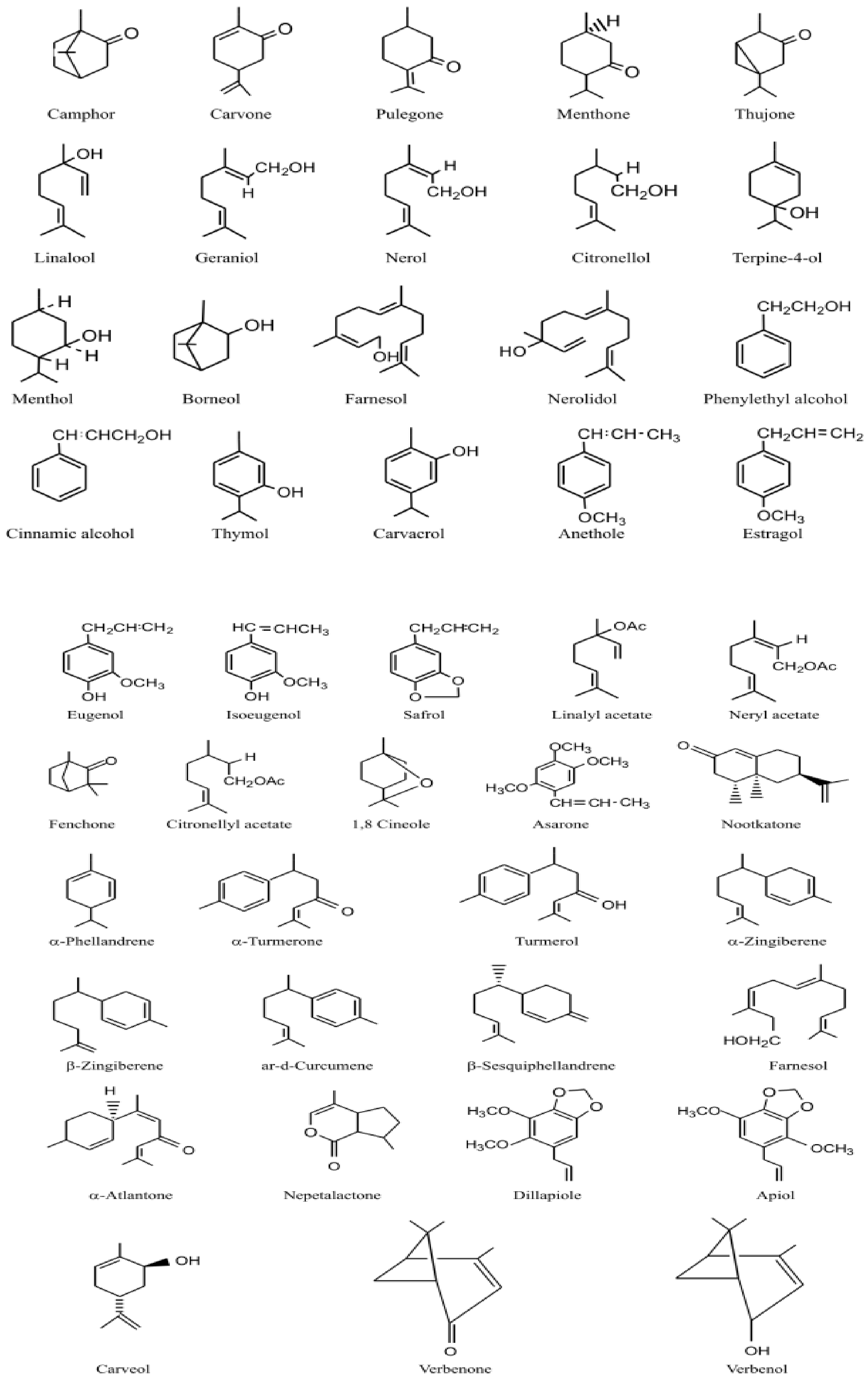



**Figure 0.2:**Chemical structures of essential oil constituents

### I.1.4. Virtues and medicinal properties

Essential oils are important secondary metabolites of plants, which have been used for centuries for their potential virtues and medicinal properties, including aromatherapy, relaxation, and natural remedies. It's important to note that the effectiveness of essential oils can vary and scientific research on their benefits and uses is still ongoing [16]. Here are some commonly recognized virtues and medicinal properties associated with certain essential oils:

- **Anti-inflammatory effect:** Many essential oils have anti-inflammatory properties, which can help to reduce swelling and pain, such as chamomile (*Matricaria chamomilla*), eucalyptus (*Eucalyptus globulus*), and lavender Eos. There is some research suggesting that lavender essential oil (EO) may be beneficial as an adjunct to intravesical therapy for interstitial cystitis (IC). [17]
- **Antimicrobial effect:** Essential oils can have antimicrobial properties, which means they can help to kill or inhibit the growth of microorganisms such as bacteria, viruses, and fungi. In recent years, several studies have shown that bacteria are susceptible to different essential oils, including those derived from *Origanum vulgare* (oregano), *Melaleuca alternifolia* (tea tree), *Cinnamomum cassia* (cassia), and *Thymus vulgaris* (white thyme). [18]
- **Relaxing effect:** Many essential oils have a relaxing effect on the mind and body, and can be used to reduce stress and promote relaxation. Lavender, chamomile and bergamot (*Citrus bergamia*) essential oils are among the most popular ones used for relaxation purposes, individually and in combination with other oils. [19]
- **Energizing effect:** There are several essential oils that are known for their energizing properties and can help boost mood and increase alertness, such as peppermint, rosemary, and lemon Eos. [20]
- **Pain relief effect:** Essential oils have been found to possess analgesic properties and can used topically to help reduce pain and discomfort. Here are a few examples of essential oils known for their potential pain-relieving effects: peppermint EO, ginger EO, and frankincense EO. [21]

- **Digestive support effect:** There are a variety of essential oils that can be used for the digestive system. For example: ginger, peppermint, coriander, star Anise, cardamom and fennel essential oils can be used to support digestive health and help to alleviate digestive issues such as bloating, gas, and constipation. [22]

- **Respiratory support:** Coughing can be caused by various factors and conditions, including respiratory infections such as the common cold, flu, or chest infections. Eucalyptus, peppermint, Rosemary, thyme and tea tree essential oils can help to support respiratory health and alleviate symptoms of respiratory. [23]

  It’s important to note that essential oils should always be used with caution and under the guidance of a qualified aromatherapist or healthcare provider. Essential oils can be powerful and may cause adverse reactions (allergies, coma, epilepsy, etc.) in some individuals (children, pregnant and breastfeeding women, elderly or allergic people), especially when used improperly or in high doses.

