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**Chemical Composition and Bioactivities of Essential Oils
from *Anacyclus valentinus L.* and *Rosmarinus officinalis L***

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Dedications

To my parents

For your hands that worked so hard,
For your heart that gave me so much,
For your eyes that were sometimes filled with tears,
May GOD protect you and grant you blessings and Grace.

To my wonderful and very dear siblings,

Wishing them a life full of success,

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In appreciation of their guidance, mentorship, and valuable support throughout my research.

To all my friends and colleagues

Finally, to all those who are very dear to me

Affectionately, **SARA.**

List of abbreviations

AFNOR: French Association for Standardization.

WHO: World Health Organization

E.O: Essential Oil

A. Valentinus: Anacyclus Valentinus

GC-MS: Gas chromatography – mass spectrometr

TLC: Thin Layer Chromatography

HPLC: High Performance Liquid Chromatography

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INTRODUCTION

Introduction

Plants have played a fundamental role in human life since ancient time. They represent an essential source of food, natural remedies, and raw materials used in various traditional and modern applications. For centuries, medicinal and aromatic plants have been widely utilized for their therapeutic properties, contributing significantly to healthcare systems and pharmaceutical research.[1]

Algeria is very rich in its natural and diverse herbs due to its vast areas and varied climates, featuring fertile soil in coastal areas, plains, mountains, and desert oases. Numerous studies have proven that Algeria contains no less than 3,500 species of plants, some of which belong to hot climates and others to temperate climates. There is no doubt that these climates and soils have a profound impact not only on the intensity of plant diversity but also on the composition of plants and giving them their special characteristics.[2].

Methods of using medicinal herbs vary, including infusions, decoctions, or using the whole plant to extract active ingredients and using them in various pharmaceutical formulations. Returning to the use of medicinal plants in treatment is a return to nature, especially since synthetic drugs have many side effects compared to these herbs. If we want to preserve our health, we must return to nature, which is the source of our life. Whenever man distances himself from nature, he causes a loss of balance in it, as each plant is considered unique in its biological characteristics, and each plant can be considered a complete chemical laboratory that produces substances beyond the energy of any other creature. Medicinal plants are characterized by a pungent aromatic scent and a distinct taste. Aromatic plants are also used to give flavor to food, and at the same time, they are used for their many medicinal benefits. Some plants have been used to maintain food quality, improve its taste, and preserve it because they contain volatile compounds[3]. The great interest in studying medicinal plants has led to the discovery of large numbers of natural chemical compounds with pharmaceutical use. It was found through a statistical study conducted to count the medicinal compounds discovered in 2002 that 40% of these drugs were obtained from a natural source. In the journey of permanent and continuous research to discover the secrets of plants in order to determine their therapeutic characteristics and understand their chemical components and the extent of their impact, we have decided in our research to study the *Anacyclus valentinus* plant (al-kartofa) and *Rosmarinus officinalis*. We have extracted its essential oil using the hydrodistillation method, determined the oil's components, and its potential activity.

This thesis is divided into four chapters:

Chapter I: A review on essential oils (bibliographic study).

Chapter II: Study plant.

Chapter III: Material and method.

Chapter IV: Results and discussion.

CHAPTER I: A review on essential oils

I. A review on essential oils

I.1 Introduction:

Since ancient times, plants have been a primary source for human needs. Humans used aromatic plants and their extracts in religious rituals, perfumery, cosmetics, and food preservation. Distillation was known to the Chinese and Indians 20 centuries before Christ, Egyptians and Arabs used aromatic plants for mummification, treating wounds with ointments, and manufacturing aromatic drinks.[4]

I.2 Definition of Essential Oils:

Essential oils are volatile aromatic substances obtained from aromatic plants through physical processes such as distillation, solvent extraction, or mechanical pressing. They are characterized by a strong fragrance and evaporate almost completely without significant residues. In plants, essential oils are synthesized and accumulated in specialized secretory tissues and can be found in various organs, including flowers, leaves, bark, wood, roots, rhizomes, fruits, and seeds.[4]

I.3 Chemical Composition of Essential Oils:

Volatile oils consist of a large number of simple and complex natural chemical compounds, which can be divided into two main groups:

- Hydrocarbons: Formed from hydrocarbon compounds (C, H).

- Oxygenated Compounds: These constitute the main aromatic part and are more soluble in water than hydrocarbons. They include alcohols, ethers, esters, oxides, ketones, phenols, and peroxides.

- Terpenes (C₅ H₈): Terpenes are a mixture of hydrocarbons and oxygenated derivatives. In some essential oils, hydrocarbons may be the dominant component, while in others, oxygenated compounds occupy the larger portion and are responsible for the scent and taste.

- Hydrocarbon compounds consist of isoprene units (C₅):

- Monoterpenes (C₁₀): Composed of two isoprene units, these are volatile compounds whose pleasant scent characterizes many plants and are used commercially in perfumes and medicine.

- Sesquiterpenes (C₁₅): Composed of three isoprene units, such as ginger oil and cade oil.

- Diterpenes (C₂₀): Composed of four isoprene units.

- Triterpenes (C₃₀): Composed of six isoprene units and are solid substances like Amyrin and Lupeol. Some triterpenes exhibit activity against microbes and insects. These terpenes are widespread in the plant kingdom and vary in their structural composition depending on the natural source. The terpenes most commonly found in volatile oils are those with low molecular weight, namely monoterpenes and sesquiterpenes.

PHENYLPROPENES

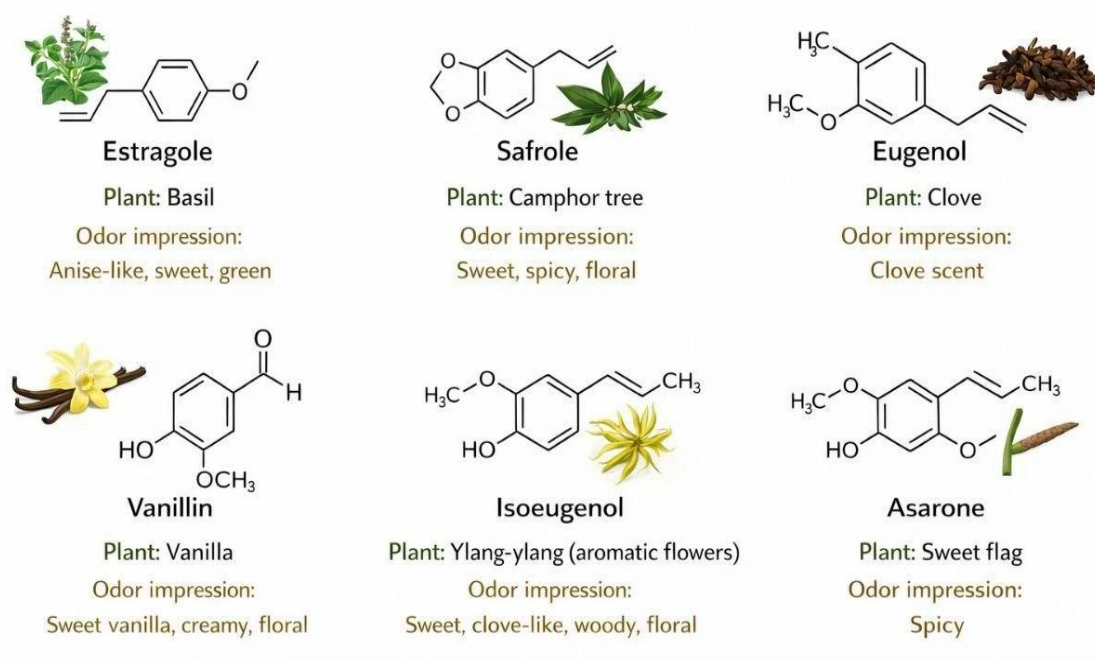
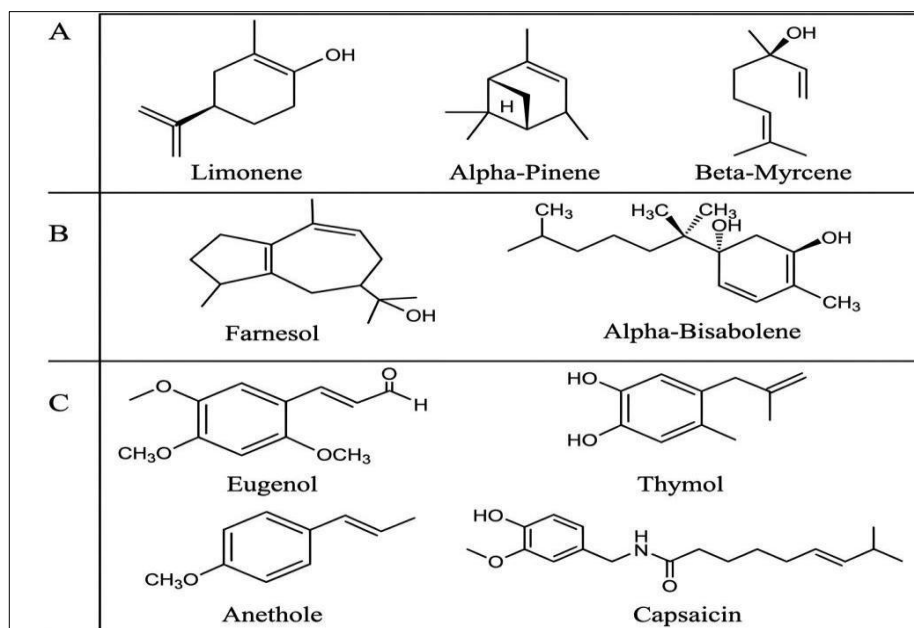


Figure (I-1): Phenylpropene compounds in some plants.

-Aromatic Compounds (Phenylpropenes): These are derivatives of the amino acid Phenylpropane, they are less common than terpenes in essential oils.

However, some plants contain high percentages of them. They consist of a carbon chain attached to a six-carbon aromatic ring and are classified according to the functional group they carry: aldehyde, ester, acid, phenolic ether, or phenol. This is especially true for some oils of the Apiaceae family (Parsley) and Cloves (Girofle). Oils containing a high percentage of the phenol molecule are characterized by therapeutic properties and are also considered antioxidants.

-Other Derived Compounds: These are compounds formed as a result of transformations in non-volatile molecules in essential oils. They may result from the degradation of terpenes or fatty acids, which often give the scent of fruits. Sulfur and nitrogen compounds are rarely present in essential oils. [5]



A: Monoterpenes/ B: Sesquiterpenes/ C: Phenylpropanoids

Figure (I-2): Structure of some compounds included in the composition of essential oils

I.4 Extraction Methods of Essential Oils:

There are several methods for extracting essential oils, and the preference between these methods varies based on the following:

- The chemical composition of the volatile oil.
- The amount of volatile oil present in the plant.

