

In partial fulfillment of the requirements for the Master's Degree (Master II) in  
Plant Breeding

**Title:**

Essential oil extraction of Lavender: Applications in  
natural care products

**Presented on:** 01/07/2025

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Université 20 Août 1955 - SKIKDA -  
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وزارة التعليم العالي و البحث العلمي  
جامعة 20 أوت 1955 - سكيكدة  
كلية العلوم  
قسم علوم الفلاحة

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العلوم الفساحية  
جامعة 20 أوت 1955 - سكيكدة

PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA  
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20th August 1955 University Skikda



Faculty of Sciences  
Department of Agronomic Sciences



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## **Dedication**

*This work is humbly dedicated to :*

*Myself for enduring all these years despite the emotional and physical exhaustion.*

*To my family, especially my mother, whose unwavering support has meant everything  
to me.*

*And to my little niece Farah , whose presence brought light to my journey.*

***Djouaida***

## Abstract :

This research provides an overview of medicinal and aromatic plants, highlighting their chemical diversity and potential uses, with a particular focus on *Lavandula stoechas*.

The experimental part of the study includes the phytochemical analysis of *Lavandula stoechas* to identify key bioactive compounds. Essential oil was extracted by hydrodistillation using Clevenger-type apparatus. The extraction yield was calculated, and the extracted oil was then subjected to organoleptic and physicochemical characterization to assess its quality and security. In addition, thin-layer chromatography (TLC) was performed.

The results revealed the presence of potent bioactive compounds in this plant, supporting its traditional uses and confirming its potential in industrial applications. The extraction yield was 0.4%. The organoleptic and physicochemical properties complied with established standards, confirming the quality of the oil. TLC analysis confirmed the presence of rosmariinic acid, Linalool, 1,8-Cineole and Fenchone compounds that contribute to the plant's high therapeutic value.

These findings underscore the importance of integrating traditional knowledge with modern scientific approaches to promote the sustainable use of natural resources in the health and wellness sectors.

**Keywords:** essential oil, *Lavandula Stoechas*, phytochemical analysis, bioactive compounds, organoleptic, physicochemical properties, Tlc, therapeutic value.

## Résumé :

Cette recherche offre un aperçu des plantes médicinales et aromatiques, en mettant en évidence leur diversité chimique et leurs utilisations potentielles, avec un accent particulier sur *Lavandula stoechas*.

La partie expérimentale de l'étude comprend l'analyse phytochimique de *Lavandula stoechas* afin d'identifier les principaux composés bioactifs. L'huile essentielle a été extraite par hydrodistillation à l'aide d'un appareil de type Clevenger. Le rendement d'extraction a été calculé, puis l'huile obtenue a été soumise à une caractérisation organoleptique et physico-chimique pour évaluer sa qualité et sa sécurité. De plus, une chromatographie sur couche mince (CCM/TLC) a été réalisée.

Les résultats ont révélé la présence de composés bioactifs puissants dans cette plante, soutenant ses usages traditionnels et confirmant son potentiel dans les applications industrielles. Le rendement d'extraction était de 0,4 %.

Les propriétés organoleptiques et physico-chimiques étaient conformes aux normes établies, confirmant la qualité de l'huile. L'analyse en CCM a confirmé la présence de l'acide rosmarinique, du linalol, du 1,8-cinéole et du fenchone, des composés qui contribuent à la grande valeur thérapeutique de la plante.

Ces résultats soulignent l'importance d'intégrer les savoirs traditionnels aux approches scientifiques modernes pour promouvoir l'utilisation durable des ressources naturelles dans les secteurs de la santé et du bien-être.

**Mots clés:** huile essentielle, *Lavandula stoechas*, analyse phytochimique, composés bioactifs, organoleptique, propriétés physico-chimiques, CCM, valeur thérapeutique.

## ملخص :

تقدم هذه الدراسة نظرة شاملة حول النباتات الطبية والعطرية، مع إبراز تنوعها الكيميائي واستعمالاتها المحتملة، مع التركيز بشكل خاص على نبات الخزامى الجبلي (*Lavandula stoechas*)

يشمل الجزء التجريبي من الدراسة التحليل الكيميائي النباتي لنبات الخزامى الجبلي بهدف تحديد المركبات النشطة بيولوجياً الرئيسية. تم استخراج الزيت الأساسي عن طريق التقطير المائي باستخدام جهاز من نوع كليفتجر. بعد ذلك، تم حساب مردود الاستخلاص، ثم دراسة بعض الخصائص الحسية و الفيزيوكيميائية للزيت المستخلص لتقييم جودته وسلامته. بالإضافة إلى ذلك، تم إجراء تحليل كروماتوغرافيا الطبقة الرقيقة (CCM).

كشفت النتائج عن وجود مركبات نشطة بيولوجياً قوية في هذا النبات، مما يدعم استخداماته التقليدية ويؤكد إمكانياته في التطبيقات الصناعية. بلغ مردود الاستخلاص 0,4%، كما أظهرت الخصائص الحسية والفيزيائية-الكيميائية مطابقتها للمعايير المعتمدة، مما يؤكد جودة الزيت. وأكد تحليل CCM وجود حمض الروزمارينيك، اللينالول، 1,8-سينول، والفينيشون، وهي مركبات تساهم في القيمة العلاجية العالية لهذا النبات.

تُبرز هذه النتائج أهمية دمج المعارف التقليدية مع المناهج العلمية الحديثة من أجل تعزيز الاستغلال المستدام للموارد الطبيعية في قطاعات الصحة والرفاهية.

**الكلمات المفتاحية:** الزيت الأساسي، الخزامى الجبلي، التحليل الكيميائي النباتي، المركبات النشطة بيولوجياً، الخصائص الحسية، الخصائص الفيزيائية-الكيميائية، كروماتوغرافيا الطبقة الرقيقة، القيمة العلاجية.

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# **General introduction**

### General introduction:

Ancient medical records reveal the existence of clay tablets dating back to between 5000 and 3000 BCE, written by the Sumerians, which show that humans used medicinal plants to help maintain and restore health. This knowledge about medicinal and aromatic plants was passed down from generation to generation, contributing to the advancement of health and life in general. Over time, these plants came to be used in various fields such as medicine production, spices, cosmetics, and more (**Inoue et al., 2019**).

Lavender plants belong to the Labiatae family, which is also known as the Lamiaceae or mint family. Lavender has been known since ancient times, as the ancient Egyptians used it in the mummification process. It was also historically used to perfume bathwater, from which its name was derived from the Latin word *lavare*, meaning "to wash." There are several species of lavender, which are distributed from the Canary Islands, Cape Verde Islands, and Madeira, across the Mediterranean Basin, North Africa, Western Asia, the Arabian Peninsula, to tropical northeastern Africa, with a disjunct distribution reaching as far as India.

Among the species of lavender are *L. latifolia*, *L. pinnata*, *L. lanata*, *L. dentata*, and *L. Stoechas* (**Lis-Balchin, 2002**). *Lavandula stoechas* is one of the well-known types of lavender that grows naturally in Mediterranean countries. It is an evergreen shrub with a height ranging from 30 to 100 cm, and in some subspecies, it can reach up to 2 meters. It is used as an insecticide and commercially in air fresheners. It is also used as a remedy for headaches, nervous irritability, colds, wound healing, rheumatic pain, and more. Its essential oil is known for its antibacterial, antifungal, and anti-yeast properties, in addition to its calming effect on the nerves. The oil is extracted from the aerial parts of the plant (**Miraj, 2016**).

This graduation thesis aims to identify the benefits extracted from the essential oil of the plant

*Lavandula stoechas*.

✓ It consists of two parts in addition to a general introduction:

✓ First, the theoretical part, which consists of three chapters:

The first chapter discusses medicinal and aromatic plants, the second chapter covers essential oils,

and the third chapter talks about *Lavandula stoechas*.

✓ Secondly, the practical part, which presents results about a phytochemical study: the organoleptic properties, physicochemical parameters, and TLC.

✓ And finally, a conclusion.



**Chapter 1:  
Medicinal and  
Aromatic  
plants**

### Chapter 1: Medicinal and aromatic plants

#### 1.1. Generalities:

The growing concern over the use of synthetic additives has led to the search for safe alternatives, and aromatic plants with their essential oils have proven to be an ideal choice. They enhance human and animal health and nutrition, and have also long been used in the production of herbs and spices. According to the World Health Organization, 80% of the world's population still relies on plants for healthcare. Even animals benefit from their use, especially as an alternative to antibiotics, particularly after the European Union imposed a ban on antibiotic-containing feed additives. The compounds found in these plants are considered a therapeutic treasure, as they possess antioxidant properties and help reduce the risk of serious diseases such as cancer and cardiovascular diseases. In general, they protect the body from damage caused by oxidative stress resulting from free radicals (**Christaki *et al.*, 2012**).

Plants have always been a fundamental support for humans in various aspects such as food, clothing, and more, and they have also entered the field of medicine, forming the basis of traditional medicine. The use of plants as medicine is not a recent development; there are written records dating back more than 2600 years BC documenting the use of *licorice*, *cedar* and *cypress* oils, *myrrh*, and poppy juice, which were used to treat various illnesses such as coughs, colds, and more.

In general, natural products constitute 50% of medicines, including anticancer drugs that have been derived from North American plants. Medicinal plants typically contain a mixture of chemical compounds that work together to enhance human health. A single plant may contain several substances that aid digestion, reduce inflammation and pain, act as antioxidants, and provide various other therapeutic effects.

Today, traditional medicine often relies on mixtures of several plants that are chemically complex, aiming to restore balance and address health conditions through a combination of natural compounds (**Gurib-Fakim, 2006**).

#### 1.2. Definition of medicinal plants:

Medicinal plants are considered a source of natural compounds known for their anti-infection properties, as well as being antibacterial drugs (**Ríos & Recio, 2005**). Medicinal plants like *Aloe vera* (Figure 1) are increasingly used around the world, as they are considered an alternative treatment for various diseases. Due to the large number of plant species with medicinal properties, some sciences have emerged, such as herbal medicine, which has led to the development of traditional medicine in some countries. The traditional uses attributed to each medicinal plant depend on the place where the plants were consumed

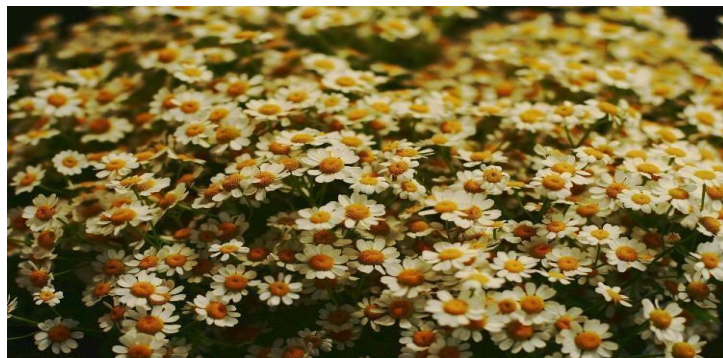
and the social groups that used them (Maldonado Miranda, 2021). Resistant diseases that have not been treated with conventional prescribed drugs make medicinal plants a promising source for discovering new drugs (J Kayombo, 2013). Medicinal plants are extremely important as a source of valuable products with both low and high molecular weights. They play a significant role in the context of heterologous expression of high-value pharmaceutical products. Moreover, they represent a future step in the field of bioengineering, which focuses on large-scale industrial production of secondary metabolites in microbial systems, as well as the production of recombinant proteins in plant cell factories (Vasilev, 2022).



**Figure 1:** *Aloe vera* plant (Smart, 2019).

### 1.3. Definition of aromatic plants:

Aromatic plants are used for their scent and flavor like *chamomile* (Figure 2), as they contain aromatic compounds, most of which are essential oils that are volatile at room temperature. Essential oils and volatile compounds are generally used in flavors and perfumes, and they also have multiple applications in various fields such as cosmetics, pharmaceuticals, and botanical pesticides. Aromatic compounds are synthesized in special structures called glands, which are located in different parts of plants, including leaves, flowers, fruits, seeds, bark, and roots. Several physical and chemical processes are used to extract these essential oils, such as steam distillation, water distillation, and cold pressing in the case of citrus fruits. Additionally, the enfleurage technique, which relies on odorless fats that remain solid at room temperature to capture aromatic compounds emitted by plants, is also used, along with solvent extraction to isolate volatile compounds (Barata *et al.*, n.d.). The therapeutic properties of essential oils obtained from aromatic plants have been known for a long time. *In silico* docking studies explained their interactions with MAO (A and B) and gamma-aminobutyric acid (GABA) receptors, which are receptors that play a crucial role in the central nervous system and are targeted by anxiolytic and sedative drugs (Wang & Heinbockel, 2018).



**Figure2:** *Matricaria chamomilla* (Hermann, 2020).

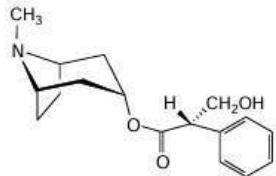
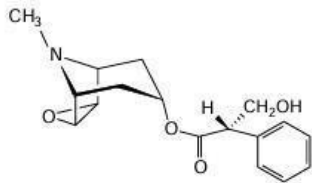
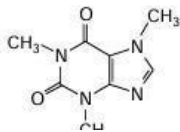
**1.4. Fields of use of Medicinal and aromatic plants:** They are used in several fields such as:

**1.4.1. Pharmaceutical industry:**

We can say that about 25% of the medicines prescribed by doctors worldwide are derived from the chemicals produced by flowering plants. If we add the compounds produced by fungi and some animals, the percentage could reach 40%. The bitter taste is often associated with the use of plants as medicine, while the white color is linked to dark liquids. The world is now increasingly recognizing plants as a source of molecules that have current or potential value in the treatment of diseases (Houghton, 2001). And when we give examples of drugs extracted from plants, we find the Pacific yew tree, from which the anticancer alkaloid paclitaxel is extracted. We also find the Madagascar periwinkle (*Catharanthus roseus*), from which vinblastine is derived, as well as the foxglove plant, which yields digoxin.

Another example is the use of plant steroids, such as those extracted from the Mexican yam (*Dioscorea spp.*), to produce steroids in a semi-synthetic manner. Plant extracts play an important role in the production of drugs that contain a single chemical compound (Lubbe & Verpoorte, 2011). Some established pharmaceuticals obtained from plants are presented in Figure 3.