### I.1.5. Physical properties

Essential oils are complex mixtures of volatile organic compounds that are derived from plants. They have a range of physical properties that are important to understand when using them in aromatherapy or other applications. Here are some of the physical properties of essential oils [24]:

- **Color:** Essential oils can range in color from clear to pale yellow, green, blue, or even brown. The color can vary depending on the plant species, geographic location, and other factors.

- **Odor:** Essential oils have a characteristic aroma that is unique to each oil. The odor can be described as floral, spicy, woody, or citrusy, among other qualities.

- **Viscosity:** Essential oils can range in viscosity from watery to thick and syrupy. The viscosity can depend on the specific chemical composition of the oil.

- **Solubility:** Essential oils are generally not soluble in water, but are soluble in alcohol, carrier oils, and other organic solvents.

- **Density:** Essential oils are less dense than water and will float on top of it. The density can vary depending on the specific gravity of the oil.

- **Flash point:** The flash point is the temperature at which an essential oil will ignite if exposed to a flame. This can vary depending on the oil, but generally ranges between 50-100°C**.**

- **Refractive index:** The refractive index is a measure of how much light is bent as it passes through an essential oil. This can be used to identify and characterize different oils.

The physical properties of essential oils are important for using them safely and effectively. For example, the viscosity of an oil can affects its ability to be absorbed by the skin, while the flash point can affect how the oil is stored and used.

### I.1.6. Extraction processes of oil essential

There are various methods used to extract essential oils, including steam distillation, solvent extraction, hydro-distillation, expression, cold pressing, carbon dioxide extraction, and enfleurage (**Fig I.3**). The choice of extraction method depends on the type of plant material being used, the desired quality of the essential oil, and the intended use of the oil.

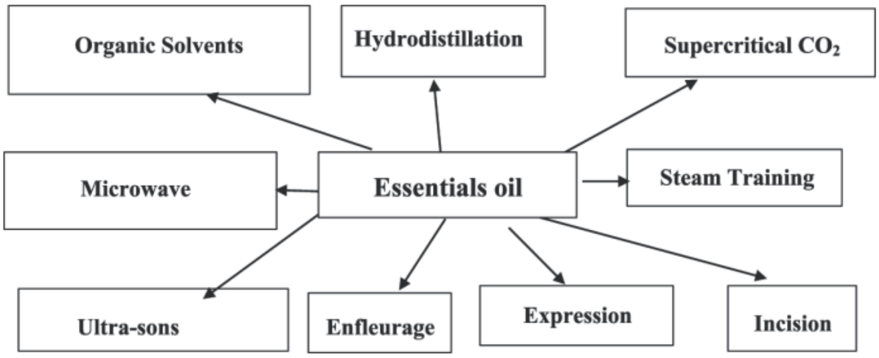



**Figure 0.3:**Processes for isolating essential oils from the plant material

Below, we will detail only the techniques that we will be using in our experimental part.

### a. Steam Distillation

**S**team distillation is a process used for extracting essential oils from plant materials. It is the most common and widely used method for extracting essential oils**.** The steam distillation process involves heating the plant material in a distillation apparatus with water. The heat causes the water to boil and produce steam, which passes through the plant material and vaporizes the essential oils. The steam and essential oils are then condensed by cooling and collected separately (**Fig I.4**). [25]

The essential oils obtained through steam distillation are highly concentrated and are often used in aromatherapy, perfumery, and flavoring. This method is suitable for plants with high oil content, such as lavender, peppermint, and eucalyptus.[25]

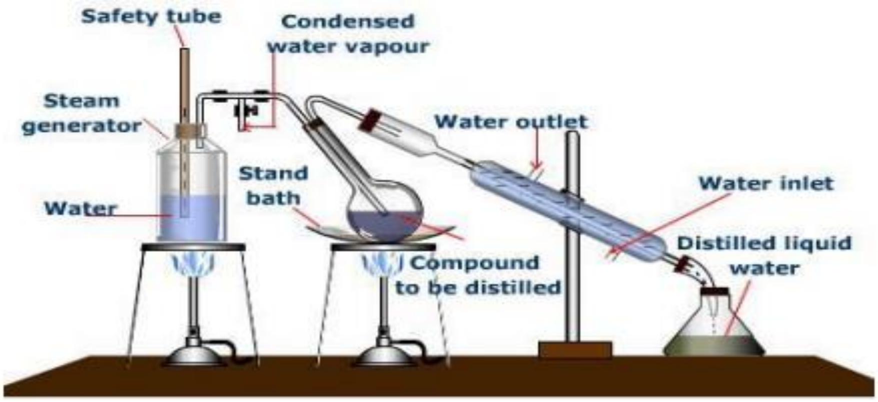



**Figure 0.4:** Schematic illustration of the Steam distillation equipment

## b. Hydrodistillation

Hydrodistillation is the oldest and simplest oils extraction method. The procedures start with immersing the plant materials directly into water inside the alembic (vessel), and whole mixture was boiled. The devices include a heating source, vessel (Alembic), a condenser to convert vapor from vessel to liquid, and a decanter to collect the condensate and to separate essential oils with water (**Fig I.5**).[25]

This extraction technique is considered as a unique method to extract plant materials like wood or flower and is frequently used for extractions involving hydrophobic natural plant material with a high boiling point. As the oils are surrounded by water, this method is able to protect essential oils to be extracted at a certain degree without being overheated. The main advantage of this extraction technique is its ability to isolate plant materials below 100°C.[25]