Using the least costly method to obtain a high-quality aromatic oil. The most important extraction methods for aromatic oils include:

I.4.1 - Steam Distillation:

Steam distillation is a widely used method for extracting essential oils from heat-sensitive aromatic plants. It lowers the boiling point of volatile compounds, preventing their thermal degradation. In this process, steam passes through the plant material placed on perforated racks without direct contact with water, allowing the essential oils to be released, carried by the steam, and then condensed for collection.[5]

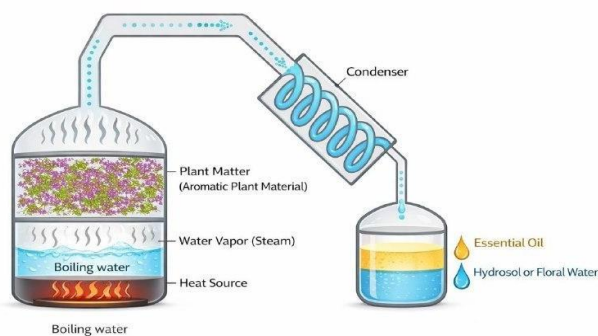
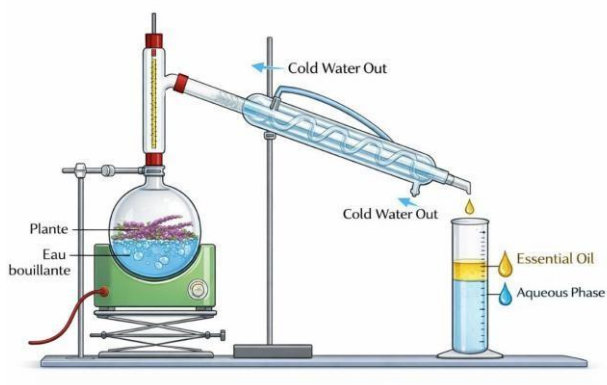


Figure (I-3): Extraction of essential oils by steam distillation

I.4.2 - Hydro-distillation:

Hydro-distillation is a traditional method of essential oil extraction standardized by AFNOR, in which the plant material is immersed in boiling water. The water and essential oil evaporate together, then condense and are separated, usually using a Clevenger apparatus. This technique is particularly suitable for dried plants rich in volatile oils and remains one of the most widely used extraction methods. However, the prolonged exposure to heat may alter some physical and chemical properties of the extracted oil.[5]



Figure(I- 4): Protocol for the extraction of essential oils by hydro-distillation.

I.4.3 -Hydro-steam Distillation (Hydro-diffusion):

This is a variation of steam distillation; the difference lies in the way the steam enters. In hydro-diffusion, steam is introduced from the top to pass through the plant material rather than from the bottom, as is the case in standard distillation systems. Condensation of the steam mixture containing the oil occurs on the grid located beneath the plant material. The advantages of this method include: reduced steam consumption, shorter processing time, and higher oil yield.[5]

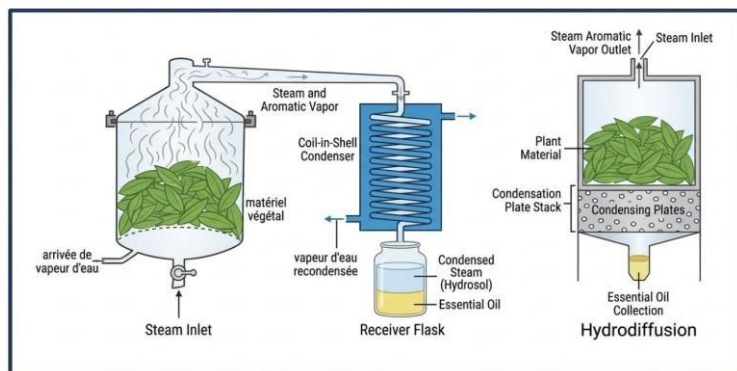


Figure (I-5): Extraction by hydro- steam distillation (hydro-diffusion).

I.4.4 -Solvent Extraction:

Solvent extraction is a modern technique used to obtain essential oils from heat-sensitive plants or those containing very low concentrations of aromatic compounds, such as jasmine and violet. The plant material is immersed in an organic solvent (e.g., hexane or petroleum ether), which dissolves the aromatic substances. After filtration and solvent evaporation under reduced pressure, the extract is purified to remove waxes and fats, yielding a fragrant oil that retains its

natural chemical properties. The solvent used should be stable, non-reactive, and easily removable due to its low boiling point.[5]

I.4.5 -Microwave Extraction:

Microwave-assisted extraction is a modern technique developed to overcome the limitations of conventional essential oil extraction methods. In this process, fresh plant material is exposed to electromagnetic microwaves, which heat the water naturally present within the plant tissues. This causes the rupture of oil glands and the release of volatile compounds. The extracted oil is then dissolved in a suitable solvent, filtered, and recovered. This method is faster, more efficient, and better suited for preserving heat-sensitive aromatic compounds.[5]

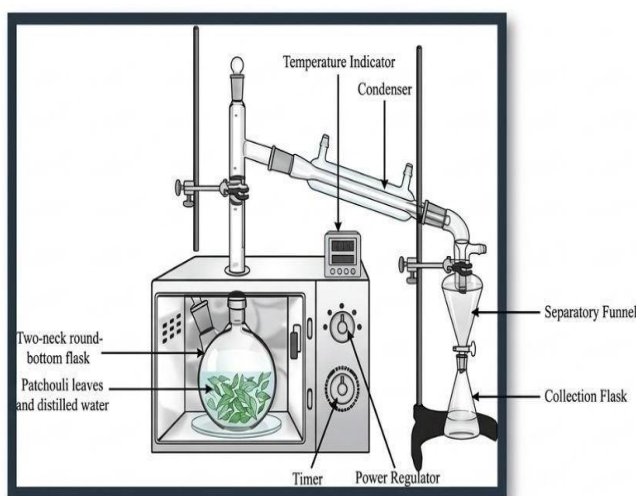


Figure (I-6): Model showing the microwave extraction method.

I.5 -Physical and Chemical Characteristics of Essential Oils:

Despite the differences in the components of volatile oils in their chemical composition, they share some common characteristics, namely:

Table (I-1): Physical and Chemical Characteristics of Essential Oils[4]

Property	Description
Color	Most oils are colorless, and a few are pale yellow if extracted by distillation. However, if extracted by expression (pressing), the oil often takes on the color of its plant.
Odor	Essential oils are characterized by a strong and fragrant aromatic scent.
Volatility	The vast majority of volatile oils evaporate completely under normal conditions, with very few exceptions.
Solubility	All oils are insoluble in water, but they dissolve in alcohol at a concentration of 95%.

Fluidity	Oils are generally considered liquid at normal room temperature, though a few may solidify when exposed to low temperatures (5°C - 8°C).
Refractive index	They possess a high refractive index which is important parameter for determining oil identity and purity.
Optical rotation	E.oils exhibit optical rotation, which is one of the most important tests for determining the type and purity of the oil.
Storage	E.oils should be stored in a cool place and kept away from flames due to their volatile and flammable nature.
Specific gravity	The specific gravity values of volatile oils vary, and most are less dense than water (lighter than water).

I.6 - Analysis of Aromatic Oils and Quality Standards:

The quality of essential oils is mainly determined by their natural aroma, flavor, color, and physicochemical properties. Most essential oils range from colorless to pale yellow, although some, such as chamomile and yarrow oils, may exhibit blue or greenish-blue colors. Evaluating physicochemical constants helps verify the purity and authenticity of the oil, as their values are influenced by factors such as plant species, environmental conditions, growth stage, and harvest time. In addition to these characteristics, advanced analytical techniques, particularly chromatographic methods such as TLC, HPLC, and GC/MS, are widely used to separate and identify essential oil components, with GC/MS being the most common and reliable method.

I.7 Toxicity of Essential Oils:

Essential oils are not products that can be used without risks. This aspect is of paramount importance, as some components of essential oils can be toxic, particularly monoterpene ketones. These components and other aromatic substances possess toxic properties when administered in large doses.

Due to the potential toxicity of certain oils to human life and health, the World Health Organization (WHO) has moved to regulate the sale of essential oils under specific conditions stipulated in Decree No.86.

Oils containing phenol are toxic to the liver, as seen in thyme and clove. Most widely used volatile oils have an LD50 ranging between 2 and 5 g/kg (e.g., anise, clove), while other oils have a lower LD50, such as basil and thyme, where it ranges between 1 and 2 g/kg. [6]

I.8 Uses of Essential Oils:

These natural products are of great importance and are used in various fields:

- Perfume and Cosmetic Industry: Many perfumes are of natural origin, and some oils form the basis of these perfumes, such as rose, jasmine, and lavender oils. Lavender oil is used to treat hair loss, while lentisk oil (Mastic) is used in treating acne.[7]
- Cleaning and Disinfection Industry: They are used as disinfectants and antifungals, antiparasitics, and antibacterials in products like soap and agricultural products such as insecticides.
- Pharmacy: Aromatic oils are used as medical preparations or pharmaceutical excipients (substances added to the medicinal dose). Notably, chamomile essential oil is used as an anti-inflammatory and a nervous system sedative. Furthermore, essential oils are used against acquired infections.[8]
- Nutrition: They have important applications as preservatives and flavor enhancers. Examples include lemon, mint, and clove oils.

I.9 - Biological Activity:

Scientific research on biological activities often increases our knowledge regarding the potential therapeutic use of oils.

Essential oils possess numerous biological activities; they are used for their therapeutic and antiseptic properties against diseases. In recent centuries, scientific studies have focused on these properties, such as antibacterial activity.

Table(I- 2): biological activity of *E. oils*.

Biological Activity	Description
Antioxidant Activity[9]	<p>Many essential oils have antioxidant properties. These are a group of compounds naturally present in the body, capable of protecting it from damage caused by unstable molecules known as free radicals. Their role consists of preventing the formation of free radicals and reactive oxygen species (ROS) within the body's cells. Natural antioxidants are used extensively worldwide and are superior to synthetic antioxidants.</p> <p>The antioxidant activity of essential oils results from certain compounds such as: Ketones and Monoterpene Aldehydes.</p>
Antimicrobial Activity[8]	<p>Essential oils have a broad spectrum of activity, as they inhibit the growth of bacteria and fungi (by preventing toxin formation) as well as yeasts (affecting the biomass).</p> <p>This antimicrobial property is primarily linked to the chemical composition of the essential oil, specifically the nature and structure of the aromatic compounds within it. Their intervention occurs at the level of bacterial reproduction, either by inhibiting it or by killing the bacteria.</p>

<p>Antibacterial Activity [9]</p>	<p>The antimicrobial effects of volatile oils have been examined on a wide range of microorganisms over the years, showing that they have the greatest impact on bacteria.</p> <p>One of the most important characteristics of essential oils is their hydro-phobicity, which allows the oil to separate the lipids in the bacterial cell membrane. This makes the cell more vulnerable to the penetration of the aromatic oil. and inhibiting their functional properties through phenolic compounds, which ultimately leads to the leakage of the cell's internal contents and its elimination.</p>
<p>Antifungal Activity[10]</p>	<p>It is well known that aromatic oils consist of several components. In fact, terpene compounds interact, and this functional group, such as phenols and aldehydes, works with membrane enzymes to disrupt the plasma membrane of yeast.</p> <p>The antifungal effect is attributed to these compounds increasing the permeability of the plasma membrane, causing the leakage of cell contents and subsequently the death of the yeast (a type of fungi).</p> <p>Active compounds can be used as protective agents against pathogenic fungi for plants and microorganisms that invade food products.</p>
<p>Insecticidal activity[6]</p>	<p>E.oils exhibit insecticidal activity against a wide range of insects. Their volatile compounds act through fumigant, contact, or ingestion toxicity, affecting the nervous system, respiration, and development of insects. this activity makes them useful as natural insecticides in agriculture, food storage, and public health pest control.</p>

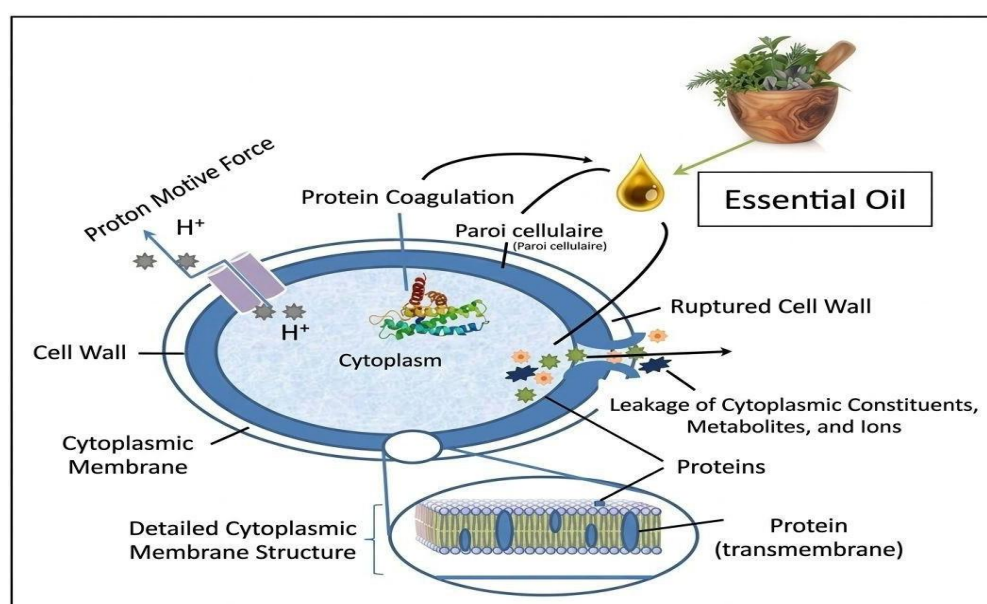


Figure (I-7): Permeability site of oils on bacterial cell membrane.