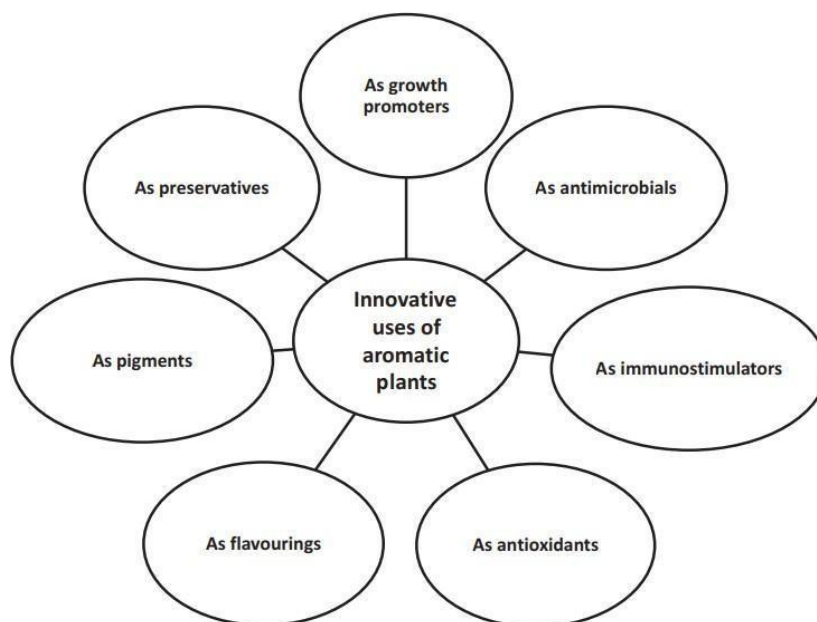
## Chapter 1: Medicinal and aromatic plants

Name	Structure	Plant Source	Use in Medicine
Atropine		Deadly nightshade ( <i>Atropa belladonna</i> ) Henbane ( <i>Hyoscyamus</i> spp.) Stramonium, jimson weed ( <i>Datura</i> spp.)	Antispasmodic for gastrointestinal tract; pupil enlargement in eye
Scopolamine		As for atropine	Preoperative sedative; antiemetic in travel sickness
Caffeine		Coffee ( <i>Coffea arabica</i> ) Tea ( <i>Thea sinensis</i> )	Central nervous system (CNS) stimulant

**Figure 3:** Some Established Pharmaceuticals obtained from plants (houghton, 2001).

### 1.4.2. Nutraceuticals and Dietary Supplements:

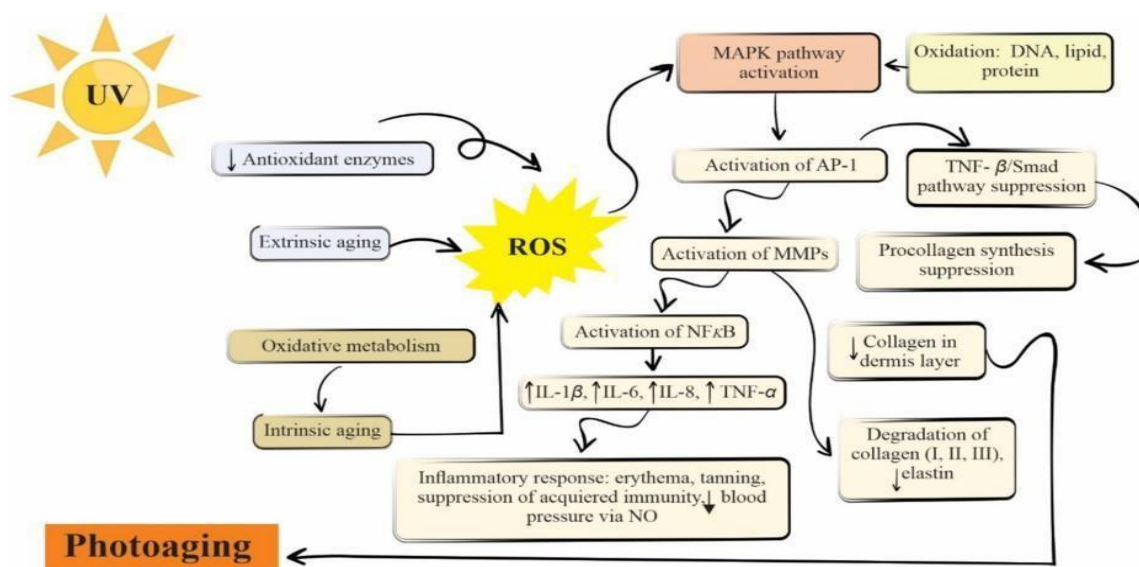
Functional foods, in addition to their nutritional effects, offer numerous benefits to the human body by improving health and well-being. Aromatic plants, with their valuable compounds, are essential for the development of these healthy foods, as they reduce the risk of chronic diseases such as cardiovascular diseases, neurodegenerative disorders, metabolic bone disorders, and cancer. (Christaki *et al.*, 2020). Thanks to recent advances in genetics, the focus has been exclusively placed on aromatic plants as dietary supplements. According to some studies, the interaction between dietary components and the genome is essential for affecting metabolic pathways and maintaining homeostasis in the human body (Simopoulos, 2010). Innovative uses of aromatic plants as natural supplements in nutrition are presented in Figure 4.



**Figure 4:** Innovative uses of aromatic plants as natural supplements in nutrition (Christaki *et al.*, 2020).

### 1.4.3. Cosmetic and personal care products:

Since ancient times, aromatic plants and essential oils have been used in skincare. Their diverse biological activities and sensory properties make them a valuable resource in the production of cosmeceutical products, thanks to their anti-aging components, which serve as an excellent alternative to synthetic products. Prolonged exposure to sunlight is considered the primary cause of aging due to the accumulation of reactive oxygen species (ROS) in the dermis. As is well known, antioxidants help counteract the oxidative stress caused by ROS. Among the constituents of aromatic plants are compounds such as flavonoids, phenolic substances, tannins, stilbenes, terpenes, and steroids, which protect against radiation, brighten the skin, promote wound healing, combat pigmentation, and regenerate tissues (Olivero-Verbel *et al.*, 2024). Diagram of the impact of ROS on the skin presented in Figure 5.



**Figure 5:** Diagram of the impact of ROS on the skin (Olivero-Verbel *et al.*, 2024).

### 1.4.4. Aromatherapy:

Aromatherapy is used in several fields, including cosmetic aromatherapy, where oils are used to make the skin healthier through specialized products for the skin, face, body, and hair. On the other hand, some people use it as a treatment through therapeutic baths, whether for the whole body or just the feet. Among its various applications, aromatherapy massage stands out, as certain oils have magical effects during massage, such as jojoba oil, almond oil, and grape seed oil, which help relax the body. Aromatherapy is also used in the medical field, as it enhances health and treats clinically diagnosed diseases. This is why René-Maurice Gattefossé, the founder of aromatherapy, used it to massage patients during surgery. Olfactory aromatherapy is considered a treatment on its own, as inhaling essential oils brings feelings of comfort, calmness, and relaxation. It also stimulates scent-related memories and reduces stress. Finally, aromatherapy is used in psychological aromatherapy, where essential oils are diffused into the air for the patient to inhale, helping improve mood and emotional well-being, leading to a sense of relaxation and energy (Ali *et al.*, 2015).

### 1.4.5. Environmental and household uses:

Industrial air fresheners are used to improve indoor air quality by eliminating unpleasant odors and purifying the air. However, their accumulation in a closed environment may cause respiratory problems due to their diverse chemical composition, leading to a deterioration in indoor air quality with continuous use.

When using certain plants such as *Azadirachta indica* (Figure 6), *Mentha piperita*, and *Aloe barbadensis*, which help combat fungi, a 60-70% reduction in vegetative structures and a 30% reduction in reproductive structures were observed after 11 days

of application. Thus, these herbal air fresheners serve as a good alternative to commercial fresheners, as they are cheaper, environmentally friendly, and pose no health risks (Lakshumanan & Velrajan, 2023). The spread of pollutants resulting from industrial activities has led to the release of toxic substances into the terrestrial ecosystem. To address this issue, phytoremediation has been developed, which relies heavily on green plants. This technique is used to remove both inorganic and organic pollutants. In phytoremediation, plants mineralize toxic organic compounds and transport them to the roots, while inorganic compounds accumulate and concentrate in the aerial parts of the plant, where they are sequestered within the harvested plant tissues (Augustina & Adriana, 2014).



**Figure 6:** *Azadirachta indica* (J.M, 2009).

### 1.5. The metabolism of plants:

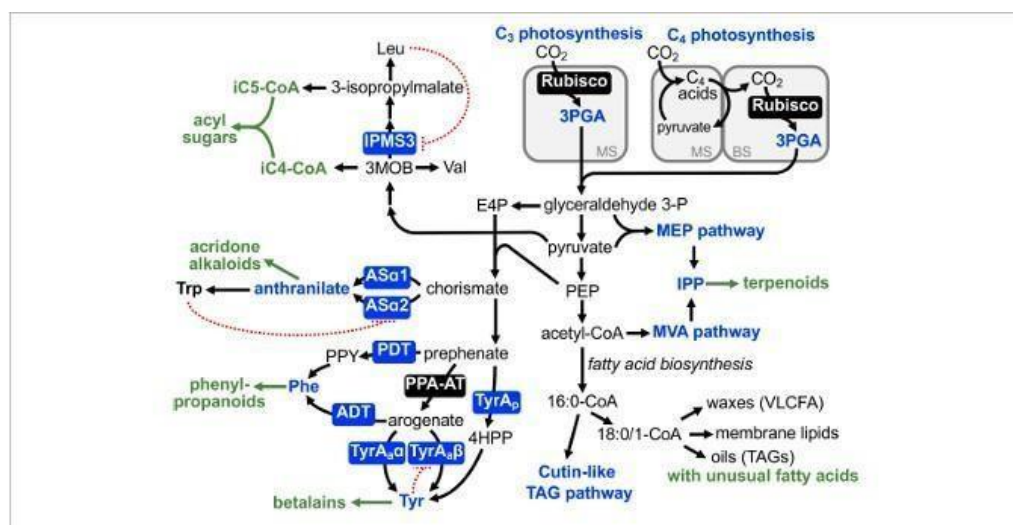
Gene duplication and functional specialization have enabled the development of plants, leading to the emergence of plant metabolic enzymes, which are one of the most important features of chemical diversity in plants (Ferne *et al.*, 2024). The plant metabolic network is one of the most complex networks in nature. It consists of two pathways: the primary pathway, which is essential for plant growth and development, and the secondary pathway, which plays vital roles in plants. These two pathways are interconnected to form a coordinated network that regulates signaling between cells, tissues, and subcellular compartments, as well as various processes in plants, such as transcriptional and post-transcriptional regulation (Lu, 2015).

#### 1.5.1. Primary metabolism:

Primary metabolism provides the essential compounds that ensure the stability of the plant cell. Despite being conserved across the plant kingdom, primary metabolic pathways have not been immune to change. Some biosynthetic pathways for certain lipids and amino acids have been altered in specific plant lineages, leading to chemical diversity based on these changes in plant metabolism (Maeda, 2019). Plants defend themselves against microorganisms by undergoing a massive reprogramming of the plant cell to activate responses that eliminate pathogens.

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These responses require a large amount of energy, which is provided by primary metabolic pathways through their carbon structures (**Bolton, 2009**). Primary metabolic processes allocate their energy for photosynthesis and plant growth. However, when plants are exposed to microorganisms, they reduce this energy allocation to conserve resources for defense responses. These metabolic pathways, for example, contribute to the breakdown of carbohydrates, amino acids, and lipids by activating specific genes, which in turn support plant defense responses (**Rojas *et al.*, 2014**). Diversification of primary metabolic pathways and enzymes are presented in Figure 7.



**Figure 7:** Diversification of primary metabolic pathways and enzymes ( **maeda, 2019**).

### 1.5.2. secondary metabolism:

Secondary metabolites are natural compounds primarily produced by bacteria, fungi, and plants.

They are low molecular weight compounds with a wide diversity of chemical structures and biological functions. Unlike primary metabolism, which produces essential compounds for growth such as amino acids and carbohydrates, secondary metabolism generates compounds that are not directly required for growth and development. The lack of involvement of secondary metabolism in growth, reproduction, and other physiological activities is due to its production at the end of the growth phase. It also mediates environmental interactions, increasing the organism's chances of survival. Additionally, it plays a crucial role in defense, such as plant resistance against herbivores. Humans utilize secondary metabolites in the production of flavorings, recreational drugs, medicines, dyes, and more. More than 214,000 secondary metabolites have been identified in scientific research, classified into five main categories: alkaloids, terpenoids, steroids, polyphenols, and fatty-acid-derived compounds, in addition to nonribosomal peptides and enzyme cofactors (**Zandavar & Afshari Babazad, 2023**).

### 1.6. Classification of Secondary Metabolites:

#### 1.6.1. The Alkaloids:

Alkaloids (Figure8) have been used since ancient times and were considered a source for the production of experimental medicines as well as poisons used in executions and hunting expeditions. They are classified into three main categories. True alkaloids are compounds derived from amino acids and contain a heterocyclic ring with nitrogen. Protoalkaloids are compounds that are not part of the heterocyclic ring but contain a nitrogen atom derived from an amino acid. Finally, pseudoalkaloids are compounds that do not derive their basic carbon skeletons from amino acids (Amirkia & Heinrich, 2014). Alkaloids are produced to protect the plant and help it survive, as they repel insects and herbivorous plants that try to feed on it and inhibit the growth of other plants. They are characterized by their bitter taste and crystalline form. Alkaloids usually contain basic nitrogen atoms and are produced by a wide variety of organisms. Due to their ability to bind to oxidative catalysts and free radicals, alkaloids act as a preventive measure against degenerative diseases. However, they are highly toxic and are used only in small quantities. Alkaloids significantly affect chemical transmitters such as acetylcholine, epinephrine, norepinephrine, gamma-aminobutyric acid, dopamine, and serotonin, which makes them have a strong impact on the nervous system. Additionally, some alkaloids have antibacterial properties, and therefore, certain ones are used in antiseptic products such as toothpaste (Roy, n.d.).

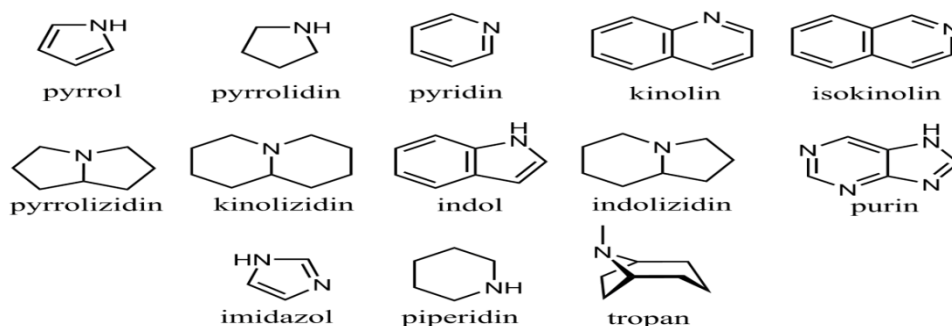


Figure8: alkaloid subgroups (Wangensteen, 2021).

#### 1.6.2 The Terpenoids:

They are also known as isoprenoids. The general name terpene was originally applied to the hydrocarbons found in turpentine oil, and they are among the most structurally diverse and numerous natural products. The suffix “-ene” indicates the presence of olefinic bonds (double bonds) (Ludwiczuk *et al.*, 2017). Terpenoids play a special role in defense and disease resistance, especially in woody plants. These compounds are derived through the formal condensation of five-carbon isoprenoid units (C5).

There are also so-called secondary metabolites or secondary products, which are part of the diverse group of terpenoids and are stored in large quantities in specialized compartments. Terpenes are considered highly important due to their ecological benefits (Croteau & Johnson, 1985). Terpenoids are among the plant antioxidants and perform

## Chapter 1: Medicinal and aromatic plants

several roles in plants, including inhibiting the growth of neighboring plants, acting as repellents or attractants in plant–plant interactions, and they are also responsible for the health benefits of fruits and vegetables due to their antioxidant properties (Graßmann, 2005). Table 1 represented classification of terpenoids.

**Table 1:** classification of terpenoids (Ludwiczuk *et al.*, 2017).

Name	No. of Isoprene Units	No. of Carbon Atoms	General Formula
Hemiterpenoids	1	5	C <sub>5</sub> H <sub>8</sub>
Monoterpenoids	2	10	C <sub>10</sub> H <sub>16</sub>
Sesquiterpenoids	3	15	C <sub>15</sub> H <sub>24</sub>
Diterpenoids	4	20	C <sub>20</sub> H <sub>32</sub>
Sesterterpenoids	5	25	C <sub>25</sub> H <sub>40</sub>
Triterpenoids	6	30	C <sub>30</sub> H <sub>48</sub>
Tetraterpenoids (carotenoids)	8	40	C <sub>40</sub> H <sub>64</sub>
Polyterpenoids	> 8	> 40	(C <sub>5</sub> H <sub>8</sub> ) <sub>n</sub>

### 1.6.3. The Flavonoids :

Flavonoids are secondary derivatives of polyphenols and are generally found in flowers, roots, bark, fruits, vegetables, and even some beverages. They are known for their multiple properties and diverse health benefits, as they possess antioxidant, anti-inflammatory, and anti-mutagenic effects. They also help regulate essential cellular enzyme functions and are considered strong inhibitors of several enzymes. Flavonoids play an important role in enabling plants to adapt to surrounding environmental conditions such as extreme heat or freezing, and they protect them from various biotic and abiotic stresses while contributing to detoxification processes.