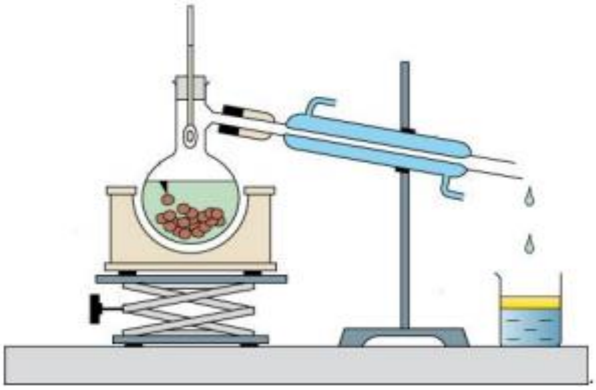


**Figure 0.5:** Schematic illustration of the hydrodistillation equipment**.**

### c. Microwave extraction

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 thermosensitive components or the extraction of essential oils for micro-analysis. Among them, microwave-assisted extraction (MAE), it is also known as microwave-assisted hydrodistillation, is an alternative method to traditional extraction processes such as steam distillation or solvent extraction. In this process, the plant material is mixed with a suitable solvent and placed in a microwave reactor. The mixture is then exposed to microwave radiation, which causes the solvent to heat up and extract the essential oil from the plant material (**Fig I.5**).

The advantages of microwave extraction for essential oils include reduced extraction time, increased yield, and improved quality of the extracted oil. Additionally, microwave extraction can be used for a wide range of plant materials, including those that are difficult to extract using traditional methods.[26]

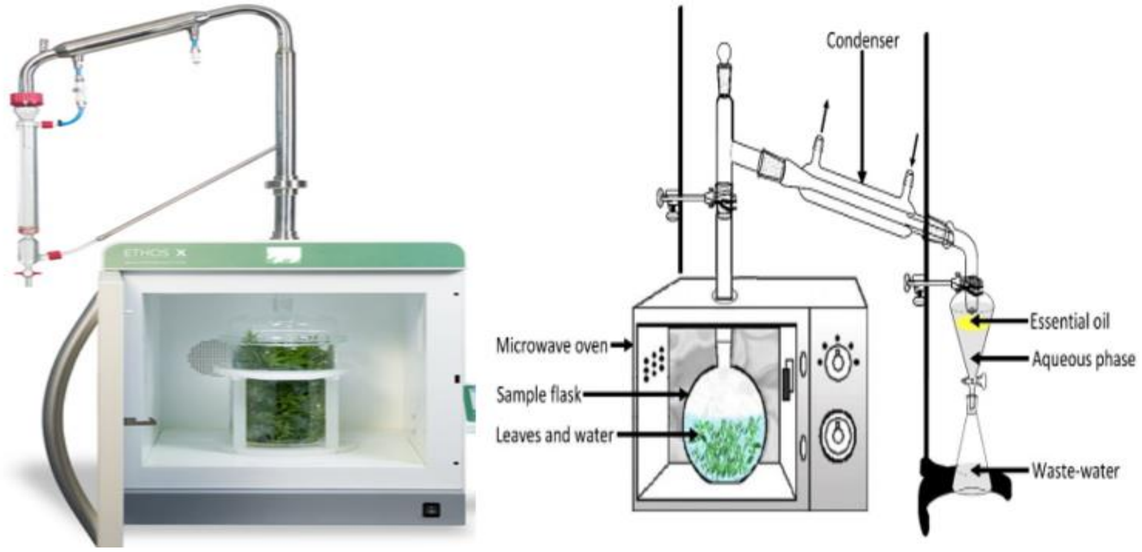



**Figure 0.6:**Schematic representation of the microwave-assisted extraction apparatus

### I.1.7. Conservation of essential oil

Essential oils are very delicate, volatile substances and need special care in order to preserve them in good condition. Independently of the method of extraction certain precautions should be taken with regard to any essential oil. [27]

- Keep them in a cool, dry place away from sunlight. Never keep them in the bathroom, the humidity could ruin them. The ideal place would be a dark, cool cupboard.
- Put the essential oils in airtight containers without rubber droppers as essential oils can dissolve the rubber, choose containers with separate droppers to facilitate accurate dosing and to avoid spillage or foreign matter from contaminating the essential oil.
- Do not leave flasks with essential oils open as these can evaporate rapidly.
- To avoid oxidation of the essential oils do not place them in used containers.
- Label each flask with the name of the essential oil and the date of extraction.

## I.1.8. Applications and used of essential oils

Due to their various properties, EOs have become a material of considerable economic importance with a constantly growing market. Indeed, they are marketed and are of great interest in various industrial sectors such as pharmaceuticals by their antiseptic, analgesic, antispasmodic, aperitif, anti-diabetic…, in food through their antioxidant activity and flavoring effect, in perfumery and cosmetics through their odoriferous property. Here are some of the main fields where essential oils are commonly used:

- Aromatherapy.
- Food industry
- Cosmetology and perfumery
- Pharmacy

## I.2. Topic 2: Selected aromatic plants

### I.2. 1. Rosemary

The name Rosemary is derived from the Latin words “ros” meaning “dew” and “marinus” meaning “sea” So, essentially, the name Rosemary means “dew of the sea.” The name was first used as a given name in the late 19th century and has been popular ever since. Rosemary is a popular herb with a long history of culinary and symbolic uses. It has been used in cooking for centuries, adding a distinct flavor and aroma to various dishes. In addition to its culinary significance, rosemary has been associated with memory and remembrance in different cultures throughout history.

#### I.2.1.1. Plant description

Rosemary (*Rosmarinus officinalis* L.) is a perennial woody herb that belongs to the mint family (Lamiaceae). It is one of the largest and most distinctive families of flowering plants, with about 236 genera and more than 6000 species worldwide. This family has an almost cosmopolitan distribution. Some genera like *Nepeta*, *Phlomis*, *Eremostachys*, *Salvia* and *Rosmarinus* have a great diversity in the Mediterranean. [28] Rosemary grows as an evergreen shrub (**Fig I.6**), reaching up to 6 feet tall, with a woody stem and the leaves are in the form of waxy, slightly curved needles. The plant produces small, blue or purple flowers that bloom in the spring and summer. It is fairly salt and drought tolerant. Numerous cultivars exist.