CHAPTER II: Study plants

II. Study plants

II.1 - Introduction:

As fundamental components of terrestrial and aquatic ecosystems, plants play a vital role in sustaining life and maintaining environmental balance. Their ability to produce organic matter through photosynthesis makes them the primary source of oxygen and energy for most living organisms. Beyond their ecological significance, plants represent an important source of biologically active compounds with diverse industrial, pharmaceutical, and cosmetic applications. In particular, aromatic and medicinal plants have attracted considerable scientific interest due to their richness in essential oils and other natural products exhibiting antimicrobial, antioxidant, and antifungal activities. Consequently, the study of plants remains a key area of research in botany, pharmacognosy, and natural product chemistry.[11].

II.2 - *Anacyclus valentines L*

II.2.1 -Definition of *Anacyclus valentinus L*

Is a medicinal and aromatic herbaceous plant belonging to the Asteraceae family. It is native to the Mediterranean basin, especially North Africa, where it grows naturally in arid and semi-arid regions. This species is well adapted to harsh environmental conditions such as drought and poor soils, and it is recognized for its richness in bioactive compounds, including essential oils and phenolic substances, which contribute to its pharmacological properties.[5].



Figure (II- 1): *Anacyclus valentines L*

Table (II-1): Botanical classification of *Anacyclus Valentinus L.*

Rank	Scientific classification	Description
Kingdom	Plantae	All plants
Division	Angiosperms	Flowering plants
Class	Eudicots	True dicots
Order	Asterales	Contain the asteraceae family
Family	Asteraceae	Composite/Daisy family
Genus	<i>Anacyclus</i>	Aromatic and medicinal genus
Species	<i>Anacyclus valentinus L</i>	Annual or perennial medicinal herb

II.2.2 Botanical Description (Detailed Morphology):

Anacyclus valentinus L is a low-growing annual herb, reaching 10–30 cm in height. It possesses a thin taproot adapted to dry soils and slender, branched stems that are often creeping or ascending. The leaves are alternate, deeply divided, and covered with fine hairs, helping to reduce water loss. Its flower heads are daisy-like capitula composed of white ray florets surrounding yellow disc florets, characteristic of the Asteraceae family. The fruit is a small achene containing a single seed, adapted for dispersal[12].

II.2.3 -Uses of *Anacyclus valentinus L*

1- Traditional and Folk Uses: although specific ethnobotanical studies on *Anacyclus valentinus L* are limited, it is reported that in Algeria this plant has been used in folk medicine and as a food condiment in certain regions. This includes traditional applications for mild ailments and general health, similar to other *Anacyclus* species in local herbal medicine.

2- Antifungal and Antimicrobial Activity: The essential oil of *Anacyclus valentinusL* has been chemically analysed and shown to contain terpenoid compounds such as δ -3-carene and spathulenol. In laboratory tests, this essential oil exhibited strong antifungal activity against several mycotoxin-producing fungal species, including *Fusarium*, *Aspergillus*, and *Penicillium*. These results suggest potential use as a natural biocontrol agent in protecting crops and reducing fungal contamination without synthetic chemicals.[10]

3- Antioxidant and Anti-Inflammatory Properties: Recent scientific studies have evaluated *A. valentinus L* essential oil and its oxygenated fraction for antioxidant activity using assays such as DPPH scavenging and β -carotene bleaching. The oxygenated fraction demonstrated significant antioxidant potential, while the essential oil itself showed good anti-inflammatory activity in vitro,

with effectiveness comparable to or exceeding diclofenac in certain tests. These findings support interest in the plant's bioactive compounds for possible pharmaceutical and nutraceutical applications.[13]

II.2.4 -Scientific Studies on *Anacyclus valentinus L*:

Anacyclus valentines L has received significant research attention in recent years due to its promising phytochemical and therapeutic properties. In this regard, the following (TableII-2) summarizes the prominent previous scientific studies that focused on determining the chemical composition of its essential oil and extracts, as well as evaluating their various biological activities

Table (II-2): Scientific Studies on *Anacyclus valentinus L*

Study	Extraction method	Composition	Biologic activity	Refrence
Evaluation of the Antioxidant and Anti-inflammatory Activity of the <i>Anacyclus valentinus L</i>. Essential Oil and its Oxygenated Fraction.[13]	Hydro-distilled using a Clevenger-type device	Oxygenated sesquiterpene compounds hydrocarbon sesquiterpenes oxygenated monoterpenes and non-terpenic compounds .	Antioxidant and Anti-Inflammatory Activity	Ainseba et al. (2024).
Chemical composition and antifungal activity of <i>Anacyclus valentinus L</i> essential oil from.[10]	Hydro-distillation	δ -3-carene spathulenol	Antifungal Activity	Algeria(2018).
Chlorogenic acid with cytotoxic activity and other constituents from <i>Anacyclus valentinus L</i>.[14]	/	chlorogenic acid and flavonoids	Cytotoxic Activity	Ramli et al(2025).
Chemical composition and antibacterial activity of the essential oil of <i>Anacyclus valentinus L</i>.[15]	Clevenger	Spathulenol Carene- 3- δ	antibacterial activity	Local Academic Work(2022).

II.3 - *Rosmarinus officinalis*

II.3.1 -Definition of *Rosmarinus officinalis*:

Rosmarinus officinalis is a woody, perennial evergreen shrub of the Lamiaceae family, indigenous to the Mediterranean. Distinguished by its fragrant, needle-like foliage, it is widely utilized across the culinary and pharmacological industries, serving as both a versatile flavoring agent and a foundational medicinal plant.[7]



Figure (II- 2): *Rosmarinus officinalis*.

Table (II-3): Botanical classification of *Rosmarinus officinalis*

Rank	Scientific classification	Description
Kingdom	Plantae	All plants
Division	Angiosperms	Flowering plants
Class	Eudicots	True dicotyledons
Order	Lamiales	Includes many aromatic plants
Family	Lamiaceae	Mint family(aromatic herbs)
Genus	<i>Rosmarinus</i>	Aromatic woody shrubs
Species	<i>Rosmarinus officinalis</i>	perennial medicinal and culinary herb

II.3.2 Botanical Description (Detailed Morphology):

Rosmarinus officinalis is a perennial evergreen shrub reaching 1–2 m in height, characterized by a bushy and highly branched habit. It has narrow, needle-like leaves that are dark green on the upper surface and whitish beneath. The stems become woody with age, while young shoots remain green and flexible. The plant produces small blue, violet, or white bilabiate flowers and possesses a well-developed root system adapted to dry, rocky environments. Its distinctive aromatic fragrance is due to the presence of essential oils.[7]

II.3.3 -Uses of *Rosmarinus officinalis*:

Rosmarinus officinalis is widely used across various fields due to its rich content of bioactive compounds and essential oils.

Table (II-4): uses of *rosmarinus officinalis*

Category	Use	Réf
Medicinal Uses	<ul style="list-style-type: none"> -Antioxidant properties (protects cells from oxidative stress) -Anti-inflammatory effects -Antimicrobial activity against bacteria and fungi -Cognitive benefits, including memory enhancement and improved concentration -Digestive aid, helping relieve indigestion and bloating -It is commonly used in the form of herbal teas, extracts, and essential oils. 	[16]
Culinary Uses	<ul style="list-style-type: none"> -Used to flavor meats (especially lamb and chicken) -Added to soups, sauces, and roasted vegetables -Used fresh or dried as a spice -Essential oils sometimes used as food preservatives 	[17]
Industrial Uses	<ul style="list-style-type: none"> -Used as a natural antioxidant in food preservation. -Applied in cosmetics and skincare products. -Ingredient in perfumes and aromatherapy oils. -Used in pharmaceutical preparations. 	[16]
Cosmetic Uses	<ul style="list-style-type: none"> -Promotes hair growth and scalp health -Used in shampoos and hair oil -Helps improve skin condition due to its antioxidant properties 	[7]
Aromatic and Traditional Uses	<ul style="list-style-type: none"> -Used in aromatherapy for relaxation and stress relief -Traditionally associated with memory and concentration -Used in cultural and herbal practices 	[7]

II.3.4 -Scientific Studies on *Rosmarinus officinalis*:

Numerous scientific studies have investigated the pharmacological and biological activities of *Rosmarinus officinalis*, confirming its importance as a medicinal plant with diverse therapeutic potential.

1. General Pharmacological Activity

A comprehensive review published in the Journal of Biomedical Science demonstrated that rosemary contains bioactive compounds responsible for multiple biological effects, including anti-

inflammatory, antioxidant, antimicrobial, and antitumor activities. These properties are mainly attributed to phytochemicals such as carnosic acid, rosmarinic acid, and carnosol.[16]

2. Antioxidant and Protective Effects

Several studies have shown that rosemary has strong antioxidant capacity. It helps reduce oxidative stress and inflammation by regulating biological pathways such as NF- κ B and MAPK signaling. This makes it effective in protecting cells against damage caused by toxins and free radicals.[18]

3. Cognitive and Neuroprotective Effects

A systematic review and meta-analysis (2022) found that rosemary significantly improves cognitive performance and memory in animal models. The results showed positive effects in both normal and cognitively impaired subjects. This supports its traditional reputation as the “herb of memory.”[19]

4. Metabolic and Therapeutic Potential

Research has explored rosemary as a potential treatment for metabolic syndrome, including its effects on blood sugar, lipid metabolism, and obesity-related conditions. Studies suggest that its bioactive compounds may help regulate metabolic processes and reduce disease risk.[18]

5. Aromatherapy and Essential Oil Studies (Recent – 2025)

Recent scientific reviews indicate that rosemary essential oil can improve cognitive function, mood, sleep quality, and pain management. Its main active compounds (1,8-cineole, camphor, α -pinene) contribute to these effects.[20]

6. Broad Biological Activities

An updated review highlights that rosemary exhibits a wide range of biological activities, including:

Antitumoral

Anti-inflammatory

Neuroprotective

Antimicrobial

Endocrine regulation effects

PMC

This confirms its role as a promising source for drug development.[21]

CHAPTER III: Material and methods

III. Material and methods

III.1 -Introduction:

The extraction of essential oils from *Rosmarinus officinalis* and *Anacyclus valentinus L* was carried out using the hydro-distillation method. Subsequently, the chemical composition of the obtained oils was investigated to identify their major constituents and characterize their phytochemical profiles. In addition, biological evaluations were performed to assess their potential medicinal properties and to provide scientific evidence of their biological activities.