Currently, there are about 6000 known types of flavonoids, and they have recently gained increasing attention for their potential use in the treatment of Alzheimer's disease. Flavonoids are divided into different subgroups based on the position of ring attachment as well as the degree of oxidation and saturation in their chemical structure. The main subgroups include flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins, and chalcones (Panche *et al.*, 2016) .

### 1.6.4. Phenolic acids :

Plants are among the natural resources that are used as both medicine and food due to their content of various compounds such as phenolic acids. As a result, they are utilized in medicine, dietary supplements, and cosmetics. They are also used as antioxidants and preservatives in food products and help in the treatment of certain types of cancer. Phenolic acids contain phenolic groups linked to aromatic rings, with a basic structure composed of a carboxyl group and one or more hydroxyl groups. Phenolic acids are divided into three categories: hydroxybenzoic acids, hydroxyphenylacetic acids, and hydroxycinnamic acids (Xie *et al.*, 2024).

### 1.6.5. Coumarins:

Coumarins are classified as polyphenols and are among the most common organic molecules due to their wide range of biological activities, including anti-inflammatory, antioxidant, anticonvulsant, antihypertensive, anticoagulant, and antimicrobial properties. They also play a neuroprotective role, among other effects. Coumarins influence cellular processes indirectly, and because of their significant therapeutic effects, they are widely used in the medical field. Coumarins have been present since ancient times, dating back thousands of years, and are extracted from various natural sources, most notably plants, as well as fungi and animals. Thanks to the tremendous advances in molecular biology and chemistry, it has become easier to extract drugs and treatments from coumarins. These compounds have demonstrated remarkable effects on the human body and have shown potential in treating several complex diseases such as inflammatory and vascular conditions, as well as cancer. Coumarins belong to a family of heterocyclic compounds and are classified into six main groups: simple coumarins, furanocoumarins, dihydrofuranocoumarins, pyranocoumarins, phenylcoumarins, and biscoumarins (**Flores-Morales *et al.*, 2023**).

### 1.6.6. Tannins:

Tannins make up between 5% and 10% of the dry weight of tree leaves. Tannins inhibit the activity of insects on the plant's leaves (**Barbehenn & Peter Constabel, 2011**). Tannins are polyphenolic compounds that are water-soluble. They are known to have antibacterial, antiviral, antifungal, and antimicrobial properties. These antimicrobial properties help increase the shelf life of food products by treating them with tannins. The effects of tannins depend on the dosage used, as they can accelerate blood clotting and may lower blood pressure. Tannins can be classified into two categories: hydrolyzable tannins and non-hydrolyzable or condensed tannins. Tannins are also used in poultry feed in low quantities and can affect egg production (**Chung *et al.*, 1998**).

### 1.6.7. Saponins:

The word "saponin" is derived from the Latin word "*sapo*," which means soap. It refers to glycosides that are surface-active and form foams in aqueous solutions, making them resemble soap. They are characterized by a variety of triterpenoids with diverse structures, and this structural diversity is the basis for most of their physical and chemical properties, among others. Therefore, they are used in several applications. Saponins are also considered active components, especially in plants used in traditional medicine, such as ginseng. Saponins are antibacterial, anti-inflammatory, and antiviral, and are used in the production of many drugs. Their primary role is to protect the plant from diseases. A prime example of this is oats, where the saponins found in its roots fight and resist soil-borne microbes (**Lambert *et al.*, 2011**).

### 1.6.8. Essential oils:

Essential oils are usually produced from aromatic plants as secondary compounds known for being volatile and strongly scented. They are generally extracted through steam distillation. These oils are known for their antiseptic properties and have various uses. They kill bacteria, viruses, and fungi, and are also used as sedatives, anti-inflammatory agents, antispasmodics, and local anesthetics. Additionally, they protect the plant from insects, making them effective natural insecticides. These plants are highly valued and mainly grow in Mediterranean countries and tropical regions (Bakkali *et al.*, 2008).

Essential oils have seen strong market growth, especially in the United States, accompanied by an increase in published evidence related to aromatherapy. Their role has expanded beyond therapeutic use, as they have gained popularity as natural self-care products. Due to the widespread use of natural oils, taking precautions has become important to avoid potential risks.

For example, *Lavandula angustifolia* oil has a calming and relaxing effect in small doses, while *L. stoechas* oil is a neurotoxin if taken orally, but is said to fight *Pseudomonas* infections when used as a topical spray. Therefore, it is crucial to identify the plant by its Latin name and avoid labeling products with generic names like “lavender oil” only (Manion & Widder, 2017).

### 1.7. Conclusion:

The integration of traditional knowledge with modern scientific methods has become increasingly popular, especially due to its vast applications in health, industry, and environmental sustainability. This is particularly evident in the use of medicinal and aromatic plants, thanks to their unique components known as secondary metabolites, such as alkaloids, terpenoids, and flavonoids. These compounds are widely used in pharmaceutical, cosmetic, and food industries, as well as in environmental applications like phytoremediation of pollution. As a result, these plants have proven to be effective in advancing medicine, biotechnology, and environmental protection. Recently, scientific research has surged to ensure their optimal and safe use.



## **Chapter 2: Essential oils**

### Chapter 2: Essential oils

#### 2.1. Generality:

Essential oils are generally extracted from oil-secreting glands, whose location varies from one plant to another; they may be found in the seeds, leaves, flowers, or other different parts of the plant. Plants that contain essential oils are usually aromatic. The oils themselves are volatile in nature and are typically lighter than water. Essential oils are formed in the cytoplasm of the intercellular spaces in the form of fine droplets. Generally, they consist of sesquiterpene hydrocarbons, monoterpenes and their oxidized derivatives, along with aliphatic aldehydes, esters, and alcohols, with a proportion ranging from 5% to 10% of non-volatile components. Essential oils are distinguished by their antibacterial constituents and, in general, act to repel various pests from plants. There are several methods for extracting essential oils, the most common being steam distillation, steam and water distillation, and combined steam and water distillation, which are traditional methods. In addition, there are modern techniques such as solvent extraction. Essential oils are used in various fields due to their active components. They are found in the field of medicine and therapy, such as oils extracted from peppermint and tea tree, among others, and are especially used for treating inflammations due to their antibacterial properties. They are also used in the agro-food sector, such as in confectionery, carbonated drinks, and alcoholic beverages. The most prominent field in which they are used is cosmetics, including the production of cosmetics, detergents, soaps, and perfumes (Naeem *et al.*, 2018).

#### 2.2. Definition:

Essential oils such as rosemary oil (Figure 9) are a complex mixture of volatile organic compounds and are considered part of the plant's secondary metabolism. They are synthesized from various parts of the plant and contain a wide range of chemical constituents such as phenols, aldehydes, and others, which makes them effective against many pathogens.

Essential oils are highly concentrated, hydrophobic, volatile, and have a strong aroma. These organic oils dissolve in weakly polar or non-polar organic solvents. They are usually colorless, clear, and have a density lower than that of water. Moreover, they oxidize easily when exposed to heat, light, or even with aging; therefore, essential oils must be stored in dark places (Wani *et al.*, 2020). Plant secondary metabolites are considered a chemical weapon due to their diverse components and properties. Essential oils alone represent a mixture of several compounds such as terpenes, sesquiterpenes, and aromatic compounds like phenols and phenylpropanoids. They are usually obtained through distillation, but some can be extracted by mechanical pressing from plant tissues or by using solvents such as hexane or supercritical carbon dioxide. Essential oils are also used in the treatment of human health disorders such as obesity, diabetes, and depression (Hoffmann, 2020).



**Figure9:** Rosemary oil (**formulatehealth, 2020**).

### **2.3. Localization:**

Essential oils are found in various parts of the plant, including flowers, leaves, underground parts of woody plants, rhizomes, seeds, fruits, wood, and bark. Aromatic plants contain specialized structures that produce these essential oils, which are distinguished by their variety and different characteristics. These oils are found in the cytoplasm of some plant secretory cells located in one or more plant organs. These include trichomes or glandular hairs, secretory cavities, internal secretory cells, and epidermal cells.

Essential oils contain more than 300 different compounds and are mainly composed of volatile organic compounds. They often belong to the large family of terpenes (**Dhifi *et al.*, 2016**).

### **2.4. Chemical composition:**

Essential oils generally consist of 20 to 60 known compounds, and in general, the main components determine the biological properties of the essential oils. The main groups are divided into two categories:

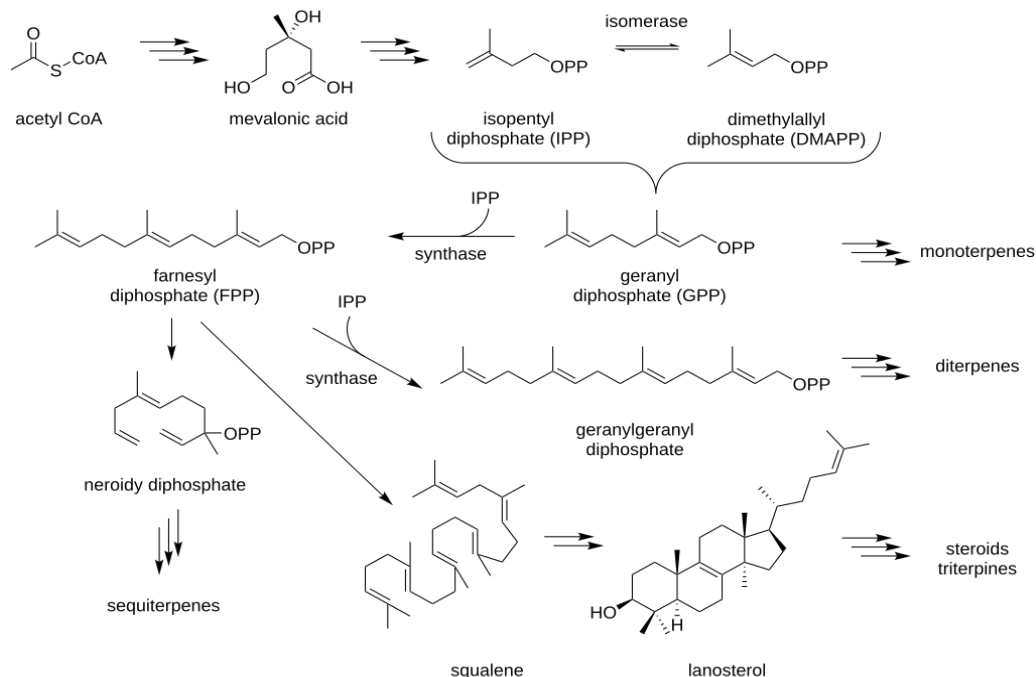
#### **2.4.1. Aromatic and aliphatic compounds:**

There are many plant sources that contain aromatic compounds, such as anise, parsley, star anise, tarragon, and others. Aromatic compounds are used in the same applications as monoterpenoids and sesquiterpenoids. They include alcohols, aldehydes, phenols, methoxy compounds, as well as methylenedioxy derivatives (**Eslahi *et al.*, 2017**).

#### **2.4.2. Terpenes and Terpenoids :**

Terpenes are compounds widely distributed in nature. They are inexpensive and readily available. Monoterpenes in plants play a defensive role against herbivores, attract pollinators, and serve as antifungal defenses. Terpenes are also used in chlorinated environments to carry out specific tasks such as cleaning aircraft parts.

Monoterpenes are among the most structurally diverse compounds, including monocyclic, bicyclic, and acyclic structures, in addition to various organic functional groups such as hydrocarbons, alcohols, aldehydes, ketones, esters, ethers, phenols, and peroxides. (Eslahi *et al.*, 2017). Terpenoid and steroid biosynthesis represented in Figure 10.



**Figure10:** Terpenoid and steroid biosynthesis (Boghog, 2024).

### 2.5. Role of essential oils:

The excessive use of pesticides has become a source of concern due to its harmful effects on the environment, in addition to causing annual losses that reach billions of dollars. Nowadays, there is a growing trend toward using natural products as a good alternative to reduce the negative impacts caused by synthetic pesticides. This is where the term "green pesticides" comes in, which refers to types of pest control derived from nature. It includes some systematic attempts to use substances such as hormones, toxins, and secondary metabolites, among others.

Furthermore, the use of biodegradable synthetic and semi-synthetic products is also considered part of green pesticides. In recent advancements, essential oils have been classified as safe green pesticides. These oils are found in the roots, flowers, leaves, and many other parts of plants. They contribute to protecting the plant from heat and cold, attracting or repelling insects, and using their chemical components as a defense mechanism. In addition, essential oils are used as flavorings, food additives, components of perfumes, cosmetics, soaps, and plastics. Thus, they are considered a good source of natural insecticides (Arshad *et al.*, 2014).

### 2.5.1. Natural function in plants:

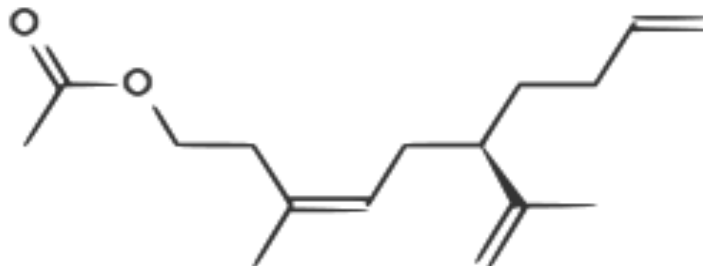
Essential oils have several roles in the plant, including:

#### 2.5.1.1. Attracting Pollinators:

Essential oils have a distinctive and pleasant aroma that attracts insects due to the compounds they release, such as monoterpenes and sesquiterpenes, which in turn make the process of pollination easier. Insects and arachnids use these compounds as part of their pheromones.

Pheromones like pheromone *cochenille californienne* (Figure 11) allow for chemical communication within the same species, and sometimes even between closely related species. They are secreted by various exocrine glands in the form of a liquid that evaporates. So far, more than one hundred types of pheromones have been identified.

Insects use some components of essential oils as primary or secondary elements in their various pheromones, thus attracting individuals of the same species. For example, larvae of a certain species of gall wasps force their host plants to change the composition of their scents, allowing them to send signals of their locations to potential mating partners. (Müller & Buchbauer, 2011).



**Figure11:** Phermone *cochenille californienne* (oiseau furtif, 2009).

#### 2.5.1.2. Defense against herbivores:

There are many herbivorous organisms such as mammals and insects, and the coexistence between plants and animals would not be possible without a self-defense mechanism in plants. Although they are rooted organisms, plants have developed remarkable strategies. They use volatile compounds to attract pollinators and contribute to both plant-plant interactions and attracting symbiotic organisms. These compounds are also used in defense against potential attacks. This defensive mechanism goes through three stages: monitoring, signal transmission, and production of defensive chemical compounds. First, the plant's monitoring system detects the attacks launched against it by recognizing specific signals.

Second, it converts them into signals through a network of signaling pathways, ultimately leading to the production of chemical substances that attract the natural enemies of the herbivorous insects. These compounds act as defense mechanisms that hinder the insects' growth and development (**Zitzelsberger & Buchbauer, 2015**).

### **2.5.1.3. Protection from microbial threads:**

In recent times, medicinal and aromatic plants have gained widespread recognition for their diverse properties and their antioxidant and antimicrobial effects. Essential oils extracted from these plants have demonstrated significant efficacy in combating free radicals, which are known to be a potential cause of various diseases such as cancer, neurodegenerative disorders, and others.

Essential oils are also known for their antibacterial, antiviral, and antifungal properties. Additionally, they are used as disinfectants, and they exhibit antioxidant, insecticidal, and antiparasitic effects. One of their notable characteristics is their hydrophobicity, which enables them to integrate with the lipid components of bacterial and mitochondrial membranes, leading to increased membrane permeability and structural disruption. This, in turn, causes the leakage of essential biomolecules and ions, resulting in the death of the bacterial cell. In this way, essential oils help protect plants from bacteria, viruses, and other pathogens.

According to recent studies, nanoformulations of essential oils have shown enhanced antibacterial activity due to their ability to reduce rapid evaporation, limit the degradation of active essential oil compounds, and improve chemical stability and solubility. These features make them a more effective antibacterial agent(**Chouhan *et al.*, 2017**).