**Figure 0.7:***Rosmarinus officinalis* L plant

### I.2.1.2. Properties and uses of Rosemary

The plant itself and the extracts and essential oils obtained from it are used in home cooking, and the cosmetic and food industries. It also finds applications in agriculture as an animal feed additive. Rosemary has been used for traditional medicinal purposes for centuries such as: [29]

- Improving digestion
- Enhancing memory and concentration
- Relieving stress and anxiety
- Relieving pain and inflammation

This plant deserves special attention, not only because of its unique taste and smell, but also due to a composition that provides health benefits. Its antimicrobial activity has been proven in relation to bacteria, fungi (including yeast) and viruses. Positive effects on the metabolism of carbohydrates and lipids, and the function of the nervous system, as well as hepatoprotective properties. [30]

### I.2.1.3. Chemical composition of Rosemary EOs

The oil content in leaves ranges between **1.0–2.5** %, depending on whether the leaves are young, or fully mature and dry. In a quantitative analysis of the chemical composition of oils from different regions (Iran, Morocco, Spain, France, Algeria, Cuba, Argentina, Italy), the following constituents were identified as shared in common: **α-pinene**, **β-pinene**, **1,8-cineole**, **camphene**, **borneol**, **camphor**, **linalool** and **β-caryophyllene**. Substances found in the majority of oils were: **β-myrcene**, **bornyl acetate**, **verbenone**, **limonene** and **sabinene**, which are terpene compounds [31].

## I.2.2. Eucalyptus

The first known use of *Eucalyptus* was in 1801, the name comes from the Greek *eucalyptos*, meaning well-covered, since the flower buds are enclosed with a cup-like membrane which is thrown off as the flower expands. On the other hand, the vernacular name of “tree with fever”, attests to its powerful medicinal properties. It is also sometimes called "blue gum tree".[32]

### I.2.2.1. Plant description

The genus *Eucalyptus* is a member of the Myrtaceae family comprising of more than 700 species. The *Eucalyptus globulus* Labill. (**Fig I.7**), commonly named as Tasmanian blue gum, is an evergreen broadleaf tree native to south-eastern Australia. Today, several countries in Asia, South America, southern Europe, and Africa, have an estimated area of planted forest around 16–19 million hectares (FAO, 2000). *Eucalyptus globulus* is a medium to large evergreen broadleaf tree, growing up to 70 m. It features reddish-brown bark that peels on the smaller branches. The leaves are a silvery to blue-green color, and they give off the plant's distinct menthol-like fragrance when bruised. The fruit is a woody capsule 0.6 to 2.5cm in diameter. [33, 34]

**Figure 0.8:** *Eucalyptus globulus* plant

### I.2.2.2. Properties and uses of Eucalyptus

Eucalyptus has been used for thousands of years throughout human history. Th Different parts of this plant are nutritionally very important and therapeutically highly valuable due to specific chemical composition as its essential oil contain. Traditionally, eucalyptus species have been used for supporting a healthy respiratory system and to soothe the muscles after exercise. The oil was used in traditional Aboriginal medicines to heal wounds and fungal infections. Teas made of eucalyptus leaves were also used to reduce fevers. Chinese, Greek, European and Ayurvedic medicine later adopted *Eucalyptus globulus* as a disinfectant and expectorant.

Present day medicinal applications of Eucalyptus globulus oil may be seen in the majority of grocery stores and pharmacies around the world including the oil's use in vapor chest rubs, over-the-counter cough and cold medications, sore throat sprays, topical pain relievers just to name a few. [12]

#### I.2.2.3. Chemical composition of Eucalyptus EOs

The essential oils obtained from the leaves, bare branches, flower buds and mature fruits of Eucalyptus globulus contain large number of highly valuable chemical compounds. The leaf oils were found to contain **1,8-cineole** (4.10–50.30%) depending upon maturity and origin of their collection site. Other major components of the leaf oils were **α-pinene** (0.05–17.85%), ***p*-cymene** (trace-27.22%), **cryptone** (0.00–17.80%) and **spathulenol** (0.12–17.00%). [17]

Due to these chemical compounds, *Eucalyptus glabrous* is found to be a potential antimicrobial, anti-fungal, anti-viral, anti-inflammatory, analgesic, anti-nociceptive and anti-oxidant agent of nature. Some recent scientific investigations have also revealed that essential oil of Eucalyptus glabrous also have anti-diabatic potentials that enhances its market value due to excessive usage in number of pharmaceutical products of traditional and advanced system of medicines. [17]

### I.2.3. Orange tree

In Rutaceae family, the *Citrus* genus has many species, some 1,600 subspecies and includes sweet orange, grapefruit, lemons, limes, etc. Oranges originated in Asia (China, Northeast India, and Myanmar). Sweet orange (*Citrus sinensis*) is a hybrid between pomelo (*Citrus maxima*) and mandarin (*Citrus reticulata*).

#### I.2.3.1. Plant description

The orange tree (**Fig I.8**) is a small to medium-sized evergreen tree that belongs to the Rutaceae family. It has a rounded crown with glossy green leaves that are oval-shaped and about 4-10 cm long. The tree grows up to a height of 5-15 meters and has a trunk that is usually thorny. The flowers are white, fragrant, and about 2-3 cm in diameter. The fruit of the orange tree is a hesperidium, which is a type of berry that has a leathery rind and pulpy interior filled with segments of juicy flesh containing seeds. Oranges are one of the most commonly grown and consumed fruits in the world, and they are rich in vitamin C and other nutrient. [35]

**Figure 0.9:***Citrus sinensis* plant

### I.2.3.2. Properties and uses of *Citrus sinensis*

*Citrus sinensis*, commonly known as sweet orange or orange, is a popular citrus fruit with a range of properties and uses. The tree is a potential source of firewood, while fruits are a good source of vitamin C. They can be eaten fresh or made into juice, marmalade or jelly. Leaf decoction with salt is taken orally for digestive tract ailments, nerve disorders, fever, asthma, blood pressure, general fatigue and vomiting. Crushed leaves or fruit juice is massaged into the skin to relieve itching. Macerated root, leaf or fruit mesoderm is taken orally for urethritis; macerated fruit mesoderm or bark decoction is taken orally for liver ailments. Peels, leaves and flowers contain fine essences of oils that may be used in manufacture of cosmetics and medicinal applications. [36]