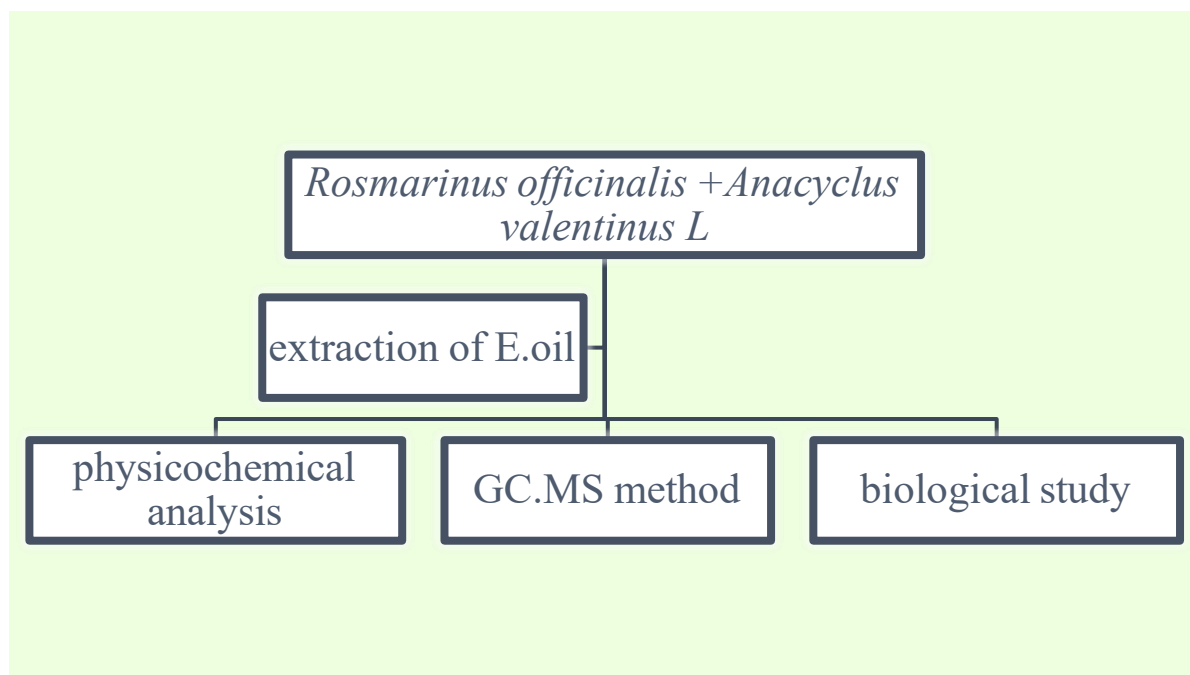


Figure (III-1): Study diagram.

III.2 Plant sample:

Aromatic and medicinal plants used in this present study were *Rosmarinus officinalis* and *Anacyclus valentinus*. The plant materials were purchased from local herbal markets in :

a- *Rosmarinus officinalis*: Kherrata(1kg).

b- *Anacyclus valentinusL*: Djelfa(2kg).

during spring 2025. A total of 1kg of *Rosmarinus officinalis* and 2kg of *Anacyclus valentinusL* were used for e.oil extraction. After collection, the samples were cleaned, shade-dried at room temperature and preserved in airtight containers until extraction.

Table (III-1): plant material used in the study

Plant species	Common name	Family	Collection site	Collection period
<i>Rosmarinus officinalis</i>	Rosemary	Lamiaceae	Kherrata	spring 2025
<i>Anacyclus valentinus L</i>	Al -kartofa	Asteraceae	Djelfa	spring 2025

III.3 Materials and tools used:

The laboratory equipment and plant materials employed throughout the different phases of this work are systematically organized. (Table III-2) provides a detailed listing of the primary materials and tools utilized for the extraction process and subsequent analyses:

Table (III-2): materials and tools used

Tools	Materials
Clevenger apparatus	Plant sample
Precision balance	
Refractometer	Distilled water
GC/MS chromatograph apparatus	

III.4- Extraction of the essential oil:

The essential oil was extracted using a Clevenger-type hydro-distillation apparatus. Hydro-distillation is based on the ability of water vapor to carry the plant essential oil.

The plant material was cut into small pieces and then placed in a 2 L glass flask, where an amount of plant material was immersed in distilled water (filling two-thirds of the flask volume at most to avoid overflowing).

The mixture was subjected to a heat source. After boiling, the water saturated with essential oil passed through a cooling system, causing the vapor enriched with oil to condense, forming small droplets that accumulated in a side arm due to the density difference between water and essential oil. The essential oil floated above the surface of distilled water. The distillation process lasted for 3 hours. After that, the essential oil was collected in a sterile, tightly sealed glass vial. this research

work was conducted in the scientific research laboratories of the Faculty of Mathematics and Material Sciences.



Figure (III-2): Hydro-distillation setup showing the accumulation of the essential oil.



Figure (III-3): Clevenger apparatus

III.5 -Physicochemical properties:

III.5.1 Evaluation of the physicochemical parameters of the essential oil:

Essential oil yield:

The oil yield was calculated as the ratio of the mass of extracted oil to the mass of dry plant material. The relationship is given as follows:

$$R(\%) = (MHE / MS) \times 100$$

Where:

R (%): Essential oil yield expressed as percentage. MHE:

Mass of essential oil (g).

MS: Mass of dry plant material used for extraction (g).

-Relative density:

Density was determined by taking a known volume of essential oil and measuring its mass at

20°C.

First, the volumetric density of the oil was calculated using the following equation:

$$\rho = m / v$$

Where:

ρ : Volumetric density of the essential oil (kg/m³).

m: Mass of essential oil (g).

v: Volume of essential oil (mL).

Then, the relative density of the oil was calculated using the following equation:

$$d_{20} = \rho_{HE} / \rho_e$$

Where:

d₂₀: Relative density of the essential oil.

ρ_{HE} : Density of the essential oil at 20°C.

ρ_e : Density of water at 20°C.

III.6-Determination of the chemical composition of the essential oil by GC/MS:

Essential oils form homogeneous mixtures whose components are difficult to separate by classical physicochemical methods. Therefore, the principal technique used for their separation is chromatography.

The chemical composition of *A. VALENTINUS L* and *R. OFFICINALIS* essential oil was studied by gas chromatography using a shimadzo GC-2030 chromatograph coupled with mass spectrometry (MS)-TQ8040NX. The analysis was performed in split injection mode with a split ratio of 100.0.

The injector, ion source, and interface temperatures were maintained at 250.0°C, 200.0°C, and 250.0°C, respectively. The oven temperature program was initiated at 35.0°C (held for 4 min), raised to 240.0°C at a rate of 10.00°C/min, and then further increased to 300.0°C at 8.00°C/min, with a final hold time of 8 min.

This technique is used for the quantitative and qualitative determination of the components of a homogeneous mixture. The operating principle of this apparatus is based on transferring the separated compounds by gas chromatography using a carrier gas to the mass spectrometer. There, the sample components are fragmented and decomposed into ions of different masses. The separation process is then performed according to their mass, and the compounds are identified by comparing the obtained mass spectra with previously known spectra.

The analysis was carried out according to a specific programmed temperature and the carrier gas used.

III.7-Biological study of essential oils:

III.7.1 Insecticidal activity:

III.7.1.1 -Material and methods:

To evaluate the insecticidal efficacy of the extracted essential oils against insect pests, the primary biological and experimental components were carefully selected. Tableau 9 systematically organizes the details of the plant materials, the target insect, and the laboratory equipment employed in this bioassay.



Figure (III-4): *Tenebrio molitor*

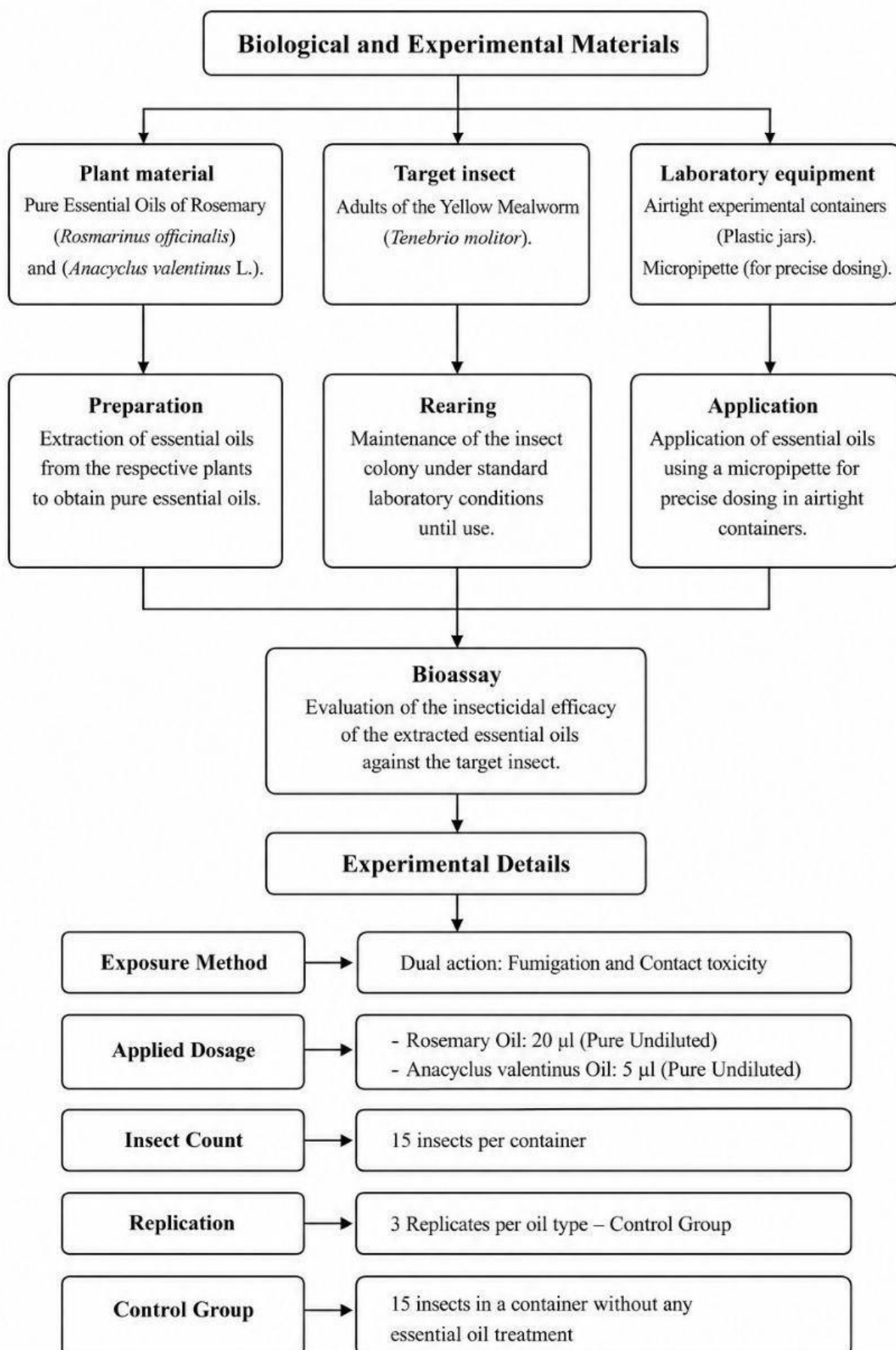




Figure (III-5): *Tenebrio molitor*.