### **2.5.1.4. Allelopathy (chemical competition):**

The increasing damage caused by synthetic herbicides has led to a shift toward alternative solutions aimed at developing biodegradable compounds. Some plants negatively affect the growth of surrounding plants, resulting in growth inhibition a chemical interaction known as *allelopathy*. In this process, the plant produces allelopathic compounds from its roots, leaves, flowers, seeds, and stems, which work to suppress weed growth.

In a study investigating the metabolism of essential oils in different plant tissues after application to dry seeds, the oils were found to be effective in inhibiting seed germination. These essential oils reduced the growth of weed seeds even at low concentrations. Other studies have also confirmed that aromatic essential oils extracted from certain medicinal and aromatic plants act as potent germination inhibitors, particularly for wheat seeds.

Moreover, these oils exhibit nematicidal (nematode-killing) properties, as well as antimicrobial and antioxidant activities. The oils used in this study contain monoterpenes, which are known to affect seeds even at low levels. Plants exposed to monoterpene vapors have been shown to suffer significant internal damage (**Azirak & Karaman, 2008**). Table 2 shows the effect of essential oils on the germination of weed species.

**Table2:** Effect of essential oils on germination of weed species (Azirak &Karaman, 2008).

Botanical name	Germination rate (%)						
	<i>Alcea pallida</i>	<i>Amaranthus retroflexus</i>	<i>Centaurea salsotitialis</i>	<i>Sinapis arvensis</i>	<i>Sonchus oleraceus</i>	<i>Raphanus raphanistrum</i>	<i>Rumex nepalensis</i>
<i>Carum carvi</i>	46.7	7.4	7.6	-0.5	0.0	-0.1	13.6
<i>Coriandrum sativum</i>	64.7	37.9	15.8	9.4	10.5	13.2	27.4
<i>Foeniculum vulgare</i>	86.7	36.7	16.3	23.0	97.3	5.6	84.2
<i>Lavandula stoechas</i>	58.3	36.6	12.7	1.0	30.4	1.3	56.1
<i>Mentha spicata</i>	46.7	2.9	10.6	1.0	5.6	-0.1	6.7
<i>Origanum onites</i>	71.7	16.5	15.4	1.1	3.2	0.1	20.2
<i>Pimpinella anisum</i>	84.7	53.7	61.3	66.2	97.8	10.9	92.0
<i>Rosmarinus officinalis</i>	73.3	59.5	5.4	3.0	87.1	2.8	40.1
<i>Salvia officinalis</i>	83.4	63.7	44.4	17.0	95.1	22.7	80.6
<i>Thymbra spicata</i>	74.5	1.1	41.5	-0.3	1.3	0.6	35.8
$\chi^2$	23.9	31.0	25.9	30.5	32.4	29.8	33.0
<i>p</i> -value	**	***	**	***	***	***	***
Control (Water)	100	100	96	100	100	88	100

\*\*\**p* <0.001.

### 2.5.2. Human use of essential oils:

#### 2.5.2.1. Therapeutic and pharmaceutical Application::

Essential oils interact with the body through three distinct mechanisms. Firstly, the biochemical (pharmacological) mechanism, where they affect hormones and enzymes through their interaction in the bloodstream. Secondly, the physiological mechanism, in which essential oils influence specific physiological functions for example, there are active compounds that are effective for women's issues, such as lactation and menstruation. Thirdly, the psychological mechanism, where neurotransmitters and chemical messengers are triggered after the olfactory area in the brain is stimulated upon inhalation of the aromatic molecules. The effectiveness of these oils may result from a single compound or a combination of several compounds (Bouayed & Bohn, 2012).

#### 2.5.2.2. Antimicrobial, antiviral and Anti-inflammatory uses:

Plants are considered the primary source for the discovery of drugs and medical preparations due to their medicinal properties. Essential oils, which are secondary metabolic components, have drawn research interest because of their antimicrobial properties and wide availability. Aromatic plants are usually extracted through hydrodistillation, and their oils typically consist of terpenoids, monoterpenes, sesquiterpenes, a variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, esters or lactones, coumarins, and phenylpropanoid derivatives. Among these components, terpenes are of particular interest. Although their mechanism of action is not fully understood, it is believed that the disruption of the cell membrane caused by the lipophilic components plays a role in their antibacterial effect. Several tests are used to evaluate the antimicrobial activity of essential oils, including agar diffusion and broth dilution methods. However, these methods may face limitations due to the volatile nature of essential oils and their poor water solubility.

Let us now briefly discuss the antimicrobial properties of essential oils for example, peppermint (*Mentha piperita*), which is used to treat various diseases, including upper respiratory tract infections and coughs. Due to its antibacterial effects, peppermint oil has been used to inhibit bacterial growth in the intestines, which is beneficial in several physical disorders such as irritable bowel syndrome (IBS), fibromyalgia, and chronic fatigue syndrome. It has been observed that peppermint oil preparations are effective in relieving symptoms (Chattopadhyay, 2010).

### 2.5.2.3. Wound Healing and Skin Infections:

Traditional, complementary, and integrative medicine (TCIM) enhances recovery from illnesses, as stated in the definition by the World Health Organization. Aromatherapy is considered a part of TCIM, and the use of essential oils promotes healing and well-being. Essential oils can be administered in three ways: orally, topically through the skin, or by inhalation. Essential oils are known for their ability to treat wounds, which highlights their importance in clinical nursing practice, as nurses are directly involved in wound management. These oils inhibit the growth of fungi, yeasts, and bacteria, in addition to their antiseptic properties. For example, tea tree oil is considered promising in wound care, while clary sage oil helps inhibit the growth and proliferation of bacteria in the wound area. Some studies have demonstrated the benefits of essential oils in the healing of surgical wounds, showing improvement in wound site recovery. Lavender oil was used in most of these studies due to its previously demonstrated promising ability in wound healing. It helps in wound contraction and exhibits antimicrobial activity. It has been used to increase the levels of transforming growth factor-beta (TGF- $\beta$ ) and type I collagen. TGF- $\beta$  promotes the differentiation of fibroblasts into myofibroblasts, which in turn contribute to wound contraction by shrinking the tissue, thus supporting clinical outcomes. Additionally, the oil blend of lavender and thymol significantly enhanced the activity of superoxide dismutase and glutathione peroxidase enzymes in various tissues. This helps inhibit the harmful effects of free radicals on cells (Nascimento *et al.*, 2022).

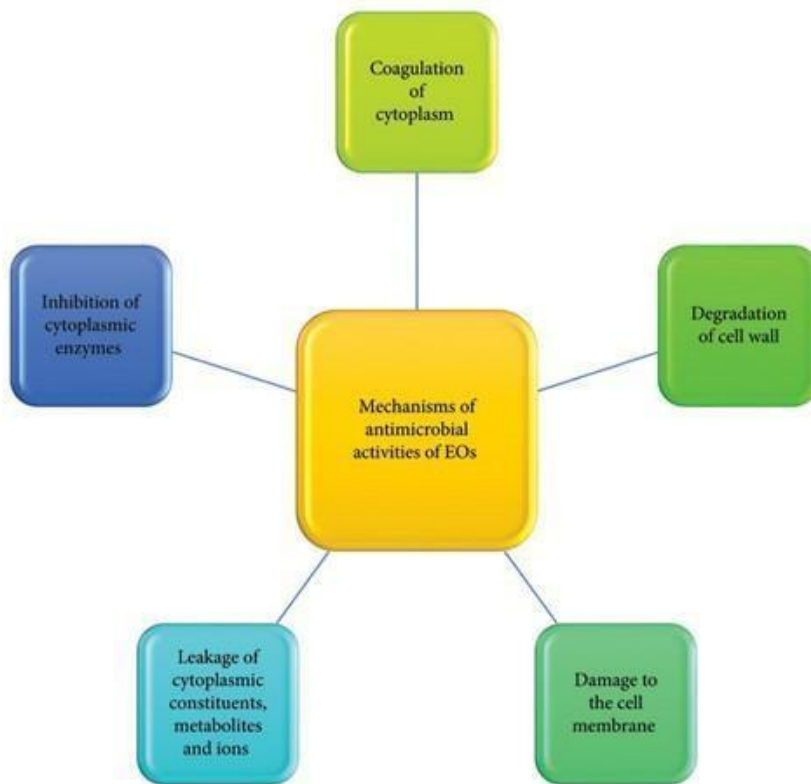
## 2.6. Industrial Application:

Due to the diverse components of essential oils, they have become an excellent means for use in various fields such as medicine, food, cosmetics, and the agricultural industry. In addition to their high marketability, they are considered a natural alternative to synthetic antioxidants, as they contain large amounts of volatile compounds that possess antioxidant and antimicrobial properties (Bolouri *et al.*, 2022).

### 2.6.1. Food industry:

With the increasing consumer awareness in recent years, there has been a growing tendency toward high-quality processed foods that are free from chemical additives. Therefore, it has become necessary to explore safe natural alternatives. Among these alternatives are essential oils, which, due to their antibacterial, antioxidant, and antifungal properties, help in preserving food for a longer time (Saeed *et al.*, 2022). Some studies have shown the antimicrobial effects of essential oils.

They damage the structure and membrane of pathogenic bacteria, leading to the leakage of intracellular contents and ultimately the death of the cells. This effect is especially prominent in Gram-positive bacteria, as they are more sensitive compared to Gram-negative bacteria. For this reason, essential oils are used in the food industry as preservatives to prevent spoilage and extend shelf life. Researchers have demonstrated, through studies on *Allium* plants, the effectiveness of their essential oils, particularly against foodborne diseases. These oils exhibit inhibitory activity against various types of bacteria and fungi, including *Escherichia coli* and *Vibrio parahaemolyticus* (Laura & Popa, 2013). Figure 12 shows different mechanisms effects of EOs and thier composents.

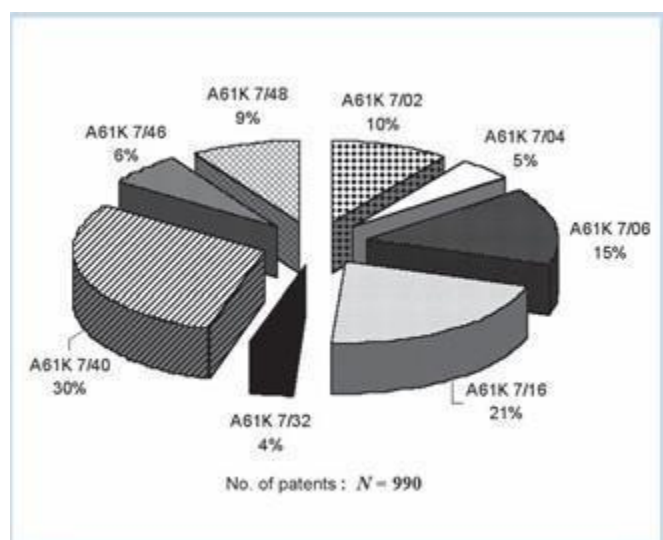


**Figure12:** Different mechanisms of antimicrobial effects of EOs and their composents (Noshirvani, 2024).

### 2.6.2. Cosmetics and Hygiene products:

Consumer demands have changed, and there is now a greater inclination towards safe, natural products, especially in the field of cosmetics. In this context, essential oils stand out, as their scent makes them a targeted choice in the production of perfumes and fragrances. Due to their many properties, such as antibacterial, anti-inflammatory, and antioxidant effects, essential oils have become popular ingredients in cosmetics and personal care products. For example, they are used as preservatives in various products such as moisturizers, lotions, skin cleansers, lipsticks, and others. However, essential oils also have some drawbacks. They are not always ideal for use due to their poor solubility in water, sensitivity to heat and chemicals, and their volatile nature, which causes them to evaporate easily (Guzmán & Lucia, 2021).

Perfume is a liquid distilled from specific parts of plants in short, essential oils. It acts in four ways: as a pharmacological, physiological, psychological, and spiritual agent. It is absorbed by the body through two main methods: via inhalation (smell) or through the skin (Vankar, 2004). Ancient civilizations used essential oils in ointments and baths for cosmetic purposes, and to this day, these oils, particularly their terpenic and terpenoid components, are among the most important natural products used in cosmetics and perfumery. After conducting a study on 990 patents related to essential oils and their components used in the cosmetic sectors, it was found that their applications are primarily focused on teeth and mouth cleaning, skin care, insect sting prevention, hair coloring, and perfume compounds (Silva-Santos *et al.*, 2004). Although essential oil components are widely used in the field of cosmetics, it is necessary to conduct thorough analyses to avoid allergic reactions that may result from improper use, thereby ensuring the development of cosmetic products based on essential oil formulations (Guzmán & Lucia, 2021). Figure 13 represented Subgroups from A61K 7/00 applied in USPTO granted patents for the use of essential oils, terpenics/terpenoids compounds in the cosmetic and perfumery sectors during 1980-2003.



**Figure13: Subgroups** from A61K 7/00 applied in USPTO granted patents for the use of essential oils, terpenics/terpenoids compounds in the cosmetic and perfumery sectors during 1980-2003 (Silva-Santos *et al.*, 2004).

### 2.7. Extraction methods:

There are several methods for extracting essential oils, including conventional and novel methods, such as:

#### 2.7.1. Conventional essential oil extraction methods:

##### 2.7.1.2. Cold Expression:

The cold press extraction method is an economical technique that costs less compared to other methods and is also environmentally friendly. This method relies on mechanical pressing and uses less energy for extraction. It is mainly applied in the case of oilseeds. Among the oils extracted by the cold press method are sunflower, grapeseed, corn, flaxseed, and others.

Consumers prefer this method because it is considered safe, and the oils extracted through cold pressing are rich in antioxidants and amino acid supplements. Although cold pressing is widely used for oil extraction, it can also be used for fruit pressing (Çakaloğlu *et al.*, 2018)

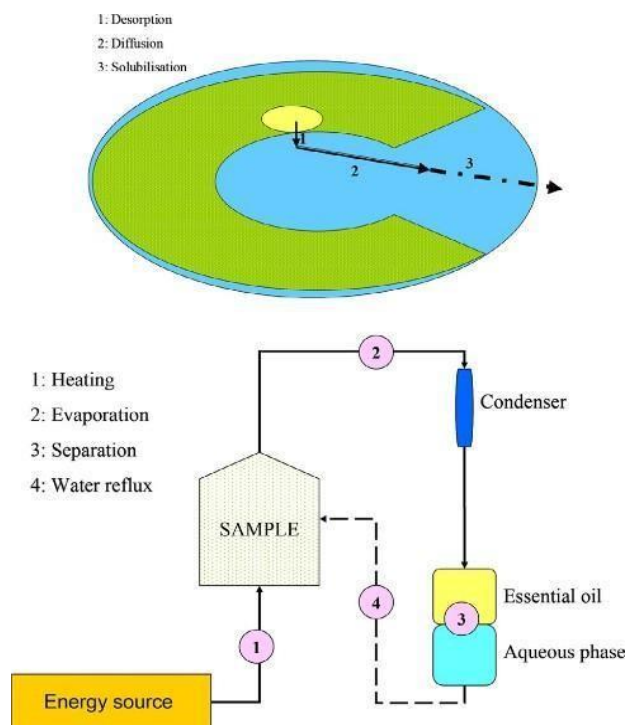
### 2.7.1.3. Solvent Extraction:

In general, solvent extraction relies on dissolving the oil using a solvent and separating it from its environment. A specific solvent is used for each plant, and in a study conducted on tobacco leaves, both solvent extraction and distillation techniques were used together to ensure the extraction of essential oil from discarded tobacco leaves (Zhang *et al.*, 2012).