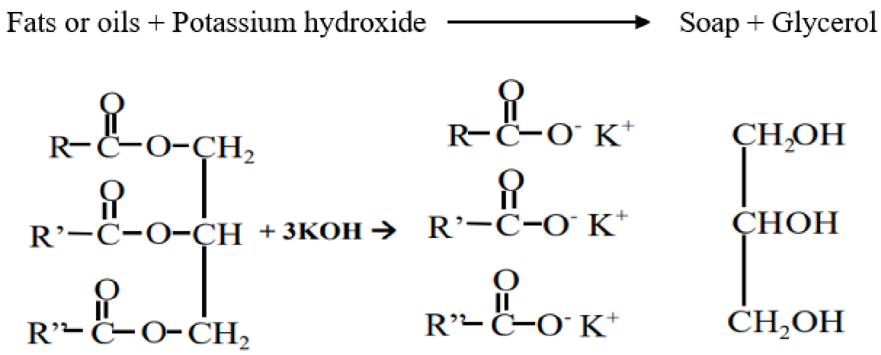
### I.2.3.3. Chemical compositions of *Citrus sinensis* EOs

Several types of chemical compounds have been identified in orange oils. The main monoterpene was limonene (89.78%), followed by myrcene (3.19). However, γ-terpinene and α-pinene appeared in amounts greater than 1%. The major oxygenated monoterpene compound was linalool (0.83%). In addition, linalyl acetate and sabinene hydrate were only present. A recent study (2023), showed that *Citrus sinensis* EOs could represent natural and safe alternatives to extend the shelf life of food products by preventing oxidation and contamination by pathogens that spoil food, meaning the sweet orange EOs can be considered as an innovative dual strategy for food preservation.[37]

## I.3. Topic 3: Saponification process

### I.3.1. What is saponification?

Saponification is a chemical reaction that involves the hydrolysis of fats or oils in the presence of an alkali (such as sodium hydroxide or potassium hydroxide) to obtain fatty acid salts during process of soap-making. The distribution of unsaturated and saturated fatty acid signifies the hardness, aroma, cleansing, lather, and moisturizing abilities of soaps. The equation for the formation of a soap is as shown below:




### I.3.2. Soap making process

Soap has been available for a long time. A soap recipe carved into a tablet from Ancient Babylon shows that soap has been available since 2200 BC. There is also evidence that the Egyptians used a soap-like substance made of animal and vegetable fats mixed with alkaline salts. Ancient Rome used pomade for their hair that was similar to soap, and Ancient China also has evidence of the use of a soap-like product.

Islamic documents from the 12th century describe the process of making soap and by the 13th century, soap making had become industrialized in the Islamic world, with production centers in Nablus, Fes, Damascus and Aleppo (Soap History, 2014). Modern soap making began in the 19th century with the work of Eugene Chevreul (person who discovered the chemical nature of soap). [38]

A soap is a cleansing agent made from the salts of vegetables and animal fat. They are a mixture of sodium or potassium salts of medium-chain and long-chain alkanoic acids (**Fig I.9**). Soaps are substances that increase the wetting power of water to remove dirt.

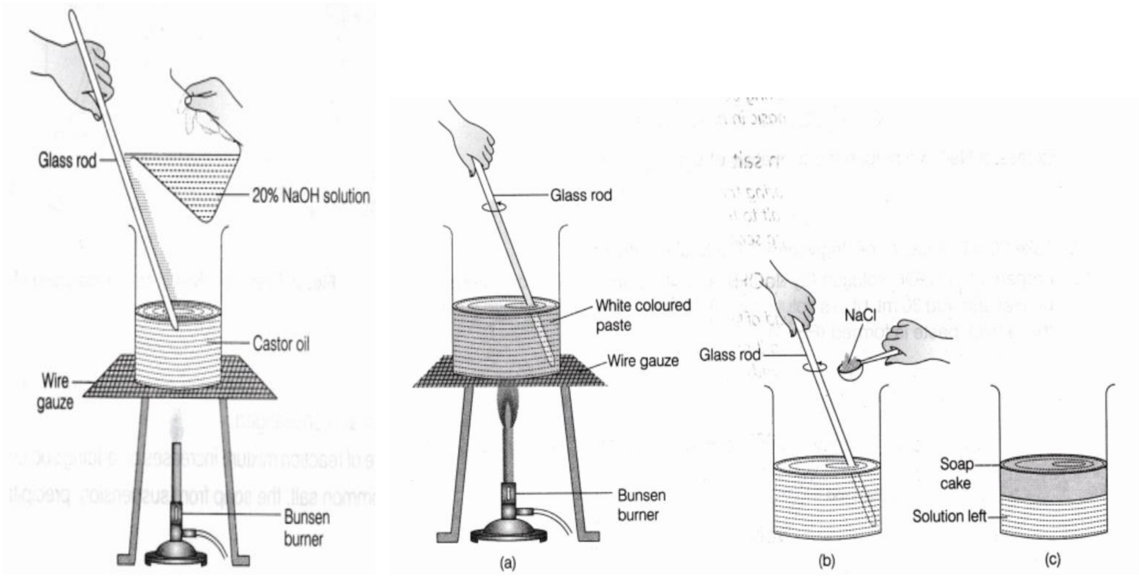



**Figure 0.10*:***Steps of preparation soap

Many things can be added to the 3 key ingredients for color, scent, texture and lather (see **Table I.1**).

**Table 0.1:**Optional ingredient added to increase soap quality.

| **Color** | **Scent** | Texture |
|---|---|---|
| • Yellow -turmeric<br>• Green-parsley<br>• Brown-cinnamon, cocoa Powder, chocolate, cloves<br>• Orange -paprika<br>• Clay – Can also be added for color | • Peppermint<br>• Spearmint<br>• Lavender<br>• Vanilla<br>• Essential Oils | • Oatmeal<br>• Flower petais<br>• Coffee grounds<br>• Tea Leaves<br>• Tapioca pearls<br>• Pippy seeds<br>• Pumice<br>• cornmeal |

### I.3.3. The Cleaning Power of Soap

The function of soaps is to remove grease and dirt by emulsifying the grease (bringing it into suspension). Dirt adheres to clothing and to skin primarily by being “glued” to these surfaces with a thin film of oil or grease; the oil (lipid) on the skin is generally secreted during perspiration. The soap removes the oil film and the dirt can be washed away. [39]