Figure (III-6): preparation of the insects



Figure (III-7): fumigation method.



Figure (III-8): contact method.

III.7.2 Antibacterial activity:

The antibacterial activity of three essential oil samples—*Rosmarinus officinalis* essential oil, *Anacyclus valentinus* essential oil, and a 1:1 (v/v) mixture of both oils—was evaluated using the disk diffusion method against three bacterial strains: *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (*S. aureus*). This assay was conducted to compare the antibacterial effectiveness of the individual oils and their combination against both Gram-positive and Gram-negative bacteria. Sterile paper disks were impregnated with 50 μ L of each sample and placed on inoculated agar plates. The plates were incubated at 37°C for 24 hours, and the inhibition zones were measured in millimeters (mm).

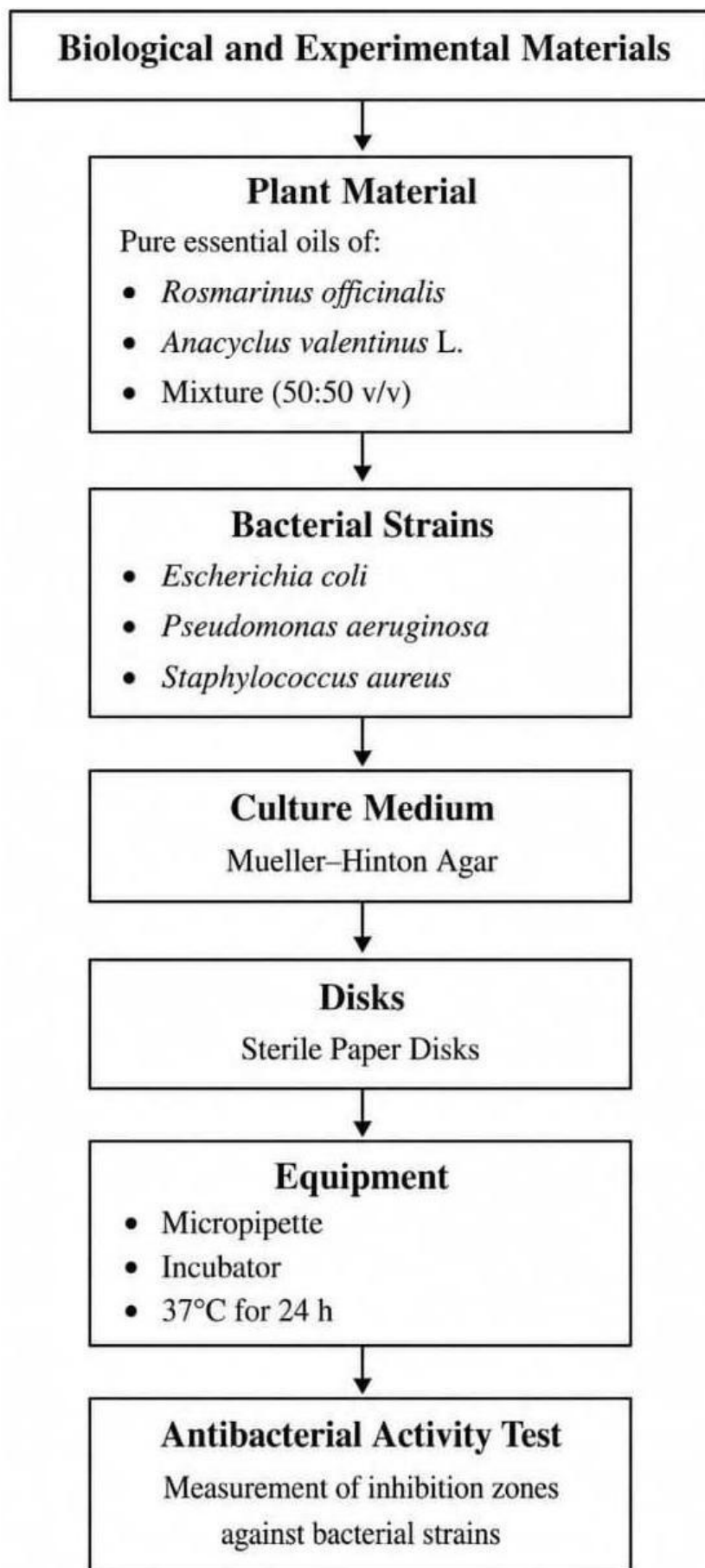




Figure (III-9): Petri dishes and test tubes for bacterial inoculation and cultivation

CHAPTER IV: Results and discussion

IV. Results and discussion

IV.1 Extractions

IV.1.1 -Essential oil yield :

The essential oil yield was calculated as a weight percentage (w/w) based on the mass of the dry plant material (2000g) used for extraction.

$$R(\%) = (\text{MHE} / \text{MS}) \times 100$$

$$R(\%) = (0.45/2000) \times 100=0.02\%$$

After 3 hours of hydrodistillation, the average yield of *Anacyclus valentinus L* essential oil was 0.02%.

$$R(\%) = (7.8/1000) \times 100=0.78\%$$

The average yield of *Rosmarinus officinalis* essential oil was **0.78%** based on the mass of the dry Plant(1000g).

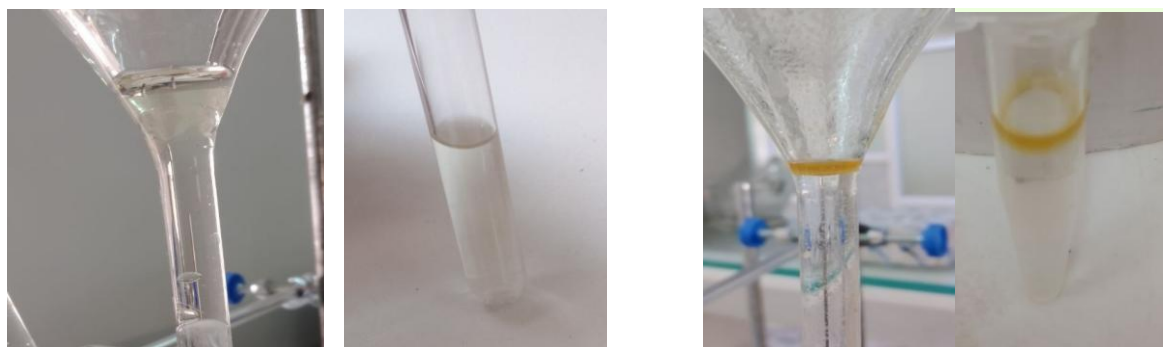


Figure (IV-1): Essential oil of *rosmarinus officinalis* **Figure (IV-2): Essential oil of *anacyclus valentinus*.**

IV.1.1.1 -Discussion of Extraction Yields

A significant difference was observed between the essential oil yields of *Rosmarinus officinalis* (0.78%) and *Anacyclus valentinus L* (0.02%). The higher yield of rosemary may be attributed to the favorable climatic conditions of the Kherrata region, which promote the synthesis and accumulation of volatile compounds. In contrast, the low yield of *A. valentinus* could be related to the semi-arid conditions of the Djelfa region, where environmental stress may limit essential oil production. Furthermore, factors such as species characteristics and the phenological stage at harvest can strongly influence oil yield. Overall, these results demonstrate the important role of environmental and biological factors in determining essential oil productivity.

IV.1.1.2 - Organoleptic Properties of the Essential Oils:

The essential oil obtained from the (*Rosmarinus officinalis*) of the Kherrata region and from the

Anacyclus valentinus L of the Djelfa samples were characterized by their specific sensory profiles. These properties provide initial indications of the oil's quality and its chemical nature:

Table (IV-1): Organoleptic Properties of the Essential Oils

<i>Anacyclus valentinus</i> L	
Color	Yellow/ Orange .
Odor	Intense and persistent, with a characteristic aromatic scent typical of the <i>Anacyclus valentinus</i> species.
Density	Lighter than water.
Aspect	A clear, limpid liquid, indicating a successful extraction and proper filtration.
<i>Rosmarinus officinalis</i>	
Color	-Colorless to very pale yellow.
Odor	- The oil possesses a very strong, fresh, and camphoraceous aroma, with characteristic woody notes.
Density	-The oil is lighter than water and was observed to be insoluble in the aqueous phase, forming a distinct layer during the collection process.
Aspect	-The oil appears as a clear, limpid, and homogeneous liquid, which indicates a high-quality extraction process free from water residues or impurities.

IV.1.1.3 -Physicochemical properties:

To evaluate the quality of the extracted *A. valentinus* L essential oil, its relative density was determined. The obtained value is presented in table(IV-2).

Table (IV-2): physicochemical properties of *A. valentinus* L

Property	Result	Value according to ANFOR standard
Relative density	0.9g/mL	0.907-0.920g/mL

The physicochemical analysis of the extracted essential oil from *anacyclus valentines* reveals its quality and compliance with international standards:

-Relative Density: According to our study,

The relative density of *Anacyclus valentinus* essential oil was determined by measuring the mass of a known volume of the extracted oil. A volume of 0.5 mL of essential oil was weighed using an analytical balance, and its mass was found to be 0.45 g. The density was then calculated using the following equation:

$$\rho = m/V$$

Where:

ρ = density (g/mL)

m = mass of the essential oil (g)

V = volume of the essential oil (mL)

Substituting the measured values:

$$\rho = 0.45/0.5 = 0.90 \text{ g/mL}$$

The relative density (d_{20}) was then calculated using the following equation:

$$d_{20} = \rho_{EO} / \rho_{water}$$

Considering the density of water at 20°C as 1.00 g/mL:

$$d_{20} = 0.90/1.00 = 0.90 .$$

Table (IV-3): physicochemical properties of *Rosmarinus officinalis* oil.

Property	Result	Value according to ANFOR standard
Relative density	0.92g/mL (calculated from our study)	0.894-0.912g/mL

According to the results shown in Table (IV-3), the relative density of the *Rosemary* essential oil was determined by measuring the mass of a known volume of the extracted oil. A volume of 8.5 mL of essential oil was weighed using an analytical balance, and its mass was found to be 7.8 g. The density was calculated using the following equation:

$$\rho = m/V$$

Where:

ρ = density (g/mL)

m = mass of the essential oil (g)

V = volume of the essential oil (mL)

Substituting the measured values:

$$\rho = 7.8/8.5 = 0.92 \text{ g/mL}$$

The relative density (d_{20}) was then calculated using the following equation:

$$d_{20} = \rho_{EO}/\rho_{water}$$

Considering the density of water at 20 °C as 1.00 g/mL:

$$d_{20} = 0.92/1.00 = 0.92$$

The obtained relative density (0.92 g/mL) falls within the AFNOR reference range (**0.894–0.912 g/mL**), indicating that the essential oil possesses acceptable physicochemical quality.

IV.2-Results of GC-MS:

The chemical composition of the essential oil was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS). The identified compounds were determined based on their retention time and mass spectra comparison with library data.