### 2.7.1.4. Distillation:

To obtain essential oil from parts of the plant, distillation (Figure 14) is usually used. This process involves exposing aromatic plants to boiling water, causing the essential oil to be released through evaporation. Since water and oil are immiscible, the oil can easily be separated at the end. The boiling temperature typically ranges from 200°C to 300°C. The water vapor rises carrying the oil, and after entering a narrow tube, it is cooled by an external source. Upon condensation, the mixture is collected in a container called a Florentine flask, where the oil separates due to its lower density compared to water and thus floats on the surface. There are four factors that determine the amount of extracted oil: temperature, operating pressure, distillation duration, and the type and quality of the plant material, with yields typically ranging between 0.005% and 10%. Three types of distillation are commonly used: water distillation, steam distillation, and water and steam distillation to obtain essential oil from parts of the plant, distillation is usually used. This process involves exposing aromatic plants to boiling water, causing the essential oil to be released through evaporation. Since water and oil are immiscible, the oil can easily be separated at the end. The boiling temperature typically ranges from 200°C to 300°C. The water vapor rises carrying the oil, and after entering a narrow tube, it is cooled by an external source. Upon condensation, the mixture is collected in a container called a Florentine flask, where the oil separates due to its lower density compared to water and thus floats on the surface.

There are four factors that determine the amount of extracted oil: temperature, operating pressure, distillation duration, and the type and quality of the plant material, with yields typically ranging between 0.005% and 10%. Three types of distillation are commonly used: water distillation, steam distillation, and water and steam distillation (Chennat & Boutekedjiret, 2015).



**Figure14:** Schematic representation of the individual steps in the extraction process of steam distillation of volatile compounds (Chennat & Boutekedjiret, 2015).

### 2.7.1 Novel “green” Extraction methods:

#### 2.7.1.1. Microwave-assisted Extraction:

This method (Figure15) uses a heating device that relies on friction, which achieves high extraction rates. It is also considered low-cost compared to other methods, while consuming less time and energy and reducing carbon emissions.

The device emits microwave waves with frequencies ranging from 300 MHz to 300 GHz, which penetrate the biomass and heat it by interacting with the polar molecules within the materials.

This process is characterized by its speed, and there are many types of this method, including vacuum microwave, microwave hydrodistillation, solvent-free microwave extraction (SFME), among others. Additionally, in this extraction method, the heating process can be controlled. Some studies have shown its effectiveness in extracting essential oils, perfumes, antioxidants, and other organic compounds (Cardoso-Ugarte *et al.*, 2013).

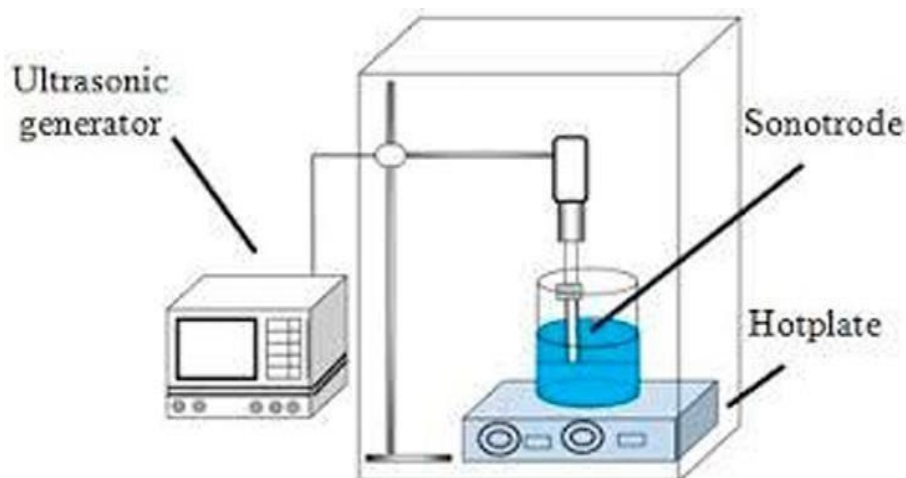


**Figure 15:** Schematic diagram of the microwave oven adaptation to perform MAE of essential oils from herbs (Cardoso-Ugarte *et al.*, 2013).

### 2.7.1.2. Ultrasound-Assisted Extraction:

After immersing the plant material from which the oil is to be extracted in water or a solvent, it is exposed to ultrasonic waves. This method is known as ultrasound-assisted extraction. It is especially used with flowers, leaves, and seeds, and relies on very long waves. These waves are used in several applications, including food preparation, and are considered a good non-thermal alternative. These waves cause changes in density, and with sufficient power, the expansion force becomes stronger than the intermolecular attraction forces, leading to the formation of bubbles. Each bubble affects the surrounding bubbles, eventually causing them to collapse.

When the bubbles collapse, they release energy, creating hot spots that raise the temperature and pressure, which can reach up to 4000 K. This property results in higher yields compared to other extraction methods (Richa *et al.*, 2020). Figure 8 shows schematic diagram of ultrasound-assisted extraction.



**Figure 16 :** Schematic diagram of ultrasound-assisted extraction (Richa *et al.*, 2020).

### **2.8. Conclusion:**

Essential oils have been used since ancient times and have always been an excellent alternative to chemical industries due to their eco-friendly nature. They are used in almost all vital areas of life, such as nutrition, health, and cosmetics. This has recently led researchers to focus more on conducting studies on essential oils in order to maximize their future benefits, while also being aware of the potential harms they may cause when used in high concentrations.



**Chapter 3:**  
*Lavandula*  
*stoeachas*

### Chapter 3: *Lavandula stoechas*

#### 3.1. Generality:

In recent decades, there has been an increase in fungal infections and inflammation-related diseases. With the growing disappointment in conventional drugs and synthetic antifungals, the search for safer alternatives has become more evident.

Among these alternatives, aromatic plants and their essential oils have been used, especially after many studies confirmed the remarkable benefits of secondary metabolites. These compounds exhibit antimicrobial, antiviral, antiparasitic, insecticidal, and antioxidant properties.

Lavender is one of the aromatic plants that contain such essential oils. Among its various species, *Lavandula stoechas* is particularly widespread in the Mediterranean region. It is commonly used in traditional medicine as a carminative (relieves gas), antispasmodic, and also for wound healing. Its essential oils are used to relieve nervous headaches and possess anti-inflammatory, antioxidant, and antibacterial properties. Additionally, they affect the viability of macrophage cells, which is why they are used for health and cosmetic purposes (Zuzarte *et al.*, 2013).

#### 3.2. History:

More than six thousand years ago, most ancient civilizations made use of plants especially aromatic ones for spiritual purposes and, more importantly, for treating diseases. With the use of extracts from aromatic and medicinal plants, such as essential oils and fruit juices, a new science known as aromatherapy eventually developed. In the 21st century, this field has witnessed significant advancements through focused research. Turning now to the Mediterranean region, it is considered a stronghold for medicinal and aromatic plants. Its people have served as a repository of inherited knowledge passed down through generations. Among the most commonly used plant families in the Mediterranean basin are the Lamiaceae, Apiaceae, and Asteraceae. Several of these plants have already been studied for their pharmacological activities, such as rosemary, sage, thyme, oregano, and lavender. The *Lavandula* genus (lavender) consists of approximately 39 species, in addition to hybrid varieties and nearly 400 registered cultivars.

Among the most well-known and widely traded species are *L. angustifolia*, *L. stoechas*, *L. (Figure17). latifolia*, and the hybrid *L. x intermedia*. These species are evergreen and are characterized by their inflorescences, typically appearing in purple, blue, dark violet, pink, or lilac colors. The *L. stoechas* variety was known to ancient Greek, Roman, and Arab civilizations, especially for the extraction of essential oils. Today, it is still widely used in the Mediterranean region due to its rich essential oil content. It is also used in herbal teas, cosmetic products, and the preparation of traditional dishes (Ez Zoubi *et al.*, 2020).



**Figure 17:** *Lavandula stoechas* (Krzysztof, 2024).

**3.3. Classification:**

Kingdom : Plantae

Phylum : Streptophyta

Class : Equisetopsida

Subclass : Magnoliidae

Order : Lamiales

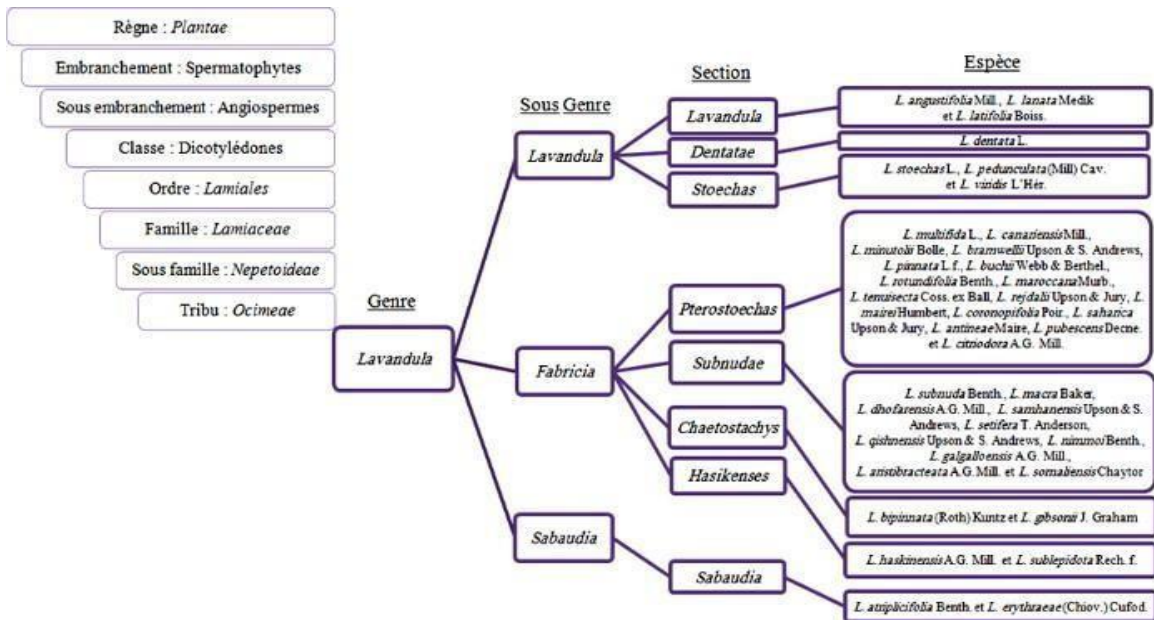
Family : Lamiaceae

Genus : *Lavandula*

Species : *Lavandula stoechas*

**According To Royal Botanic Gardens, Kew, (n.d).** Taxonomy of the genus *Lavandula* appear in Figure 18.

## Chapter 3: *Lavandula stoechas*



**Figure 18:** Taxonomy of the genus *Lavandula* (d'après Upson & Andrews, 2004).

### 3.4. Origin of the name:

The Romans used lavender in ancient times to perfume their bathwater, and the word *lavender* is generally derived from the verb "to wash." It may also come from the word *lavando*, or trace back to the Latin *lavare*. In the past, lavender was used to scent freshly washed laundry, and dried flower sachets were placed in wardrobes to perfume clothes and repel moths. It is also likely that the names *Lavandula* and *lavender* are derived from the Latin word *livere*, which means "pale" or "bluish" (Benabdelkader, 2012).

### 3.5. Botanical description: Resumed in Table 3.

- **General overview:**

*Lavandula stoechas* L., which belongs to the Lamiaceae family, is generally an evergreen shrub that grows up to one meter in height and occurs naturally in Mediterranean countries. It is characterized by its violet, spike-shaped flowers (Lynda *et al.*, 2023). Table 1 shows Summary of *Lavandula stoechas* Botanical Description .

- **Habit and size:**

Its height ranges from 10 to 39 cm, and its leaf dimensions are between 10.5 to 25 × 2 to 6 to 7 mm. The floral peduncle measures between 0.3 and 3.5 cm, which may be shorter than or equal to the length of the spike. The inflorescence spike is 1 to 3 to 3.5 cm long. The fertile bracts measure 4 to 8 × 5 to 10 mm, while the upper sterile bracts measure 8 to 18 × 2 to 4 to 8 mm (Küçük *et al.*, 2019).

- **Stems:**

The stem (Figure 19) is typically erect or ascending, branched, and ranges in length from 10 to 39 cm. It is densely covered with woolly hairs. When taking a cross-section of the herbaceous stem, we found that it consists of the epidermis, covering hairs, glandular hairs, endodermis, the tissue surrounding the vascular bundle, vascular system, cambium, medullary rays, and pith. In addition to the herbaceous stem, there is also the woody stem, which is composed of secondary phloem, cambium, medullary rays, and the pith region (Küçük *et al.*, 2019.)



**Figure 19: *Lavandula stoechas* stem (Marshall, 2024).**

- **Leaves:**

This plant generally exhibits a variety of leaf shapes; sometimes the leaves (Figure 20) are found only at the base of the stems and may be either lobed or unlobed. Their color ranges from deep bluish-gray to green or pale brown. As for the tender lavenders such as *Lavandula stoechas* and *L. dentata*, they tend to be more green than gray in appearance (Chu & Kemper, 2001).

- **Flowers:**

Its flowers (Figure 20) are also diverse, with colors ranging from blue to violet, and they have prominent floral bracts at the tops of the inflorescences (Chu & Kemper, 2001).



**Figure 20:** *Lavandula stoechas* leaves and flowers (Starr & Starr, 2012).

- **Roots and fruits:**

The root (Figure21) is branched in its upper part, while the lower lignified branches are densely branched; therefore, it is a woody taproot. These branches also bear numerous small buds. The corolla is bilabiate (two-lipped), its color ranges between blue and purple, and it is covered with fine hairs. As for the fruit(Figure5), it is enclosed in the persistent calyx (Fakhriddinova *et al.*, 2020)



**Figure 21:** Fruits and roots of *Lavandula stoechas* (Adams, 1970).

**Table 3:** Summary of *Lavandula stoechas* Botanical Description

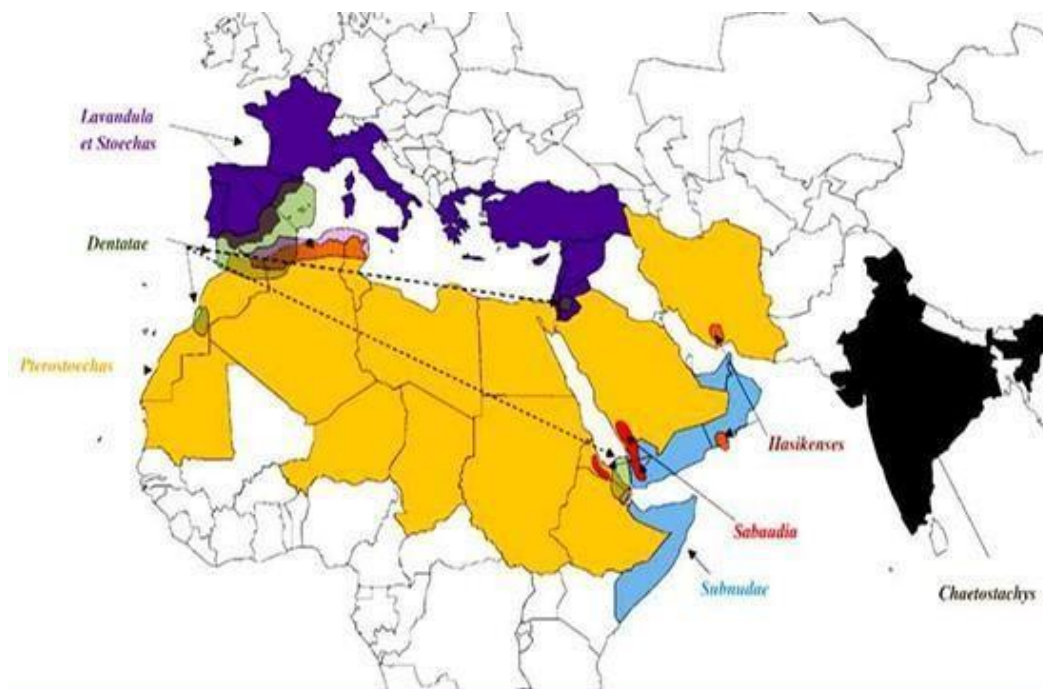
Feature	Details
Family	Lamiaceae
Common Name	<i>Lavandula stoechas</i>
Height	10 to 39 cm
Leaf Dimensions	10.5 to 25 × 2 to 6-7 mm
Flowers	Colors range from blue to violet, with prominent floral bracts at the tops of the inflorescences
Stem	Erect or ascending, branched, 10 to 39 cm in length, densely covered with woolly hairs
Fruit	Enclosed in the persistent calyx

### 3.6. Geographical distribution:

The geographical distribution of lavender (Figure 22) extends from the Canary Islands to Cape Verde, and includes central and southeastern India, the Mediterranean region, the Indian subcontinent, and the Arabian Peninsula. Among the most well-known species in the Mediterranean region are *Lavandula angustifolia* and *Lavandula stoechas*, along with several hybrid varieties. The wild species *Lavandula stoechas* thrives in hot, dry, and sunny climates.