The cleansing action of soap or detergents is determined by its polar and non-polar structures in conjunction with an application of solubility principles (**Fig I.10**). The long hydrocarbon chain is of course non-polar and hydrophobic (repelled by water). The "salt" end of the soap molecule is ionic and hydrophilic (water soluble). The use of such compounds as cleaning agents is facilitated by their surfactant character, which lowers the surface tension of water, allowing it to penetrate and wet a variety of materials (**Fig I.11**). [39]

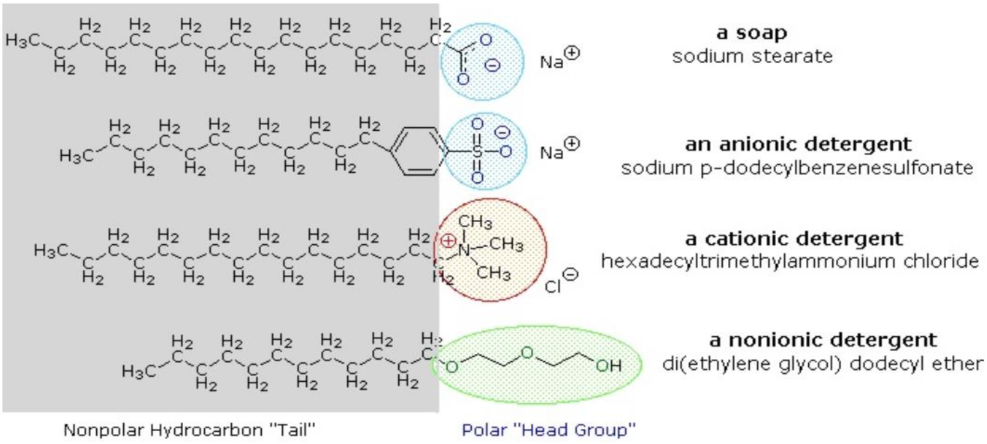



**Figure 0.11:**Soap and detergent molecules with different polar structures.

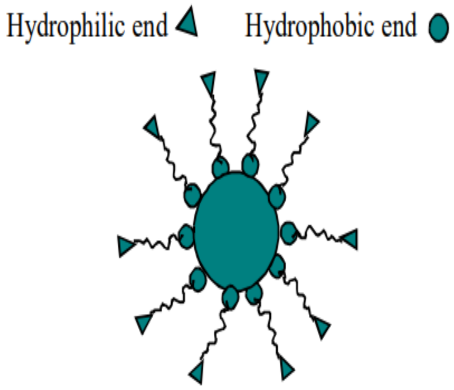



**Figure 0.12:**Soap molecules surround the oil droplet “A soap micelle”

### I.3.4. Qualities of Soap

When creating soap recipes, ingredients can be adjusted in order to control the qualities of the soap produced. Here are some key qualities of soap:

- **Hardness:** The hardness value describes how hard the soap is. Different fats create soaps with different hardness values. The higher the hardness value, the harder the soap will be.


- **Emulsification:** Soap acts as an emulsifying agent, meaning it can disperse and suspend oils or other substances in water. This property helps to break down and wash away oil-based substances, making soap effective for cleaning greasy or oily surfaces.

- **pH Balance:** Soap is typically formulated to have a pH level that is closer to the slightly alkaline side (around pH 9-10). This pH balance ensures compatibility with the skin's natural pH, helping to maintain the skin's acid mantle and minimize irritation.
- **Fragrance:** Soap can be scented with various fragrances to provide a pleasant and refreshing experience during use. Fragrances can range from floral and fruity scents to herbal or citrus notes, enhancing the sensory aspect of using soap.

# Chapter II

# Material and methods

The research work presented in this study was conducted in the laboratory of the Department of Matter sciences, Faculty of Exact Sciences and Sciences of Nature and Life, University of Biskra.

## II.1. Isolation of Volatile Oils

The air-dried plant materials (entire leaves and finely ground) were subjected to hydro-distillation for 4 h using Clevenger-type apparatus and simple steam distillation. It should be noted that the microwave extraction method that we wanted to use is unfortunately not available.

### II.1.1.Plant materials

Both plant *Rosmarinus officinalis* L. and *Eucalyptus globulus* Labill. (**Fig II.1**) were collected in the region of Ain Zaatout and Djemorah, respectively, in January 2023. The leaves are first dried before being packaged for subsequent use.

**Figure 0.1:**Fresh leaves of plants; Rrosemary (on the left) and Eucalyptus (on the right).

Orange peels were obtained from cleaned and dried oranges. They were separated from the endocarpthe and cut into small pieces (**Fig II.2**). The peels were softly dried and stored in the refrigerator until treatments.

**Figure 0.2:**Orange peels

### II.1.2.Extraction protocol

Samples, 100 g of dried plant (leaves, peels and their powder) were immersed in 1 L of distilled water. The extraction was carried out during 4 h from the first drop of distillate until the amount of essential oils stabilized. The different steps are shown below:

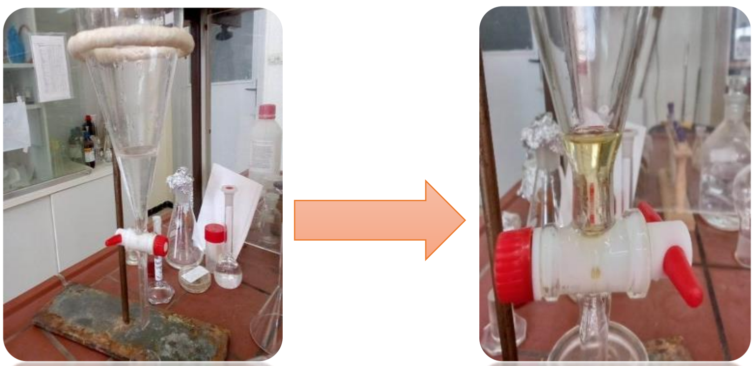
| | | |
|---|---|---|
|  |  |   |
| Rosemary leaves | Eucalyptus leaves | Orange peels |
|  |   |  |
| Rosemary powder | Eucalyptus powder | Orange powder |

**Hydro-distillation apparatus**

**Clevenger apparatus**

**Distillate obtained**

Afterwards, the essential oil is separated using a separatory funnel. The oil phase (upper portion) was dried over anhydrous sodium sulfate, filtered and stored at + 4 °C until analyzed.