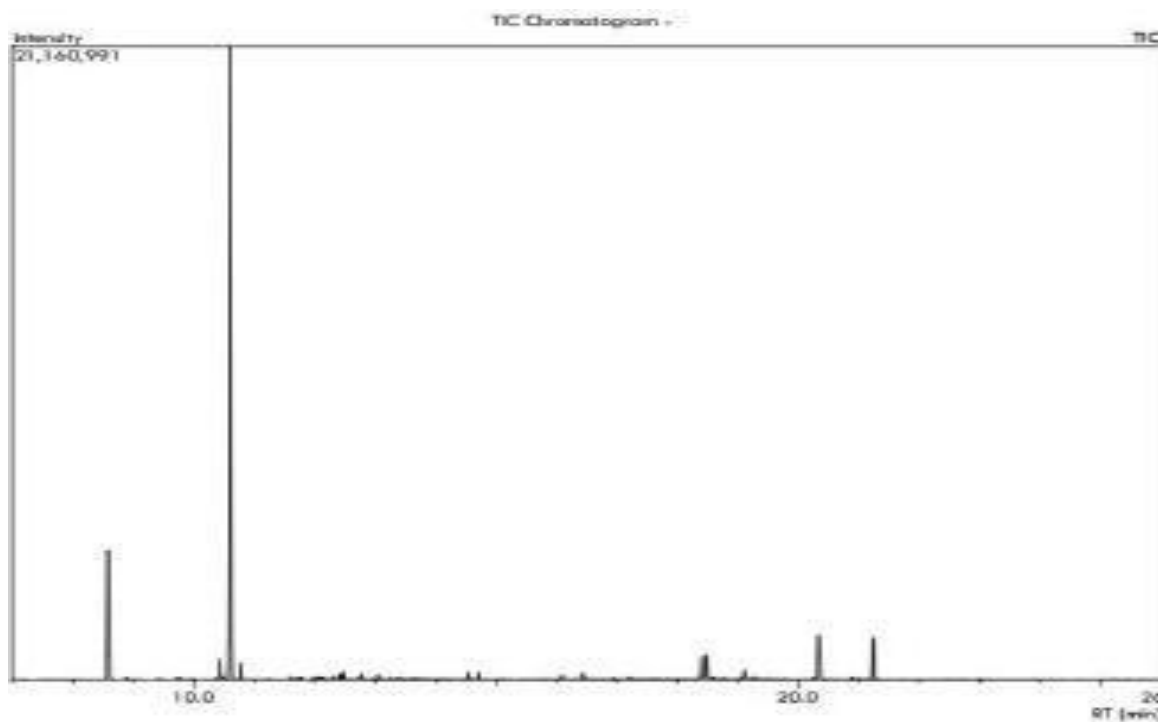
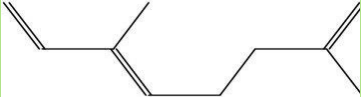
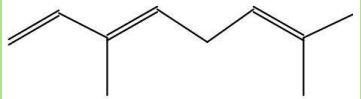
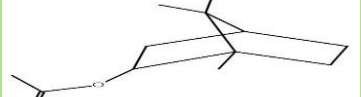
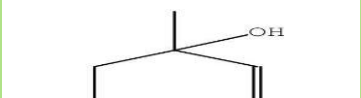
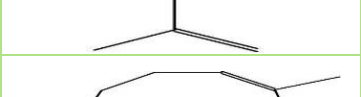


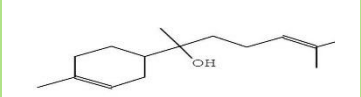

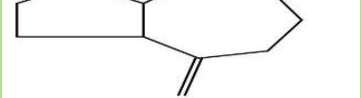
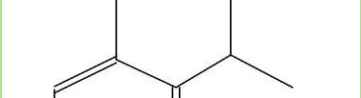


Figure (IV-3): GC chromatogram of *Rosemary oil*.

Table (IV-4): Main chemical constituents of *Rosmarinus officinalis* essential oil identified by GC–MS analysis.

N	R. time	Area	Compounds	Mass/Base peak	Structure
1	8.577	12.01	2-Pinene	255/93.10	
2	8.879	0.20	Camphene	229/93.10	
3	10.436	1.87	D-Limonene	312/68.10	
4	10.484	0.26	Eucalyptol	243/81.10	

5	10.607	60.22	(3E)-3,7-dimethylocta-1,3,7-triene	255/93.10	
6	10.781	1.41	beta.-Ocimene	274/93.10	
7	14.547	0.58	Bornyl acetate	232/95.10	
8	9.743	0.24	1,7-Octadien-3-ol, 3,7-dimethyl-	220/81.05	
9	16.440	0.53	Caryophyllene	282/91.10	
10	19.272	0.33	Ledol	349/73.05	
11	19.544	0.11	alpha.-Bisabolol	312/69.05	
12	18.402	2.17	1H-Cycloprop[e]azulene-7-ol, decahydro-1,1,7-trimethyl-	349/73.05	
13	10.355	0.13	o-Cymene	251/119.10	
14	14.711	0.53	Phenol, 2-methyl-5-(1-methylethyl)	304/135.10	
15	16.098	0.38	Benzene, 1,2-dimethoxy-4-(2-propenyl)-	312/193.10	

16	19.111	0.98	Benzene, 1,2,3-trimethoxy-5-(1-propenyl)-, (E)	312/193.10	
17	18.783	0.15	5-Oxatricyclo[8.2.0.0(4,6)]dodecane, 4,12,12-trimethyl	273/79.05	
18	12.739	0.25	Furan, 2-methoxy	274/98.10	
19	20.272	0.17	Triethanolamine triacetate	258/87.05	

The GC–MS analysis of rosemary essential oil revealed a complex mixture of volatile compounds belonging mainly to three chemical groups: monoterpenes, sesquiterpenes, and aromatic compounds. Monoterpenes represented the dominant class, including α -pinene, β -pinene, camphene, limonene, 1,8-cineole, β -ocimene, linalool, and bornyl acetate.

This predominance is characteristic of rosemary essential oil and contributes to its typical aromatic profile.

Among the identified compounds, the compound (3E)-3,7-dimethylocta-1,3,7-triene was found in high proportion, indicating that the oil is particularly rich in monoterpene hydrocarbons. However, this result differs from most published studies on *R. officinalis* e.oil [23], where 1,8-cineole, linalool, and bornyl acetate are usually reported as the major constituents. This discrepancy may be attributed to analytical factors during GC-MS analysis, such as possible **carry-over** from a previous sample or slight contamination of the instrument, which may have influenced the automatic identification of compounds. Oxygenated monoterpenes such as 1,8-cineole, linalool, and bornyl acetate were also detected, reflecting the chemical diversity of the oil. The sesquiterpene fraction was represented by compounds such as β -caryophyllene, ledol, α -bisabolol, and cycloprop[e]azulene derivatives. Although present in lower amounts than monoterpenes, these compounds contribute to the overall complexity of the essential oil composition.

In addition, several aromatic compounds were identified, including o-cymene, methyl eugenol, carvacrol, and E-asarone. These constituents, despite occurring in relatively smaller proportions, further enrich the chemical profile of the oil.

Short conclusion:

The GC–MS analysis of rosemary essential oil showed a chemically diverse composition dominated by monoterpenes, which constituted the major fraction of the oil. The most abundant compounds included (3E)-3,7-dimethylocta-1,3,7-triene, α -pinene, β -pinene, 1,8-cineole, and linalool. Smaller

amounts of sesquiterpenes and aromatic compounds were also detected, contributing to the complexity of the oil. Overall, the predominance of monoterpenes is characteristic of rosemary essential oil and may be associated with its biological and aromatic properties.

Chemical Composition of Essential Oil

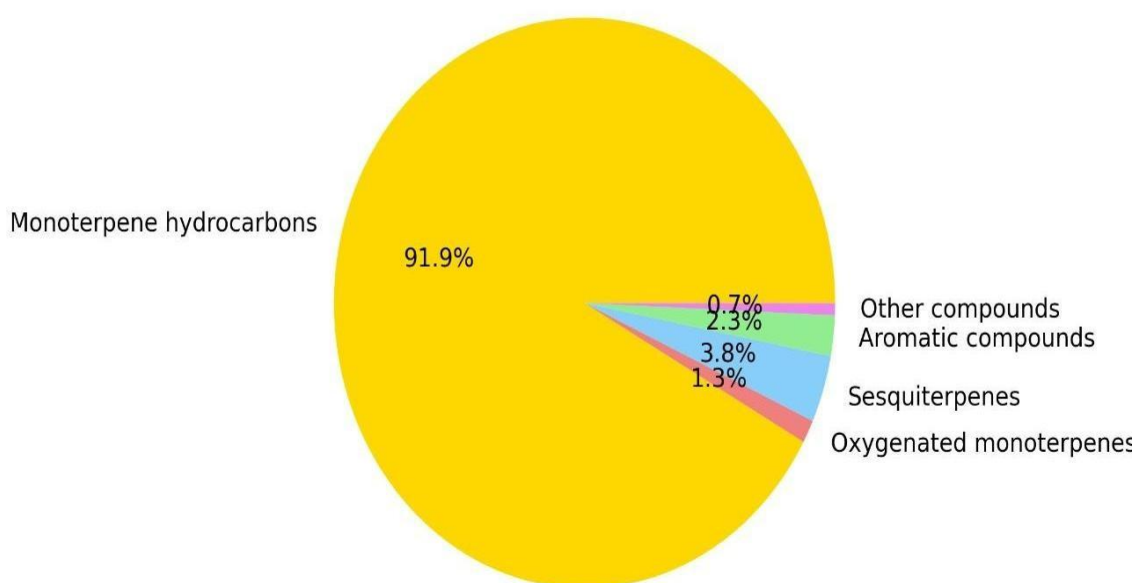


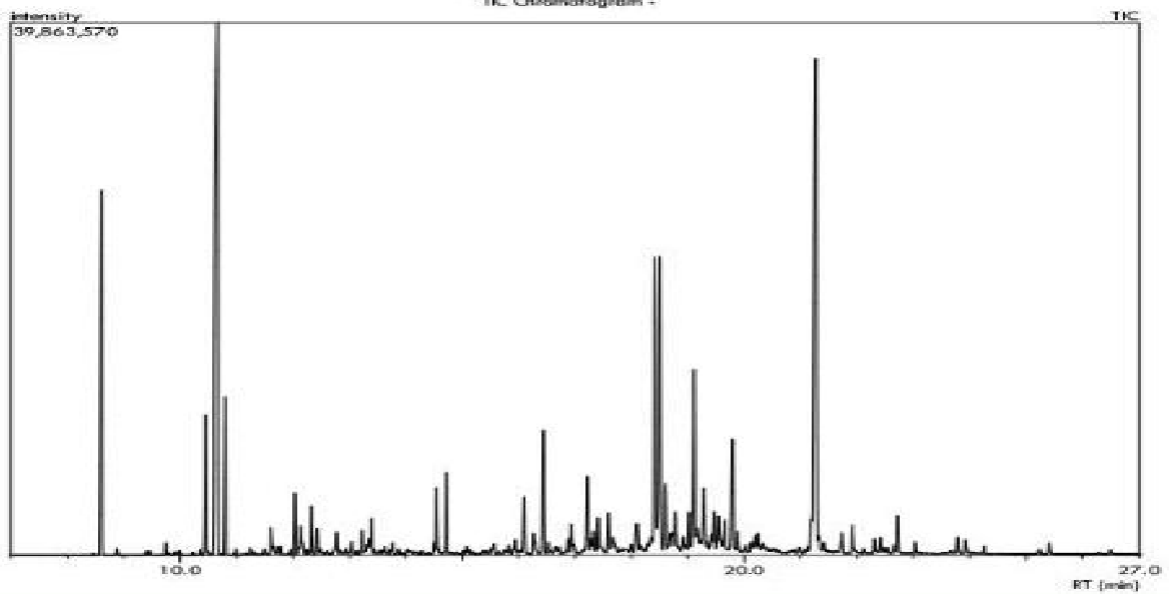
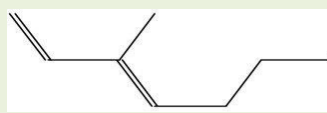
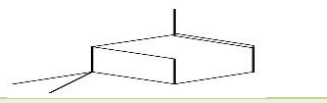