The plant grows within a Lauretum-type phytoclimatic zone, typically at altitudes of 600 meters above sea level, though it may also be found sporadically at elevations up to 1000 meters.

It is commonly distributed in degraded areas and shrubby grasslands, and it is often found in association with *Cistus* species. This lavender species usually grows in siliceous (silica-based) soils (Bella *et al.*, 2015).



**Figure 22:** Schematic representation of the distribution area of the sections of the genus *Lavandula* (Guitton,2011)

### 3.7. Uses:

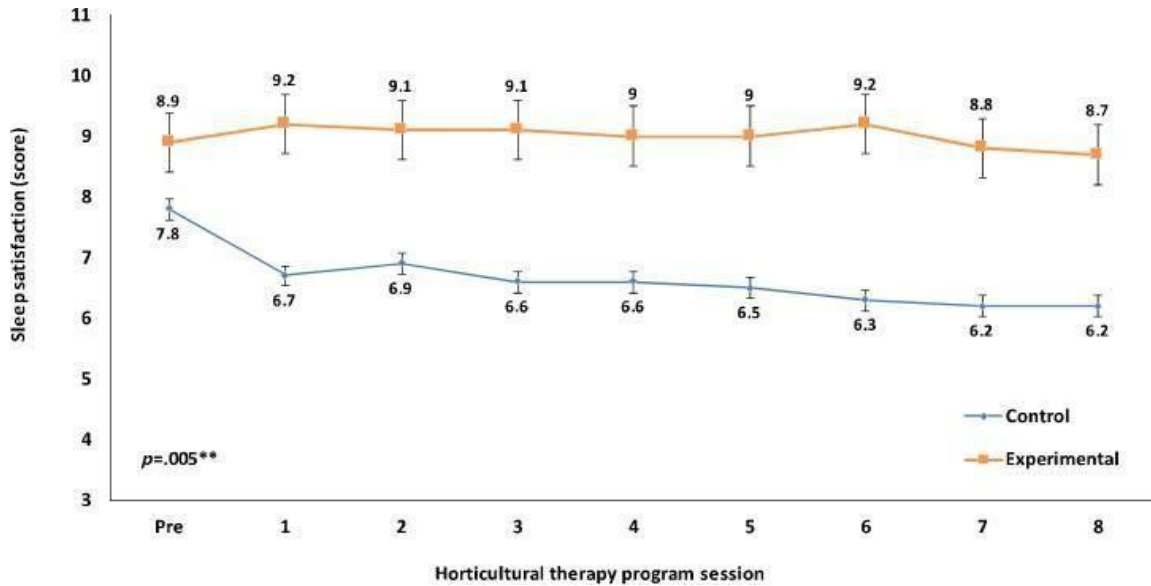
- **Medicinal and pharmaceuticals uses:**

This plant has been used, especially in the Maghreb countries, as a treatment for rheumatism, diabetes, depression, and also for headaches. It is also used in Pakistan to treat epilepsy and migraines. Additionally, its oil is used as an antiseptic to treat wounds. A study conducted on *L. stoechas* showed its anti-inflammatory effects, as it reduces the vitality of inflammation-causing cells by up to 63% after just three hours of incubation. It is also a strong antioxidant due to its high content of 1,8-cineole and camphor, which help to scavenge free radicals. According to a study conducted on male diabetic rats, this plant contributed to protecting the liver and kidneys from oxidative stress. Not to forget its antibacterial properties, a recent study on eight pathogenic bacterial strains confirmed its antibacterial activity, especially against *L. monocytogenes* and *S. aureus* (ŞahiNler *et al.*, 2022).

- **Aromatherapy and relaxation:**

Hospice patients with terminal cancer experience sleep disturbances due to poor pain management, which leads to depression and anxiety. Some plants have been shown to affect emotional functions because of their compounds, such as alkaloids, which particularly affect the nervous and digestive systems, among others. Lavender, in particular, is known for its calming effects and stress-relieving properties. To study pain and sleep satisfaction, a study

Was conducted using *Lavandula stoechas* by having patients inhale its scent through various activities such as cutting, rubbing, or touching the plant. A significant difference in pain intensity was observed, with a reduction in the program from 3.5 to 1.2, showing a positive result in pain relief (Figure23). After completing 8 sessions over eight days to measure sleep satisfaction in both groups, a noticeable difference was observed in the group using lavender, demonstrating its effectiveness in improving sleep satisfaction (**Ra et al., 2018**).



**Figure 23:** Changes in sleep satisfaction by session of the control and experimental groups. Error bars mean standard deviation. \*\*Significant at  $p < .01$  by Mann-Whitney test (**Ra et al., 2018**)

- **Cosmetic and personal care:**

Lavender oil, extracted from several species of this plant, including *L. latifolia*, *L. stoechas*, *L. x intermedia*, and *Lavandula angustifolia*, is known for its antibacterial and antifungal properties. It has been proven effective in treating burns, as it promotes cell growth, helps in wound healing, and reduces stretch marks, pimples, and sores, as well as sunburns. This makes it a valuable ingredient in the production of creams, perfumes, and soaps. Moreover, lavender oil prevents immediate allergic reactions, as confirmed by its use on mice and rats, and it also inhibits ear swelling responses (**Aburjai & Natsheh, 2003**). Table 4 shows MIC and MBC of *Lavandula stoechas* Essential Oil against the Test Bacteria.

**Table 4:** MIC and MBC of *Lavandula stoechas* Essential Oil against the Test Bacteria (Khavarpour *et al.*, 2019).

Microorganism	<i>Lavandula stoechas</i> flowers	
	MIC	MBC
<i>Staphylococcus aureus</i>	1/64	1/32
<i>Bacillus subtilis</i>	1/32	1/16
<i>Escherichia coli</i>	1/32	1/32
<i>Salmonella enteritidis</i>	1/8	1/4

MIC: Minimum inhibitory concentration (as oil dilution)

MBC: Minimum bactericidal concentration (as oil dilution)

- **Genetically Modified Lavender in Cosmetics:**

Essential oils, particularly those produced by the lavender plant, are composed mainly of monoterpenes, including linalool, which is one of the most abundant and scent-determining constituents of these oils. Although it is present in relatively small quantities, linalool contributes a sweet and pleasant aroma. Despite the cloning of several genes encoding terpene synthase enzymes, many important enzymes including S-linalool synthase have not yet been characterized in these plants. Therefore, a study was conducted that described the use of RNA-Seq technology, along with other complementary sequencing data, to clone and functionally characterize the cDNA of the low-expression enzyme S-linalool synthase (LiS-LINS) from *Lavandula × intermedia*. The data indicate that the production of S-linalool is at least partially regulated at the transcriptional level of the LiS-LINS gene. The cloned LiS-LINS cDNA may be used in the future to enhance the oil composition in lavender and other plants through metabolic engineering (Adal *et al.*, 2019). Essential oils constitute a heterogeneous group of plant derived products that have long been used for cosmetic, disinfectant, and therapeutic purposes. Among these oils, lavender oil is notable for its properties and has been particularly used as an ingredient in bath salts and cleaning agents (Sabara & Kunicka-Styczyńska, 2009).

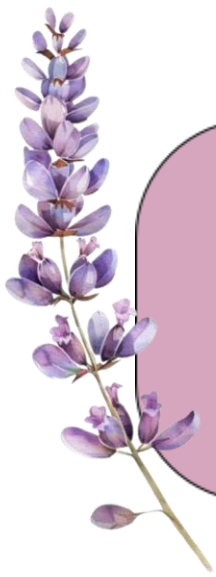
### 3.8. Conclusion:

*Lavandula stoechas* is a unique plant, not only a decorative shrub characterized by its purple flowers, but also a medicinal and aromatic plant known since ancient times, especially in the Mediterranean region. It has various uses in medicine, cosmetics, and even relaxation. Its oil, which contains 1,8-cineole and camphor, is distinguished by its antibacterial, antioxidant, anti-inflammatory, antifungal, and disinfectant properties, making it effective for treating several ailments such as rheumatism, diabetes, headaches, and promoting wound

### Chapter 3: *Lavandula stoechas*

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healing. It also plays a major role in the fragrance industry and is known for its calming and stress-relieving effects. Studies have shown its effectiveness in pain relief and improving sleep quality. Additionally, it has cosmetic benefits such as treating burns, reducing stretch marks, treating skin inflammation, preventing allergic reactions, and reducing swelling, making it safe for personal care products. Therefore, *Lavandula stoechas* is an important plant in both traditional practices and modern science, and research continues to highlight its potential to improve human health and well-being.



# **Chapter 4: Materials and methods**

## Chapter 4 : Materials and methods

### Chapter 4: Materials and Methods

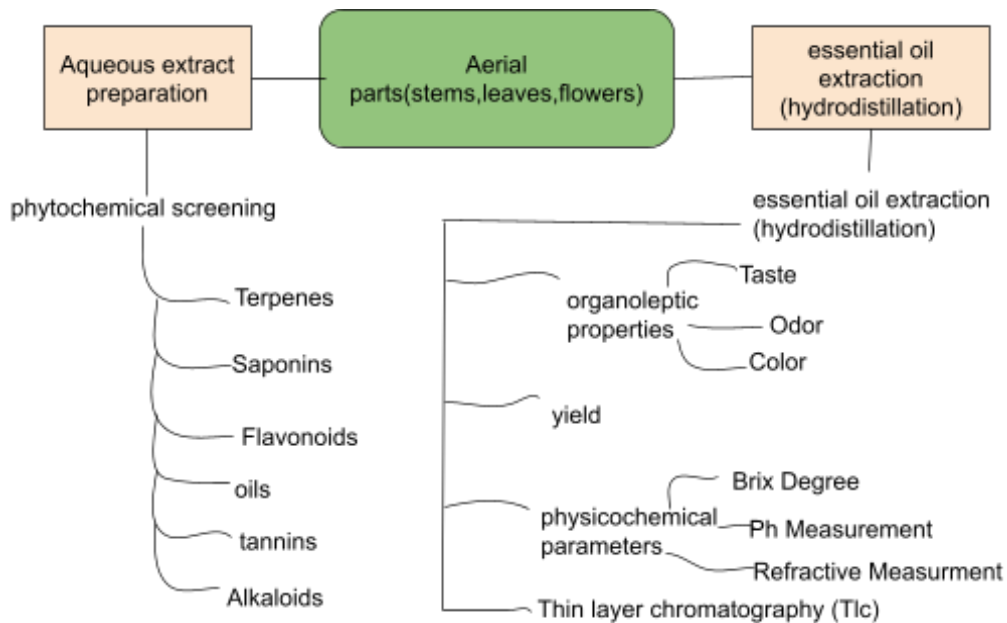
This study was conducted in the laboratory of the Department of Natural Sciences (Plant Physiology Laboratory) at the Higher Normal School of Technological Education in Skikda (ENSET-Skikda).

#### 4.1. Objective:

The plant used in this work is French lavender or *Lavandula stoechas* (aerial parts). The objective of this study is to:

- Phytochemical study: to detect some of its active substances (saponins, tannins, flavonoids, terpenes, and alkaloids),
- Extraction of its essential oil by hydrodistillation,
- Calculation of the extraction yield,
- Physicochemical characterization of the extracted oil: to assess its quality and safety, in order to confirm its suitability for use in the industrial sector.

The experimental protocol is summarized in the following Figure 24:



**Figure 24 :** Summary of the experimental protocol.

## Chapter 4 : Materials and methods

### 4.2. Materials used:

The materials used (equipment and reagents) are presented in Table 5 as follows:

**Table 5:** Materials Used.

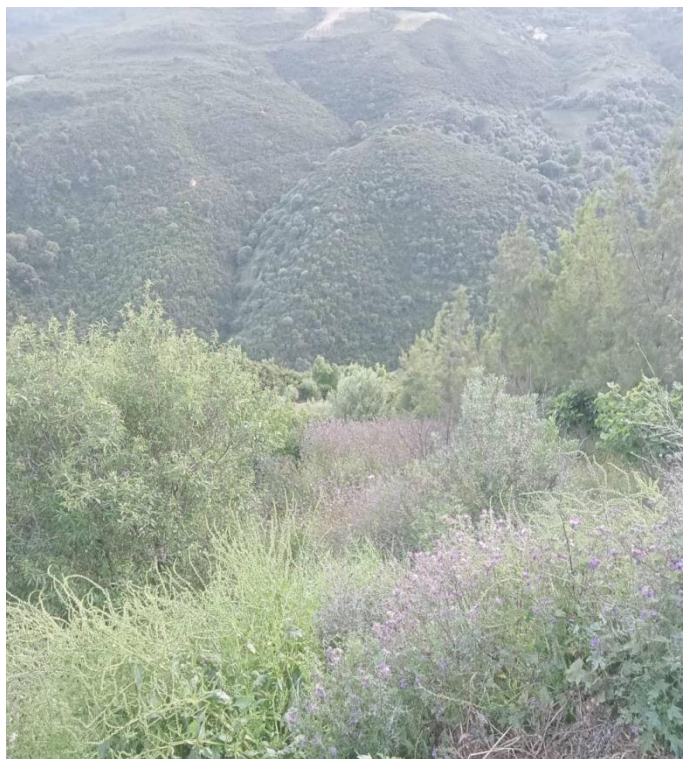
Equipment and Tools	Reagents and Solutions	
Beaker	Distilled water	
Test tubes and Eppendorf tubes	Wagner's reagent: 1 g potassium iodide + 0.635 g iodine, and complete the volume to 100 mL with distilled water	
Precision balance	Chloroform	
Electric grinder	Salts	FeCl <sub>3</sub>
Pipette	Acids H <sub>2</sub> SO <sub>4</sub>	
Filter paper, aluminum foil, and pH paper	HCl	
Vortex		
Clevenger apparatus		
Refractometer		
Bunsen burner		

### 4.3. Choice of the plant:

- ✓ The availability, The elevated yield;
- ✓ The therapeutic effects;
- ✓ Its antibacterial, anti-inflammatory, antifungal, antioxidant, and other beneficial properties.

### 4.4. Sampling area:

The plant used in this work was harvested between February and May 2025. A green, elevated, and lush mountainous area covered with fine plants and wild grasses, It is located in a mountainous area (approximately 400–600 m above sea level), near Oum-Toub in the Skikda province of Algeria (Figure 25).



**Figure 25:** Location of flower collection.

### **4.5. Plant preparation:**

After harvesting, the aerial parts of *Lavandula stoechas* were cleaned, washed with tap water to remove any foreign matter such as soil, and then dried for 10 days in a dry place away from direct sunlight in order to preserve the integrity of the molecules as much as possible. They were then ground into a fine powder using an electric grinder (Figure 26)



**Figure 26:** Comparative images showing the sample before and after grinding.

### **4.6. Phytochemical study or screening:**

Phytochemical screening is a method used to identify the presence of different groups of chemical families in a given plant. The characterization tests are partly based on qualitative analysis, either through the formation of insoluble complexes using precipitation reactions or through the formation of colored complexes using coloration reactions (**EL-Haoud *et al.*, 2018**).

#### **4.6.1. Preparation of the aqueous extract:**

The aqueous extract was prepared by adding 10 g of plant powder to 100 ml of boiling distilled water, which was left to infuse for 15 minutes. It was then filtered (Figure27).