### II.1.3. Analysis of essential oils

#### II.1.3.1. Physical analyses

The refractive index (20 °C) and density (20 °C) of essential oils were determined following standard methods. An Abbe refractometer (**Fig II.3**) was used for the determination of refractive index of the essential oils tested.

**Figure 0.3:**Abbe refractometer.

#### II.1.3.2. Chemical analyses

The acid index, also known as the acid value or acid number, is a measure of the acidity or free fatty acid content of a substance, was calculated to determine the acidity of our EOs. To measure the acid index, we are followed the standard titration method (**Fig II.4**). Therefore, the EO was diluted with EtOH solvent and titrated by slowly adding solution of potassium hydroxide (0.01 M) from a burette while stirring the mixture continuously. The volume of the base solution required to reach the endpoint was recorded (the phenolphthalein indicator will change color at the endpoint, indicating neutralization). The acid index is given by the following formula:

**Acid Index (mg KOH/g) = (Volume of Base Solution × Normality of Base Solution × 56.1) / Sample Weight**

**Figure 0.4:** Standard test method for determination of acidity in essential oils by titration

### II.1.3.3. Chromatographic analysis

The essential oils were analyzed using a thin-layer chromatography (TLC). TLC plates were developed in different elution systems and after were visualized by using Jodie reagent ($I_2$).

## II.2. Preparation of Aromatic Soap

Preparation of fragrant soap was performed by using rosemary, eucalyptus, and orange essential oil with olive and coconut oils in aqueous hydroxide solution during the saponification process. To avoid volatilization of the essential oils, we added some drops at the end of saponification.  The method could be summarized as follows:

**Ingredients:**

- 50 grams of Olive Oil
- 50 grams of Coconut Oil
- 34 grams of Water
- 15.9 grams of NaOH (Sodium hydroxide)
- 25 grams of Beeswax
- 34 grams Camel milk
- Spirulina powder
- Somes drops essential oils

1. Measure the Olive Oil and Coconut Oil and place them in a suitable container.


2. Carefully weigh the NaOH (Sodium Hydroxide) and place it in a separate container. Add water to the NaOH (Sodium hydroxide) safely.


3. Slowly pour the NaOH solution into the oils, stirring continuously. Use a stick blender to mix the oils and NaOH solution until they reach trace, which is a thick, pudding-like consistency.


4. Add the Beeswax, some drops of essential oils, Camel milk and spirulina powder to the mixture and stir well to incorporate it.




5. Pour the soap mixture into a soap mold and smooth the top with a spatula.


6. Once the soap has released from the mold, carefully lift it out of the mold using your fingers or a utensil like a spatula or butter knife and place it on a clean surface, ready for further curing or packaging.

7. After removing the soap from the mold, it is recommended to let it cure for an additional period to harden and dry fully. This curing period can vary depending on the recipe and personal preference, but typically ranges from a few weeks to several weeks.

# Chapter III Results and discussion

## III.1. Extraction

### III.1.1. Extraction yield

In order to study the extraction yield of the three plants (leaves and peels), the experiments were performed on whole and crushed plant material. The extraction yield refers to the amount of EOs obtained from a plant material. It is expressed as a percentage and can be calculated using the following formula:

**Extraction Yield (%) = (Weight of EO Obtained / Weight of Plant Material Used) x 100**

The obtained results are presented in the following tables:

**Table 0.1:**Calculated extraction yield (%) of different oil samples obtained by hydro-distillation method.

| | Rosemary | | Eucalyptus | | Orange peels | |
|---|---|---|---|---|---|---|
| | **crushed** | **leaves** | **crushed** | **leaves** | **crushed** | **pieces** |
| **Wight of oil (g)** | 0,6 | 0,59 | 0.6 | 0.4 | 5.5 | 0.6 |
| **oil yield (%)** | 0,6 | 0,59 | 0.6 | 0.4 | 5.5 | 0 .6 |

**Table 0.2:**Calculated extraction yield (%) of different oil samples obtained by Clevenger.

| | Rosemary | | Eucalyptus | | Orange peels | |
|---|---|---|---|---|---|---|
| | **crushed** | **leaves** | **crushed** | **leaves** | **crushed** | **Pieces** |
| **Wight of oil (g)** | 1 | 1.3 | 0.8 | 0.6 | 4.6 | 0.6 |
| **oil yield (%)** | 0,6 | 0,59 | 0.6 | 0.4 | 5.5 | 0.6 |

## III.1.2. Physical and chemical characteristics of EOs

Physical values of different oil samples were shown in Table III.3. The density of each essential oils (Rosemary, Eucalyptus and orange peels) was around 0.82 and 0.88 g/mL, their refractive index was about 1.469-1.488, and the acid index (AI) was between 0.4 and 2.35. From the values of refractive and acid index were very close to each other in the same EOs given by literature data.

**Table 0.3:** Physical, chemical and organoleptic properties of essential oils from Rosemary, Eucalyptus and orange peels. (Leaves and powder)

| | Rosemary | | Eucalyptus | | Orange peels | |
|---|---|---|---|---|---|---|
| | **crushed** | **leaves** | **crushed** | **leaves** | **crushed** | **Pieces** |
| **Density (g/mL)** | 0.88 | | 0.80 | | 0.82 | |
| **AI (20°C)** | 0.42 | 0.67 | 1.68 | 2.35 | 0.729 | 0.561 |
| **Ref** [40] | 0.5-2 | | 2.25 - 2.93 | | 0.5-2 | |

| | | | |
|---|---|---|---|
| **IR (20°C)** | 1.469 | 1.488 | 1.470 |
| **Ref** [41] [42], [43] | 1.467 | 0.8 | 2.8 |
| **Appearance** | Clear | Clear | Clear |
| **Color** | Colorless to pale yellow | Colorless | Colorless to pale yellow |
| **Odor** | Herbal, woody, and slightly camphorous | Fresh, camphorous, and slightly medicinal | Sweet, citrusy, and refreshing |

### III.1.3. Interpretation of chromatograms

The TLC-profiles of all samples (**Fig III.1**) showed the same component of EOs obtained by both methods (Clevenger and hydro-distillation) from the same material plants (Rosemary, eucalyptus and orange peels).