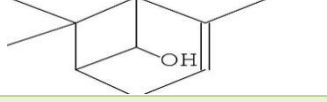
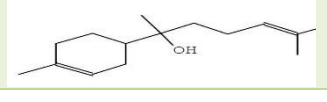
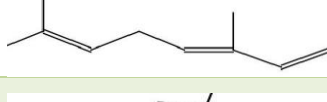

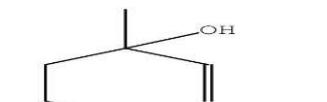



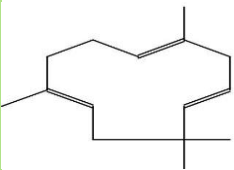
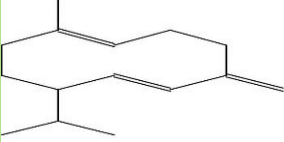
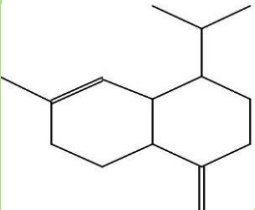
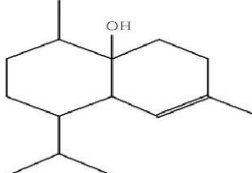
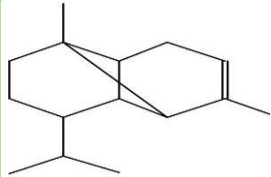
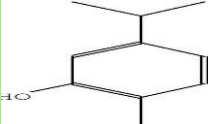
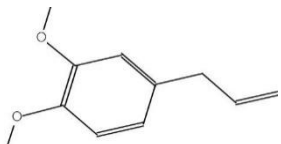
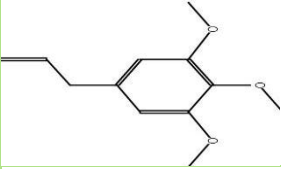
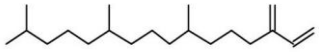
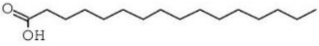
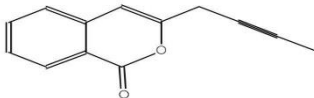
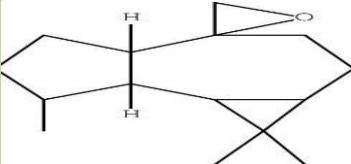
Figure (IV-4): GC chromatogram of *A.valentinus L*

Table (IV-5): Main chemical constituents of *A.valentinus L* essential oil identified by GC-MS analysis.

N	R. time	Area	Compounds	Mass/ Base peak	Structure
1	10.65 8	20.45	3E)-3,7-dimethylocta-1,3,7-triene	255/93.10	
2	8.600	7.17	2-Pinene	255/93.10	
3	10.44 6	2.13	D-Limonene	312/68.10	
4	19.78 9	2.09	trans-Chrysanthenol	228/69.10	
5	19.54 7	1.01	alpha-Bisabolol	319/69.05	
6	10.76 7	2.33	1,3,6-Octatriene, 3,7-dimethyl-, (E)	238/93.05	
7	14.54 8	1.06	Bornyl acetate	232/91.10	
8	9.750	0.19	1,7-Octadien-3-ol, 3,7-dimethyl-	220/82.10	
9	12.77 1	0.35	endo-Borneol	201/93.10	
10	12.42 6	0.41	(+)-2-Bornanone	264/95.10	
11	18.15 5	0.28	Piperitenone oxide	225/161.1 5	

12	16.878	0.21	alpha-Humulene	276/119.10	
13	17.216	1.32	(-)-Germacrene D	274/95.10	
14	16.985	0.15	gamma-Muureylene	225/161.15	
15	18.975	0.15	Cubenol	276/119.10	
16	15.835	0.13	Copaene	214/119.10	
17	14.713	1.27	Phenol, 2-methyl-5-(1-methylethyl)-	304/132.10	
18	16.100	0.94	Benzene, 1,2-dimethoxy-4-(2-propenyl)-	300/178.10	
19	19.116	3.99	Benzene, 1,2,3-trimethoxy-5-(1-propenyl)-, (E)-	332/208.15	
20	21.122	0.17	Neophytadiene	316/89.05	
21	22.313	0.25	n-Hexadecanoic acid	334/73.05	
22	21.739	0.41	Capillarene	316/89.05	

23	18.785	0.97	alloaromandendrene oxide-(1)	294/67.05	
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The GC-MS analysis of the essential oil of *Anacyclus valentinus*L revealed a diverse chemical composition dominated by monoterpenes. The major compound identified was

(3E)-3,7-dimethylocta-1,3,7-triene (20.45%), followed by α -pinene (7.17%).

However, this result differs from most published reports on *Anacyclus* essential oils[24] that shows that the major compounds are alpha-Humulene and (-)-Germacrene D. This discrepancy may be attributed to analytical factors during GC-MS analysis, such as possible carry-over from a previous sample, slight contamination of the instrument, or limitations of the automatic compound identification. Therefore, the detected compound may not necessarily represent the true major constituent of the essential oil.

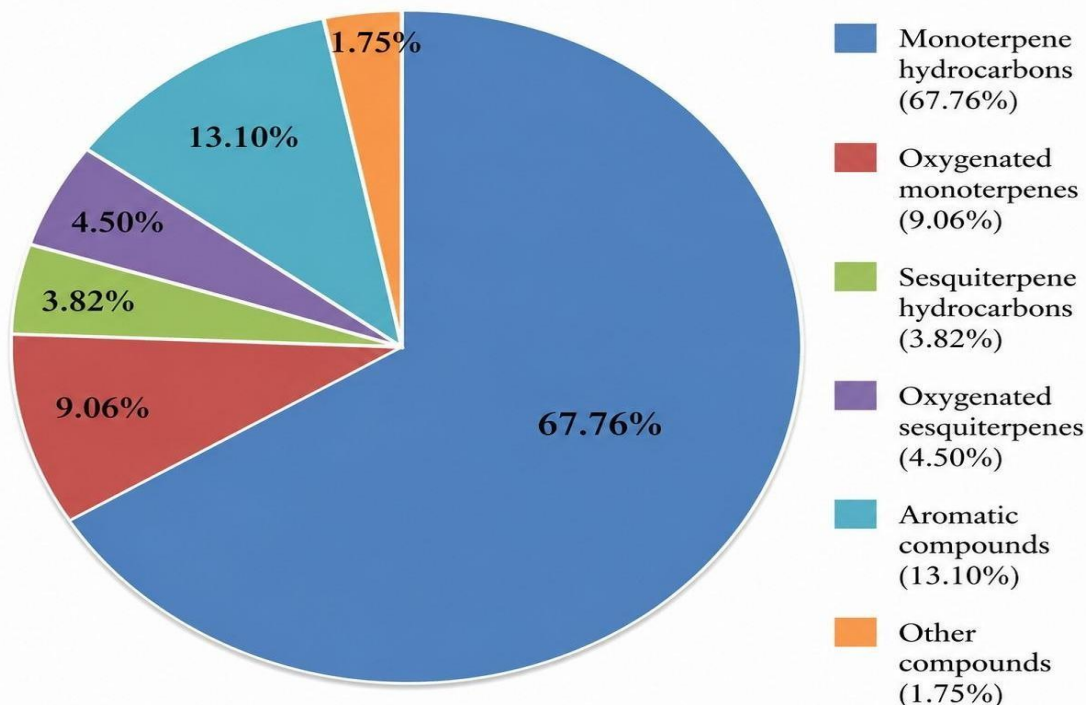
Other important monoterpenes detected included D-limonene, β -ocimene, bornyl acetate, and camphor.

The oil also contained several sesquiterpenes, such as β -caryophyllene, α -bisabolol, and germacrene D, as well as aromatic compounds including carvacrol, methyl eugenol, and E-asarone.

Overall, the essential oil was characterized by a predominance of monoterpenes, followed by sesquiterpenes and aromatic compounds. This composition is generally consistent with previous reports on species of the Asteraceae family, although quantitative variations may occur due to environmental conditions and extraction methods.

The GC-MS analysis of *Anacyclus valentinus* essential oil revealed a complex chemical profile dominated by monoterpenes, with (3E)-3,7-dimethylocta-1,3,7-triene (20.45%) and α -pinene (7.17%) as the major constituents. Other monoterpenes, together with several sesquiterpenes and aromatic compounds, contributed to the overall composition of the oil. The predominance of monoterpenes indicates that the essential oil possesses a characteristic volatile profile, while the presence of sesquiterpenes and aromatic compounds enhances its chemical diversity and may contribute to its biological activities.

Relative Percentage of Chemical Classes in *Anacyclus valentinus* Essential Oil



IV.3 -Results of the insecticidal activity:

The evaluation of the insecticidal activity of *Rosmarinus officinalis* and *Anacyclus valentinus* essential oils against *Tenebrio molitor* adults revealed varying degrees of toxicity. The results, recorded over a period of 72 hours, indicate that both oils possess significant bio-pesticidal properties. The effectiveness of the treatment was found to be dependent on both the plant species and the duration of exposure. The following section details the mortality rates observed and compares the potency of the two essential oils.

Table (IV-6): Mortality rate of *Tenebrio molitor* adults treated with oils by fumigation method.

<i>Anacyclus valentinus</i> L						
24h						
	A1	A2	A3	TA1	TA2	TA3
Male	02	03	01	00	00	00
Female	01	02	00	00	00	00
48h						

Male	04	05	05	01	00	00
Female	03	02	02	00	00	00
72h						
Male	05	06	05	02	01	02
Female	03	03	04	00	00	00
<i>Rosmarinus officinalis</i>						
24h						
	R1	R2	R3	TR1	TR2	TR3
Male	04	03	02	00	00	00
Female	02	02	02	00	00	00
48h						
Male	08	06	08	00	00	00
Female	07	09	07	00	00	00
72h						
Male	08	06	08	01	02	00
Female	07	09	07	00	00	00

IV.3.1 - Insecticidal Activity of *Rosmarinus officinalis* Essential Oil

The insecticidal bioassay against *Tenebrio* adults showed that *Rosmarinus officinalis* essential oil exhibited toxic effects throughout the exposure periods (24, 48, and 72 h). Mortality increased with exposure time, whereas the control groups showed little or no mortality, confirming that the observed effects were due to the essential oil.

The insecticidal activity of *R. officinalis* may be related to its chemical composition revealed by GC–MS analysis. This oil was dominated by (3E)-3,7-dimethylocta-1,3,7-triene (60.22%), **together** with α -pinene, D-limonene, eucalyptol, and β -ocimene. These monoterpenes are known for their high volatility and their ability to penetrate the insect cuticle and interfere with the nervous and respiratory systems, leading to mortality.

Furthermore, the activity of *R. officinalis* may result from synergistic interactions among its major monoterpenes, particularly α -pinene, D-limonene, and eucalyptol, which have been widely reported

as bioactive compounds with insecticidal and repellent properties. The abundance of these volatile constituents likely contributes to the overall toxicity of rosemary oil against *Tenebrio* adults.

IV.3.2 - Insecticidal Activity of *Anacyclus valentinus* L Essential Oil

The insecticidal bioassay against *Tenebrio* adults showed that *Anacyclus valentinus* essential oil induced mortality that increased progressively with exposure time (24, 48, and 72 h). The control groups showed little or no mortality, confirming that the observed effects were attributable to the essential oil.