**Figure 27:** representing the aqueous extract of *Lavandula stoechas*.

### 4.6.2. Phytochemical tests:

After the preparation of the aqueous extract, each test was carried out as follows:

#### 4.6.2.1. Saponins:

- Take 10 sterile test tubes with a capacity of 20 ml and number them from 1 to 10.
- Place from 1 to 10 ml of aqueous extract in each tube respectively (Table 6).
- Complete the volume of all the tubes to 10 ml with distilled water.
- Shake the tubes well for 15 minutes.
- Observe the foam formed (EL-Haoud *et al.*, 2018).

## Chapter 4 : Materials and methods

**Table 6:** Detection of Saponins.

Tube No	1	2	3	4	5	6	7	8	9	10
Aqueous extract (ml)	1	2	3	4	5	6	7	8	9	10
Distilled water (ml)	9	8	7	6	5	4	3	2	1	0

The foam index (I) is determined using the following formula:

$$I = 1000 / N$$

Here, N represents the number of the tube in which the foam height corresponds to 1 centimeter (EL-Haoud *et al.*, 2018).

### 4.6.2.2. Tannins:

1 ml of extract is placed in a test tube, then 1 ml of distilled water and 2 drops of FeCl<sub>3</sub> solution (ferric chloride) with 1% concentration are added. The appearance of a dark green or blue-green coloration indicates the presence of catechin or gallic tannins, respectively (EL-Haoud *et al.*, 2018).

### 4.6.2.3. Flavonoids:

1 ml of extract is placed in a test tube, then a few drops of FeCl<sub>3</sub> with a 10% concentration are added. The appearance of a yellow coloration indicates the presence of flavonoids (Shaikh and Patil, 2020).

### 4.6.2.4. Terpenes:

1 ml of extract is placed in a test tube, then 2 ml of chloroform and 3 ml of H<sub>2</sub>SO<sub>4</sub> (sulfuric acid) are added. The appearance of brown reddish coloration indicates the presence of terpenes.

### 4.6.2.5. Alkaloids (Wagner's test):

Mix a few milliliters of the aqueous extract with 1 to 2 drops of Wagner's reagent. The appearance of a red or brown coloration indicates the presence of alkaloids (Shaikh and Patil, 2020).

## Chapter 4 : Materials and methods

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### 4.6.2.6. Oils:

Little quantity of plant extract is pressed in between 2 filter papers. Oil stain on the paper if it is present in the extract (**Shaikh and Patil, 2020**).

### 4.7. Extraction and study of the essential oil:

#### 4.7.1. Extraction principle:

The extraction was carried out by simple hydrodistillation using a Clevenger type apparatus (Clevenger, 1928), as shown in Figure. This method involves directly immersing the plant material to be processed (either intact or possibly ground) in water, which is then brought to a boil. The heterogeneous vapors are condensed on a cold surface, and the essential oil separates due to the difference in density (**Bruneton, 1995**).

#### 4.7.2. Extraction steps:

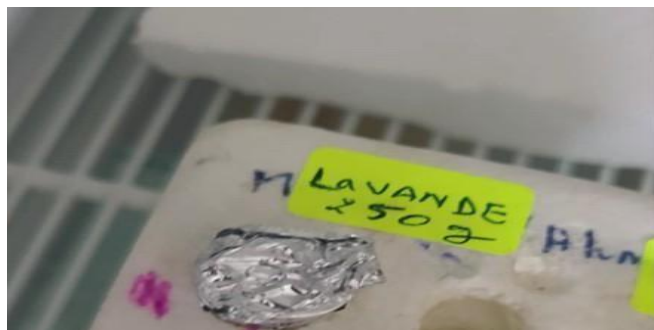
- Prepare 140 grams of plant material (dried leaves cut into small pieces), and place it in a flask with 1000 milliliters of distilled water (the contents of the flask should not exceed three-quarters full to prevent overflow during boiling)
- The entire setup is then brought to a boil. Once boiling is reached, the cells burst and begin to release their essential oil contents, which are subsequently carried with the water vapor to the condenser.
- The vapors loaded with oil that pass through the condenser condense and fall into a separating funnel
- The water and the oil separate due to their difference in density
- The essential oil will then be collected.



**Figure 28:** Distillation apparatus

### 4.7.3. Preservation of the oil:

Most of the constituent molecules of essential oils are unsaturated, which makes them unstable and prone to degradation. The extracted essential oil was stored at a temperature of approximately 4°–6°C, in a tightly sealed tube covered with aluminum foil to protect it from air and light. Figure 29 represented Storage of Extracted Essential Oil under Refrigeration Conditions.



**Figure 29:** Storage of Extracted Essential Oil under Refrigeration Conditions.

### 4.7.4. Extraction yield:

According to the **AFNOR** standard (1986), the essential oil yield (EOY) is defined as the ratio between the mass of essential oil obtained after extraction (EOmass) and the mass of the dry plant material used (DM). It is given by the following formula:

$$\text{EOY} = (\text{EOmass} / \text{DM}) \times 100$$

Where:

**EOY:** Essential oil yield in percentage (%).

**EOmass:** Mass of the essential oil in grams.

**DM:** Dry plant material mass in grams.

### 4.8. Organoleptic characteristics:

The organoleptic analysis of lavender essential oil involves evaluating its properties such as appearance, smell, and color:

- ✓ **Aspect:** The appearance of an extract depends on the components it contains, which may appear in solid or liquid form.
- ✓ **Odor:** The sense of smell is a highly sensitive chemical sense, and the ability of perfumers to classify and characterize chemical substances allows them to accurately measure natural products. Their perception can detect concentrations as low as ten-millionths of a gram per liter of air.
- ✓ **Color:** The color of an essential oil depends on the compounds it contains. Some solvents have the ability to extract many pigments, which intensifies the color of a given oil.

### 4.9. Evaluation of some physicochemical parameters of essential oils of

#### *Lavandula stoechas:*

The extracted essential oil was subjected to a series of physicochemical analyses, which are as follows:

#### 4.9.1. Ph:

It measures the chemical activity of hydrogen ions (H<sup>+</sup>) in a solution (Djousse *et al.*, 2022). It is a coefficient that indicates whether a solution is acidic, basic, or neutral. According to a standard scale, a solution is considered acidic if its pH is below 7, basic if its pH is above 7, and neutral if it is equal to 7. The most commonly used method is pH paper, as follows:

## Chapter 4 : Materials and methods

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- A few drops of the solution to be tested (essential oil) are taken using a pipette.
- They are then placed on the pH paper (Figure 30).
- The paper takes on a specific color, which is compared with the reference colors on the container that held the pH paper.



**Figure 30:** Universal pH Indicator Paper Reel.

### 4.9.2. Refractive Index:

The refractive index of an essential oil is the ratio between the sine of the angle of incidence and the sine of the angle of refraction of a light ray of a specific wavelength, passing from air into the essential oil maintained at a constant temperature of 20°C (AFNOR, 2000).

The measurements were carried out using an Abbé refractometer (Figure 31), Prisma-CETI convex (Figure 31).



**Figure 31:** Abbe Refractometer in use

### 4.9.3. Brix Scale:

It is used to measure, in degrees Brix ( $^{\circ}\text{B}$  or  $^{\circ}\text{Bx}$ ), the sucrose content in a liquid that is, the percentage of soluble dry matter. The higher the Brix value, the sweeter the sample.

The device used for this measurement is a refractometer (Figure 32) (the same instrument used for measuring the refractive index).



**Figure 32:** Abbe Refractometer

### 4.10. Thin Layer Chromatography (TLC):

Thin layer chromatography (TLC) is an analytical method known for its simplicity and versatility. The advantage of thin layer chromatography lies in the wide range of revealing agents that can be applied to it, making it possible to detect a large number of compounds in the samples (Deschepper, 2017). It was carried out according to the following steps:

- Initially, a TLC plate with dimensions of 3 cm by 7 cm was prepared. A baseline (known as the deposition line) was drawn 0.5 cm from the bottom edge of the plate.
- A drop of essential oil was deposited on the baseline using a capillary tube.
- The plate was placed into a chamber (Figure 33) containing the eluent (Dichlorometane), ensuring that the level of the eluent did not exceed the baseline.
- The chamber was then covered with Parafilm and left to allow the eluent to

migrate (develop the chromatogram)

- The plate was removed from the chamber, and the solvent front was marked.



**Figure 33:** UV chamber.

### 4.11. UV-Vis spectrophotometry:

UV-Visible spectroscopy (UV-Vis) (Figure 34) is used to measure the absorbance of light by a sample in the ultraviolet (UV) and visible (Vis) regions of the electromagnetic spectrum. This technique is based on the principle that different substances absorb light at specific wavelengths.

As a result, valuable information can be obtained regarding the concentration and properties of the studied substance by analyzing the absorbance at these wavelengths (Dubey *et al.*, 2024).



**Figure 34:** UV-Vis Spectrophotometer.

# **Chapter 5: Results and discussion**



### Chapter 5: Results and Discussion

This study focused on the extraction of essential oils from the plant *Lavandula stoechas*, the calculation of yield, and the analysis of its organoleptic and physicochemical characteristics.

#### 5.1. Phytochemical Screening:

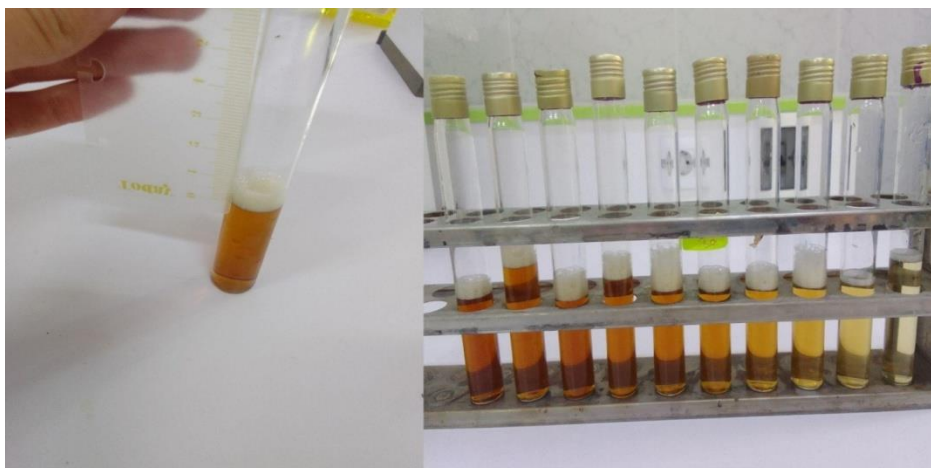
In this practical study, the active compounds present in the aqueous extract of *Lavandula stoechas L.* leaves were identified based on the appearance of foam for saponins, color changes for each of the other compounds (tannins, flavonoids, terpenes, and alkaloids), and the presence of an oily spot for essential oils.

##### 5.1.1. Saponins:

Foam formation was observed after shaking each of the tubes (from 1 to 10), indicating the presence of saponins in the aerial parts of the lavender plant (*Lavandula*) (Figure 35).

The foam thickness was 1 cm in tube number 5, so the corresponding foam index was  $1000/5$   
= 200.

The foam thickness was 1 cm in tube number 5; therefore, the corresponding foam index was  $1000/5 = 200$ .



**Figure 35:** The tubes showed saponin foam formation, with tube number 5.

##### 5.1.2. Tannins:

A color change was observed in the aqueous extract after some time following the addition of the  $\text{FeCl}_3$  solution, turning to a dark green shade. This indicates the presence of catechic-type tannins in the extract and therefore in the aerial parts of the lavender plant (Figure 36).



**Figure 36:** The aqueous extract after the addition of the  $\text{FeCl}_3$  solution.

### 5.1.3. Terpenes:

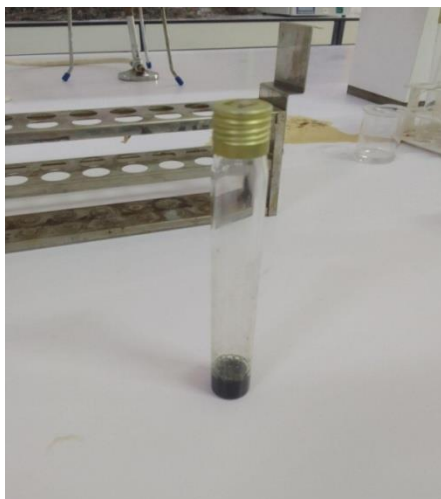
The appearance of a red or brown color was observed in the aqueous extract following the addition of 3 ml of sulfuric acid, indicating the presence of terpenoids in the plant (Figure 37).



**Figure 37:** A test tube indicating the presence of Terpenes.

### 5.1.4. Flavonoids:

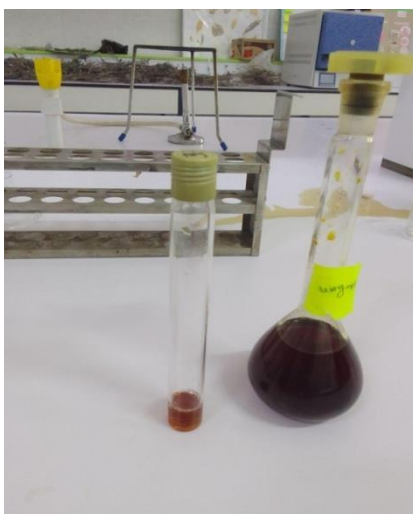
A color change was observed in the aqueous extract after some time following the addition of  $\text{NH}_4\text{OH}$  and  $\text{H}_2\text{SO}_4$  solutions, turning to a yellow hue. This indicates the presence of flavonoids in the extract and therefore in the leaves of the studied plant (Figure 38).



**Figure 38:** A test tube indicating the presence of Flavonoids.

### 5.1.5. Alkaloids:

The appearance of a red or brown color was observed in the aqueous extract following the addition of 1 to 2 drops of Wagner's reagent, indicating the presence of alkaloids in the plant (Figure 39).

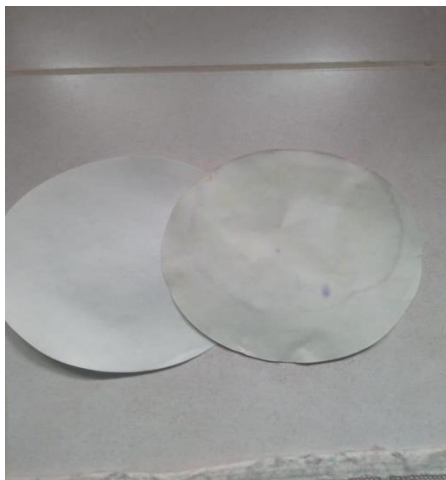


**Figure 39:** A test tube indicating the presence of Alkaloids.

## Chapter 5: Results and Discussion

### 5.1.6. Essential Oils:

The appearance of oily spots (yellow-colored spots) on the filter paper compared to distilled water confirms the presence of essential oils in the aqueous extract of lavender (Figure 40).



**Figure 40:** Filter papers indicate the presence of oil droplets.

The following table 7 summarizes the phytochemical screening of the aqueous extract from the aerial parts of *Lavandula stoechas*:

**Table 7:** the results of the phytochemical screening

Components	Coloration	Results
<b>Tannins</b>	Dark green	+
<b>Flavonoids</b>	Dark green	+
<b>Alkaloids</b>	Light brown	+
<b>Terpenes</b>	Reddish brown	+
<b>Saponins</b>	Presence of foam	+
<b>Essential oils</b>	<b>Oily (yellow) spots</b>	+

+ Presence

The phytochemical analysis of the aqueous extract from the aerial parts of *Lavandula stoechas* revealed the presence of saponins, tannins, flavonoids, terpenes, alkaloids, and essential oils. These results partially align with those reported by **Boufellous *et al.*, (2017)**, who also observed the presence of tannins, flavonoids, terpenes, and essential oils in the aqueous extract, but noted the absence of alkaloids, unlike in our study. In contrast, the findings of **Boulahia *et al.*, (2020)** are more consistent with our observations, showing the presence of tannins, alkaloids, saponins, and flavonoids in the aqueous extract. Finally, the study by **Ez Zoubi *et al.*, (2016)**, conducted on a hydroethanolic extract, demonstrated the presence of tannins, flavonoids, and terpenes.