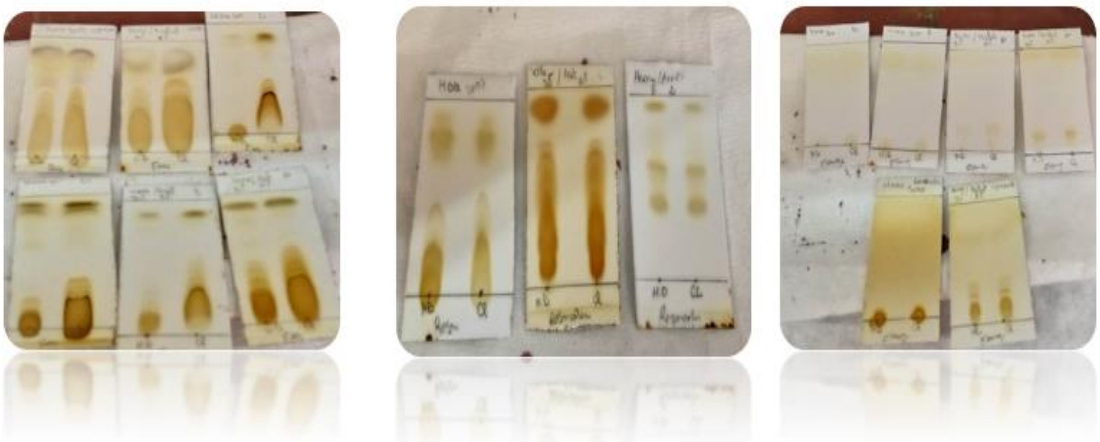


**Figure 0.1:**Chromatograms of EOs (Rosemary, eucalyptus and orange peels) obtained by two methods (Clevenger and hydro-distillation)

## III.2. Soap characterization

Fragrant soaps were prepared for sake of refreshment and good smell during hand washing or shower. Oil-solid soaps were prepared using olive and coconut oils. The results showed that the obtained solid soap exhibited a good emulsifying power (**Fig III.2**) with pH level that is closer to the slightly alkaline (**8-9**). In the current work, olive and coconut oil and its mixture with some essential oils and other ingredients such as Beeswax, Camel milk and spirulina were chosen for making aromatic, fragrant, and antibacterial soap because of these excellent antibacterial known agents.

**Figure 0.2:**Results of emulsifying power and pH level of different aromatic soap.

# General Conclusion

# Conclusion

This work consists in studying two methods of extraction of the essential oils of *Rosmarinus officinalis* L., *Eucalyptus globulus* Labill. and *Citrus sinensis*: Simple hydrodistillation and Clevenger hydrodistillation. Several parameters have been studied: the yield of extraction, and the physicochemical properties of essential oils. Consequently, the best EOs yields were obtained by using Clevenger apparatus and powdered plant materials, compared to those obtained by simple hydrodistillation of whole leaves or peels.

During our study, we conducted a qualitative analysis of the essential oils derived from rosemary and eucalyptus leaves, and orange peels. The results show that our EOs are in good condition and of good quality. Indeed, we observed that the essential oils obtained exhibited physical, chemical and organoleptic properties such as appearance, color, odor, density, acid index (IA), and refractive index at 20°C which aligned with literature data.

Additionally, we have carried out chromatography analysis (TLC) to determine the richness of the obtained essential oils in secondary metabolites.

Essential oils have shown promise as antiviral agents against several pathogenic viruses. Fragrant soaps were prepared for sake of refreshment and good smell during hand washing or shower. The concept of fragrant soap was extended to produce antimicrobial soap by adding herbal extracts/ herbal essential oil during saponification reaction. For that raison, olive and coconut oils mixed with isolated EOs and other natural ingredient were chosen for making soap as practical application of essential oils.

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# Abstract

This master's thesis focuses on the extraction of essential oils from three aromatic and medicinal plants, using two methods. In our work, we aimed to study the effect of extraction method on the quality and weight yield of EOs. In fact, the results indicate that essential oils extracted from, *Rosmarinus officinalis* L., *Eucalyptus globulus* Labill. and *Citrus sinensis* were compatible with those of previous studies, reported in literature. All essential oils were extracted using Clevenger-type apparatus and simple hydrodistillation.

The findings of this research provide valuable insights into the extraction methods and properties of essential oils derived from rosemary and eucalyptus leaves and orange peels. Furthermore, the integration of these essential oils in soap production highlights their potential as natural additives, offering fragrance and potential therapeutic benefits.

**Keywords:** Essential oils, Clevenger, hydro-distillation, Rosemary, Eucalyptus, Orange peels, soap.

**الملخص**

تركز رسالة الماستر هذه على استخراج الزيوت العطرية من ثلاث نباتات عطرية وطبية باستخدام طريقتين مختلفتين. يهدف عملنا هذا على دراسة تأثير طريقة الاستخراج على جودة الزيوت العطرية وكمية الانتاج. في الواقع، تشير النتائج إلى أن الزيوت العطرية المستخرجة من الإكليل الجبل (*Rosmarinus officinalis* L.)، وشجرة اليوكاليبتوس (*Eucalyptus globulus* Labill.) ، وقشور البرتقال (*Citrus sinensis*) متوافقة مع تلك الموجودة في الدراسات السابقة الموثقة في المراجع العلمي.

تم استخراج جميع الزيوت العطرية باستخدام جهاز من نوع Clevenger وطريقة التقطير البسيطة. توفر نتائج هذا البحث رؤى قيمة حول طرق الاستخراج وخصائص الزيوت العطرية المستمدة من أوراق إكليل الجبل وأوراق اليوكاليبتوس وقشور البرتقال. وعلاوة على ذلك، فإن دمج هذه الزيوت العطرية في إنتاج الصابون يسلط الضوء على إمكانية استخدامها كمواد إضافية طبيعية توفر العطر والفوائد العلاجية المحتملة .

**الكلمات الرئيسية:** الزيوت العطرية، جهاز Clevenger ، التقطير، إكليل الجبل، اليوكاليبتوس، قشور البرتقال، الصابون.