The chemical composition of *A. valentinus* essential oil revealed by GC–MS showed the presence of (3E)-3,7-dimethylocta-1,3,7-triene (20.45%) as well as other bioactive constituents such as α -pinene, D-limonene, and β -ocimene. In addition, this oil contained sesquiterpenes, including (-)-germacrene D, α -humulene, and alloaromadendrene oxide-(1). These compounds are generally less volatile than monoterpenes and may exert toxic effects on insects.

The biological activity of *A. valentinus* essential oil can be associated with the presence of these bioactive constituents and their individual or combined effects on insect physiology, including interference with nervous and metabolic functions, which contributes to mortality in *Tenebrio* adults.



Figure (IV- 5): Result of fumigation method (rosemary oil)



Figure (IV-6): Result of fumigation method (*A.valentinus* oil)

Table (IV-7): Mortality rate of *Tenebrio molitor* adults treated with rosemary E. oils by contact method.

<i>Rosmarinus officinalis</i>						
24h						
	R1	R2	R3	TR1	TR2	TR3
Male	10	12	08	00	00	00
Female	05	03	07	00	00	00
48h						
Male	10	12	08	00	00	00
Female	05	03	07	00	00	00
72h						
Male	10	12	08	00	00	00
Female	10	03	07	00	00	00

**Figure(IV-7): Result of contact method (rosemary oil)**

IV.4 Evaluation of the Insecticidal Activity of Rosmarinus Essential Oil via Contact Method:

The high insecticidal activity of *Rosmarinus officinalis* essential oil observed in the contact bioassay can be explained by its chemical composition identified through GC–MS analysis. The oil was characterized by a predominance of monoterpenes, particularly (3E)-3,7-dimethylocta-1,3,7-triene, α -pinene, β -pinene, 1,8-cineole, and limonene. These compounds are known for their high volatility and their ability to penetrate the insect cuticle, resulting in rapid toxic effects.

The complete mortality observed in males within the first 24 hours suggests a strong and immediate

toxic action of these monoterpenes. In particular, α -pinene and 1,8-cineole have been reported as major contributors to the insecticidal properties of rosemary essential oil due to their effects on the insect nervous system, leading to paralysis and death.

Moreover, oxygenated monoterpenes such as linalool and bornyl acetate may enhance the effectiveness of the oil through synergistic interactions with the major constituents. The presence of carvacrol, a phenolic compound with recognized insecticidal activity, may further contribute to the observed mortality.

Sesquiterpenes such as β -caryophyllene and α -bisabolol may also participate in the overall biological activity of the oil. Therefore, the strong insecticidal effect of *Rosmarinus officinalis* essential oil is likely the result of the combined action of its major and minor constituents, particularly the monoterpene-rich fraction, which was responsible for the rapid mortality recorded during the contact exposure assay.

-Results of the antibacterial activity:

To evaluate the antibacterial potential of the isolated volatile oils and their synergistic effects, an in vitro screening was conducted against both Gram-positive and Gram-negative bacterial strains. (TableIV-8) presents the experimental findings regarding the inhibition zone diameters (mm) generated by Rosemary essential oil, *Anacyclus valentinus* essential oil, and their 50:50 (v/v) mixture.

Table (IV-8): Inhibition zone diameters (mm) of essential oils against different bacterial strains.

Bacterial strain	<i>Rosemary essential</i>	<i>Anacyclus valentinus L</i>	Mix (50:50v/v)
<i>Staphylococcus aureus</i>	5mm	22 mm	21mm
<i>Pseudomonas aeruginosa</i>	10mm	16 mm	15mm
<i>Escherichia coli</i>	15mm	12mm	15mm

- The antibacterial activity of the essential oils is strongly correlated with their **chemical composition identified by GC–MS analysis**.
- *Rosmarinus officinalis* oil showed **low activity against *Staphylococcus aureus* (5 mm)**, which may be due to the dominance of a single compound **(3E)-3,7-dimethylocta-1,3,7-triene (60.22%)**, with a relatively less balanced synergistic profile.
- However, it showed **moderate activity against *Pseudomonas aeruginosa* (10 mm)** and **higher activity against *E. coli* (15 mm)**, likely due to the presence of bioactive monoterpenes such as **α -pinene, D-limonene, eucalyptol, and β -ocimene**.

- *Anacyclus valentinus* exhibited the **highest activity against *Staphylococcus aureus* (22 mm)** and strong activity against *Pseudomonas aeruginosa* (16 mm).
- This higher activity may be related to its **more diverse chemical profile**, including **sesquiterpenes such as germacrene D and α -humulene**, which can enhance membrane disruption and antibacterial synergy.
- The **50:50 (v/v) mixture** showed **intermediate activity**, suggesting a **dilution or interaction effect** between the two oils:
 - *S. aureus*: 21 mm (high activity retained from *A. valentinus*)
 - *P. aeruginosa*: 15 mm
 - *E. coli*: 15 mm
- Overall, the antibacterial activity is influenced not only by the major compounds but also by **synergistic interactions between monoterpenes and sesquiterpenes** identified by GC–MS.
- *A. valentinus* *L* appears to have a more effective antibacterial profile, likely due to its **chemical diversity and higher proportion of active sesquiterpenes**, while *R. officinalis* activity is more dependent on its dominant monoterpene.

CONCLUSION

Conclusion

Conclusion

In conclusion, this study aimed to extract essential oils from two plants, (*Rosmarinus officinalis*) and (*Anacyclus valentinus*L), then chemically characterize them and evaluate some of their biological activities, particularly antibacterial and insecticidal effects.

The results of gas chromatography–mass spectrometry (GC-MS) analysis showed that the studied essential oils are characterized by a diverse and rich chemical composition in terpenoid compounds. The essential oil of (*Rosmarinus officinalis*) was dominated by monoterpenes, with several major compounds identified, including (E)-3,7-dimethylocta-1,3,7-triene, α -pinene, β -pinene, camphene, limonene, 1,8-cineole, β -ocimene, linalool, and bornyl acetate, in addition to some sesquiterpenes such as β -caryophyllene and α -bisabolol, and some aromatic compounds. The essential oil of *Anacyclus valentinus* L also showed a diverse chemical composition containing a mixture of monoterpenes and sesquiterpenes, including germacrene D, α -humulene, and other compounds that contributed to the chemical fingerprint of the oil.

Insecticidal activity tests against *Tenebrio molitor* showed that both essential oils exhibited significant biological activity, with mortality rates increasing with exposure time in both fumigation and contact methods. The essential oil of rosemary demonstrated high effectiveness, which can be attributed to its richness in monoterpenes known for their ability to affect the nervous and respiratory systems of insects. The essential oil of *Anacyclus valentinus* also showed insecticidal activity, which is attributed to the presence of several bioactive compounds and their individual or synergistic effects. Regarding antibacterial activity, the results showed that the essential oil of *Anacyclus valentinus* L exhibited the best activity against *Staphylococcus aureus* with an inhibition zone of 22 mm, and also showed good activity against *Pseudomonas aeruginosa* with an inhibition zone of 16 mm. The essential oil of rosemary showed variable activity depending on the tested bacterial strain, while the mixture of both oils maintained antibacterial activity at different levels. These results indicate that biological activity is closely related to the chemical composition of essential oils and the synergistic interactions among their various constituents.

Based on the obtained results, it can be concluded that the essential oils extracted from rosemary and *Anacyclus valentinus*L represent a promising natural source of bioactive compounds and have significant potential in controlling pathogenic microorganisms. This study also highlights the importance of valorizing local medicinal and aromatic plants and utilizing them to develop environmentally friendly alternatives to conventional chemicals and pesticides.

For future research directions, it is recommended to conduct further studies aimed at isolating and identifying the compounds directly responsible for the observed biological activities, investigating their mechanisms of action, expanding the range of biological assays to include other bacterial and insect species, and evaluating their effectiveness under field and industrial application conditions

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ملخص:

هدفت هذه الدراسة إلى تحديد التركيب الكيميائي وتقييم الفعالية البيولوجية للزيوت العطرية المستخلصة من نبتتين طبيبتين هما: إكليل الجبل و القرطوفة تم استخلاص الزيوت العطرية باستعمال جهاز كلينفجر بطريقة التقطير المائي، حيث بلغ مردود الاستخلاص 0.78% بالنسبة لزيت إكليل الجبل و0.02% بالنسبة لزيت القرطوفة تم تحليل التركيب الكيميائي للزيوت العطرية باستعمال تقنية كروماتوغرافيا الغاز المقترنة بمطيافية الكتلة حيث تم التعرف على 19 مركباً كيميائياً في زيت إكليل الجبل و23 مركباً في زيت القرطوفة. وقد أظهرت النتائج أن المركب السائد في كلا الزيتين هو (3E)-3,7-dimethylocta-1,3,7-triene). أما فيما يتعلق بالفعالية البيولوجية، فقد أظهرت النتائج أن الزيتين العطريين يمتلكان نشاطاً مضاداً للحشرات، مع تفوق ملحوظ لزيت إكليل الجبل مقارنة بزيت القرطوفة. كما بينت اختبارات النشاط المضاد للبكتيريا أن زيت القرطوفة أظهر فعالية جيدة ضد السلالات البكتيرية: (*Escherichia coli*, *Pseudomonas aeruginosa* , *Staphylococcus aureus*) في المقابل، أظهر زيت إكليل الجبل نشاطاً مضاداً للبكتيريا تجاه السلالات المختبرة باستثناء (*Staphylococcus aureus*) التي لم يسجل ضدها أي تأثير تثبيطي تشير هذه النتائج إلى أن الزيوت العطرية لنباتي إكليل الجبل و القرطوفة تمثل مصادر واعدة للمركبات الحيوية الفعالة، مما يؤهلها للاستخدام في مجالات مكافحة الحشرات وتطوير بدائل طبيعية مضادة للكائنات الدقيقة

الكلمات المفتاحية: الزيوت العطرية، روزماري، القرطوفة، GC-MS التركيب الكيميائي، النشاط المضاد للبكتيريا، النشاط المضاد للحشرات، النباتات الطبية.

Abstract

This study aimed to investigate the chemical composition and biological activities of essential oils extracted from two medicinal plants, *Rosmarinus officinalis* and *Anacyclus valentinus*L. The essential oils were obtained by hydrodistillation using a Clevenger apparatus. The extraction yields were 0.78% for *R. officinalis* and 0.02% for *A. valentinus*L.

The chemical composition of the essential oils was analyzed using Gas Chromatography–Mass Spectrometry (GC–MS). A total of 19 compounds were identified in the essential oil of *R. officinalis*, whereas 23 compounds were detected in *A. valentinus*. The major constituent identified in both essential oils was (3E)-3,7-dimethylocta-1,3,7-triene.

The biological evaluation revealed that both essential oils exhibited insecticidal activity, with *R. officinalis* showing a stronger effect than *A. valentinus* L. Antibacterial assays demonstrated that the essential oil of *A. valentinus* L exhibited good activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. In contrast, the essential oil of *R. officinalis* showed antibacterial activity against the tested bacterial strains except *Staphylococcus aureus*, for which no inhibitory effect was observed.

These findings suggest that the essential oils of *R. officinalis* and *A. valentinus* L are promising sources of bioactive compounds and could be considered as potential natural agents for insect control and antimicrobial applications.

Keywords: Essential oils; *Rosmarinus officinalis*; *Anacyclus valentinus* L; GC–MS; Chemical composition; Antibacterial activity; Insecticidal activity; Medicinal plants.