The diversity of results observed in this study compared to the other cited studies is due to several natural factors. These variations can be attributed to multiple parameters, such as the time of harvest and the environmental and climatic conditions of the plant's growing region, including soil salinity and acidity, as well as temperature. Moreover, the geographical origin of the plant whether from different regions of Algeria can also influence the results. Lastly, the extraction techniques used, along with the devices and equipment employed, play a crucial role in the variability of the observed data.

### 5.7. Extraction yield:

At the end of the extraction, which lasted 30 minutes, the *Lavandula stoechas* plant yielded an extraction rate of 0.4% (Table 8). Lavender oil is a volatile essential oil; therefore, despite the low yield obtained, it is considered satisfactory due to the low oil content in the plant material.

**Table 8:** Final Extraction Yield (%) of Essential Oils from *Lavandula stoechas*.

Parameter	Results
Weight of Plant Material (g)	150
Weight of Oil (g)	0,6
Final Yield (%)	0,4

Our results are similar to those obtained by **Boualleg and Bousnobra (2021)**, who used the same plant with hydrodistillation (Clevenger method) and reported a yield of 0.35%. In contrast to our findings, the extraction yield of the same plant harvested from different regions of Algeria, as reported in the studies by **Benabdelkader (2012)**, **Laiche and Mechri (2023)**, and **Djendi *et al.*, (2023)**, was 0.34% to 1.63%, 2%, and 1.83% respectively, using hydrodistillation.

It can be concluded that the harvesting period of the plant impacts the yield, which may be attributed to the influence of climatic conditions (sunlight or photoperiodism, temperature, and humidity) on the plant material. According to **Jouault (2012)**, not all plants are equal in the amount of oil they contain.

### 5.8. Organoleptic Properties:

The results of the organoleptic properties of our essential oils (color, odor and appearance) are summarized in the Table 9. These results are consistent with those reported in several other studies (**Benabdelkader, 2012; Bachiri et al., 2016; Boualleg and Bousnobra, 2021; Laiche and Mechri, 2023; Djendi et al., 2023; Djouma et al., 2024**).

**Table 9:** Organoleptic Properties of *lavandula stoechas*.

Property	Appearance	Odor	Color
<b>Results</b>	Liquid Clear	Strong Very aromatic	Light yellow
<b>AFNOR (2000)</b>	Clear	Characteristic lavender odor Very slightly camphorated	Light yellow

### 5.9. Physicochemical Characteristics:

The results for pH, refractive index, and Brix index (Table 10) evaluated in this study are presented in the Table 10.

**Table 10:** Physicochemical characteristics of essential oils from *Lavandula stoechas*.

Properties	pH	Refractive Index	Brix Index (%)
<i>L. stoechas</i>	6	1,4692	71,5

#### 5.9.1. pH:

The pH analysis of *Lavandula stoechas* essential oils, measured using pH paper, was 6, indicating slight acidity (Figure 41). Our results are similar to those of **Boualleg and Bousnobra (2021)**, who reported a pH of 5.

Essential oils generally have a slightly acidic pH. This acidity is mainly due to the presence of oxygenated compounds such as phenols. These compounds can release protons ( $H^+$ ) in water, thereby lowering the pH of the solution. As a result, preparations containing essential oils often have a pH between 4 and 6, which reflects their slightly acidic nature. According to **Naouel (2015)**, essential oils with a pH below 7 helps preserve food products.



**Figure 41:** pH Determination of *Lavandula stoechas* Essential Oil.

### 5.9.2. Refractive Index:

The study of the refractive index of *Lavandula stoechas* essential oils revealed a value of 1.4692 (Figure 42). In other studies (**Boualleg and Bousnobra, 2021; Slimani and Maghnini, 2023**), the refractive index was estimated at 1.4824 and 1.461, respectively.

The refractive index is an important physicochemical parameter for the analysis of essential oils. It measures how light propagates through the oil, which reflects its composition and purity.

Variations in the refractive index can indicate differences in the concentration of volatile constituents or the presence of impurities. Thus, this index is commonly used for quality control, identification, and standardization of essential oils.

### 5.9.3. Brix Index:

For the Brix index of *Lavandula stoechas* essential oils, the recorded value is approximately 71.5 (Figure 42). When applied to essential oils, the Brix index helps estimate the concentration of soluble solids, which may include sugars, phenolic compounds, and other dissolved constituents. The Brix value can provide insights into the purity, density, and overall composition of the oil, thereby contributing to the quality control and standardization of essential oil-based products.



**Figure 42:** Abbe Refractometer Showing Brix scale and Refractive index.

### 5.10. Thin Layer Chromatography (TLC):

The results of the TLC (Figure 43) analysis of the essential oil extracted from *Lavandula*

*stoechas* by hydrodistillation (Clevenger) are summarized in Table 10 and Figure This includes information on the retention factors (Rf) of the chemical constituents and their behavior under UV light (254 nm).



**Figure 43:** TLC Plate After Removal from the UV Chamber (254 nm).

The obtained chromatogram shows 4 very distinct spots, with different retention factors (Rf), leading us to conclude that the oil of *Lavandula stoechas* is composed of several components. The comparison between the results of the chromatograms indicates (Table 11):

The four visible spots suggest the presence of 4 major chemical species. The major compounds could be: Rosemarinic acid , Linalool , 1,8-Cineole and Frenchone (Wagner& Bladt, 1996) .

## Chapter 5: Results and Discussion

**Table 11** : TLC analysis of essential oil components: Identification and cosmetic applications based on Rf values

Spots	Observed Rf	Theoretical Rf Range	Proposed Compound	Interpretation	Cosmetic use
1	0.14	0.10 - 0.20	<b>Rosmarinic acid</b>	The observed Rf value of 0.14 falls within the expected range for Rosmarinic acid, suggesting its presence in the sample.	<b>Rosmarinic acid</b> is widely used in skincare products for its antioxidant, anti-inflammatory, and anti-aging properties. It helps to soothe irritated skin and reduce redness, making it valuable in products aimed at sensitive or inflamed skin ( <b>Boualleg, 2021</b> ).
2	0.24	0.15 - 0.25	<b>Linalool</b>	The Rf value of 0.24 is consistent with the expected range for Linalool, indicating its likely presence in the sample.	<b>Linalool</b> , a naturally occurring compound in lavender, has calming properties, often included in moisturizers, serums, and fragrances. It helps in reducing stress and anxiety and has antimicrobial effects, making it ideal for use in products for both relaxation and acne treatment ( <b>Bakkali et al., 2008</b> ).
3	0.38	0.35 - 0.45	<b>1,8-Cineole</b>	The observed Rf of 0.38 is within the expected range for 1,8-Cineole, confirming its identification in the oil.	<b>1,8-Cineole</b> (Eucalyptol) is frequently used in skincare for its antibacterial, antifungal, and decongestant properties. It can help in cleansing the skin and promoting clear skin by fighting acne-causing bacteria ( <b>El-Ghorab et al., 2010</b> ).
4	0.56	0.54 - 0.58	<b>Fenchone</b>	The Rf value of 0.56 matches the theoretical range for Fenchone, supporting its probable presence in the sample.	<b>Fenchone</b> , known for its cooling and refreshing properties, is used in products designed for oily skin. It helps regulate sebum production and can provide a refreshing, toning effect. Fenchone is also included in formulations targeting skin irritation or conditions like acne ( <b>Tassou et al., 2007</b> ).

Rosmarinic acid is a powerful antioxidant found in rosemary and other herbs. It is widely used in skincare products for its ability to calm irritated skin, reduce redness, and protect against environmental stressors such as UV radiation and pollution. Its anti-inflammatory and antioxidant properties make it an excellent ingredient in anti-aging skincare products. Additionally, it has been shown to help improve skin tone and texture (Boualleg, 2021).

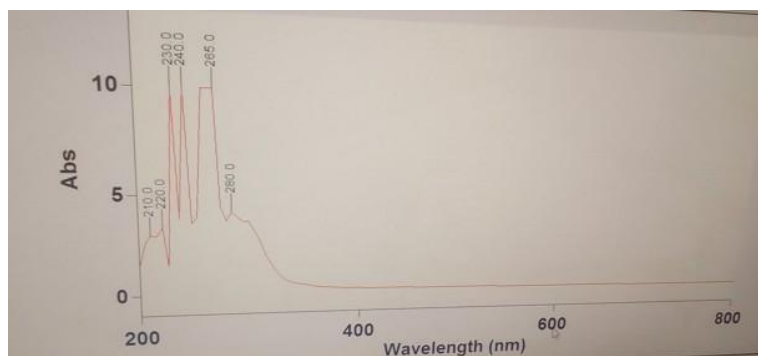
Linalool is a naturally occurring terpene alcohol found in lavender, basil, and other plants. It has calming and soothing properties, often used in aromatherapy to reduce stress and anxiety. In cosmetics, linalool is used in moisturizers, serums, and facial masks due to its ability to relax the skin and promote a calming effect. It also has antimicrobial properties that make it an excellent ingredient for acne treatment products. Its sweet fragrance is also a key feature in perfumes and scented products (Bakkali *et al.*, 2008).

Eucalyptol, or 1,8-Cineole, is often found in eucalyptus, rosemary, and tea tree oil. Its antimicrobial properties make it an excellent ingredient for acne-fighting products, while its ability to promote clear skin is especially helpful for individuals with oily or acne-prone skin. Additionally, 1,8-Cineole is known for its cooling and refreshing effects, making it popular in toners and face mists. It can also help to soothe skin irritation and reduce inflammation, providing a calming effect for sensitive skin (El-Ghorab *et al.*, 2010).

Fenchone is a compound found in fennel and some rosemary varieties. It has antiseptic and cooling properties, often used in formulations targeting oily and acne-prone skin. By regulating sebum production, fenchone helps prevent breakouts, making it a valuable ingredient in products for oily skin. It also contributes to the refreshing, toning sensation found in many facial cleansers and masks, offering a cooling effect to soothe irritation and reduce redness (Tassou *et al.*, 2007).

### 5.7. UV-Vis spectrophotometry

The analysis for this part is not comprehensive due to the chemical complexity of essential oils and the inability of UV-Vis to separate, identify, and quantify each component in the *Lavandula stoechas* essential oil. However, it is considered essential for determining the oil's fingerprint and evaluating its quality. Figure 43 shows the absorption spectrum of the pure essential oil of *Lavandula stoechas*. The essential oil absorbs in the UV range with wavelengths between  $\lambda=210$  nm and  $\lambda=280$  nm.



**Figure 43:** UV-Vis Spectrum of *Lavandula stoechas* Essential Oil.

**Table 11:** results of UV-Vis Spectrum analysis

Wavelength (nm)	Absorbance (Abs)	Description
210	~8.5	High absorbance peak
220	~8.0	Significant absorbance, slightly lower than 210 nm
230	~9.0	High absorbance peak
240	~9.5	Highest absorbance peak observed in this region
265	~8.0	Distinct absorbance peak, indicating a chromophore
280	~4.5	Absorbance decreasing, indicating a decline in chromophore concentration or different chromophores absorbing at this wavelength

The ultraviolet-visible (UV-Vis) spectrum reveals absorption by chromophoric molecules or molecular components responsible for light absorption. The presented spectrum demonstrates strong UV absorption by the essential oil, particularly between 200 nm and 300 nm, with a general decline in absorption beyond 300 nm. This pattern is characteristic of essential oils in general, which represent complex mixtures of volatile organic compounds (e.g., terpenes, terpenoids, and aromatic compounds) (Tzakou *et al.*, 2007).

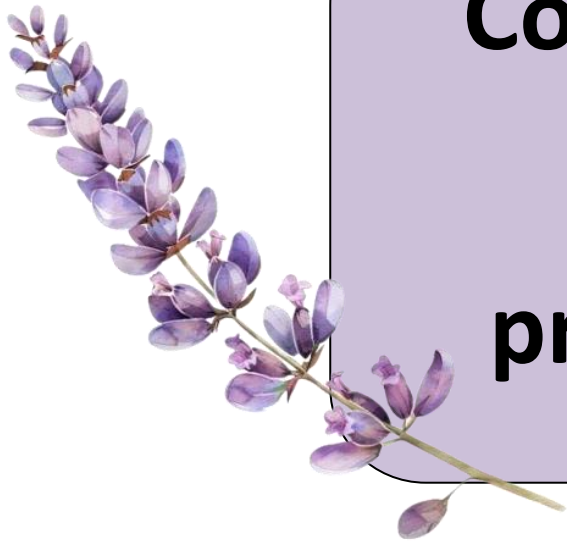
## Chapter 5: Results and Discussion

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The analytical results reveal several distinct absorption peaks, indicating the presence of multiple chromophores (light-absorbing groups) in the essential oil. A prominent absorption peak is observed at approximately 210 nm with an absorbance value of 8.5 units, followed by another peak at 220 nm (~8.0 units). Additionally, there is a notable peak at 230 nm (~9.0 units) and a maximum absorption peak at about 240 nm (~9.5 units). These high absorbance values in the lower UV spectrum region strongly suggest the presence of conjugated double bonds and carbonyl groups, which are well-known components of terpenoid compounds commonly found in essential oils (**Pretsch *et al.*, 2000**).

At 265 nm (~8.0 absorbance units), additional absorption features appear, indicating the presence of supplementary chromophores contributing to the overall absorption profile. Furthermore, absorption is observed at 280 nm (~4.5 absorbance units), showing a noticeable decline compared to other peaks. This suggests a transitional phase where different functional groups or concentrations of light-absorbing species become less prominent. While UV-Vis spectroscopy provides valuable preliminary quality assessment and detects gross adulteration in essential oils, its utility is limited by the inherent chemical complexity of these natural products. Essential oils consist of numerous volatile compounds, resulting in overlapping absorption peaks in UV-Vis spectra that represent combined contributions from multiple constituents (**Baser & Buchbauer, 2010**).

UV-Vis spectroscopy *cannot* provide a comprehensive characterization of the essential oil's composition, including the identification and quantification of its primary and secondary components (e.g., linalool, linalyl acetate, camphor) in *Lavandula stoechas*. Therefore, complementary analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) are *indispensable* and *essential* for complete profiling (**Adams, 2017**).



# **Conclusion And prospects**

### Conclusion and prospects

The fields of cosmetics, food, medicine, and even traditional medicine all without exception are connected by a common factor: the use of medicinal and aromatic plants. The reason behind choosing them lies in their numerous and incredibly beneficial active compounds. In the past, they were the foundation of healing and traditional medicine in general, and today they have become a basis for the development of drugs and treatments. We must also not forget their psychological impact, which makes them stand out from other treatments, as they do not only affect the physical aspect. Recently, they have become a focus of research aimed at further uncovering their complex chemical compositions and even genetically modifying them to maximize their benefits across all the aforementioned fields.

In this research, we focused on the plant *Lavandula stoechas*, which contains essential oil in its aerial parts. This oil includes active compounds such as tannins, saponins, terpenes, flavonoids, alkaloids, and essential components. The extraction yield was estimated at 0.4%. The TLC (Thin Layer Chromatography) technique identified the presence of four main components: fenchone, 1,8-cineole, linalool, and rosmarinic acid.

*Lavandula stoechas* is distinguished by its active components with antiseptic and nerve soothing properties. It is used in various fields, especially in the cosmetics industry and in relaxation therapies, which makes its benefits twofold as it affects both the psychological and physical aspects. Moreover, its full chemical composition is not yet fully known due to the lack of applied research, which adds another dimension to its potential, as it may represent a unique breakthrough and an exceptional point of convergence between several fields.

#### Prospects and Future Development:

We can use *Lavandula stoechas* essential oil in several innovative applications, including:

- ✓ **Textile industry:** It can be used to enhance the properties of clothing materials.
- ✓ **Pain and anxiety relief:** Especially beneficial for reducing discomfort and emotional distress in patients with chronic illnesses, particularly cancer patients.
- ✓ **Natural cosmetics:** Its application in natural beauty products is expected to gain great success, as it offers both internal and external restoration benefits.
- ✓ **Safe genetic modification:** Its role in safe genetic engineering could represent a major breakthrough, potentially amplifying its multiple benefits, particularly in the cosmetic field.



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